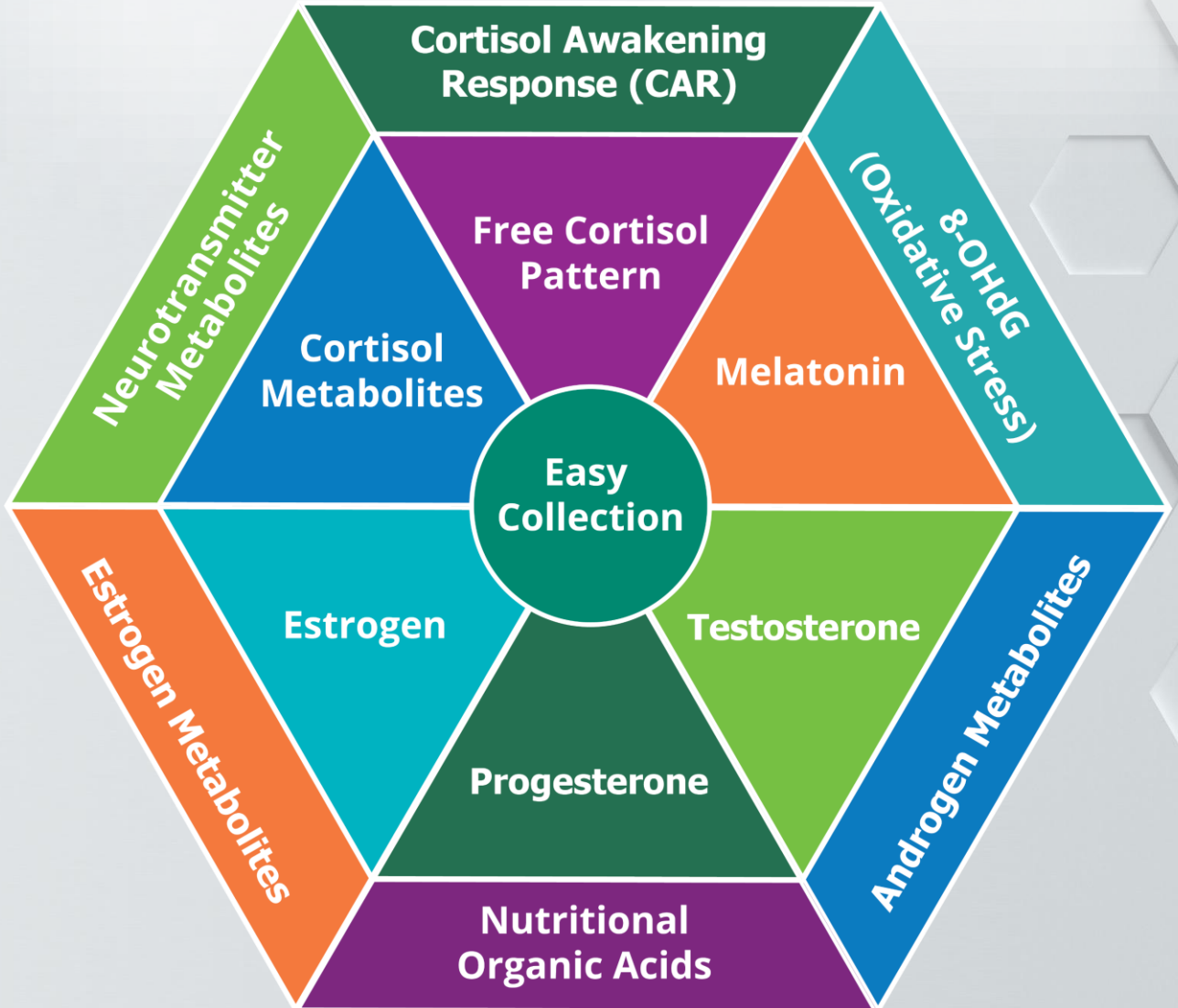
PRECISION ANALYTICAL INC.

SIMPLY · BETTER · TESTING



dutchtest

Dried Urine Test for Comprehensive Hormones

Newman *et al. BMC Chemistry* (2019) 13:20
https://doi.org/10.1186/s13065-019-0539-1

BMC Chemistry

METHODOLOGY ARTICLE

Open Access

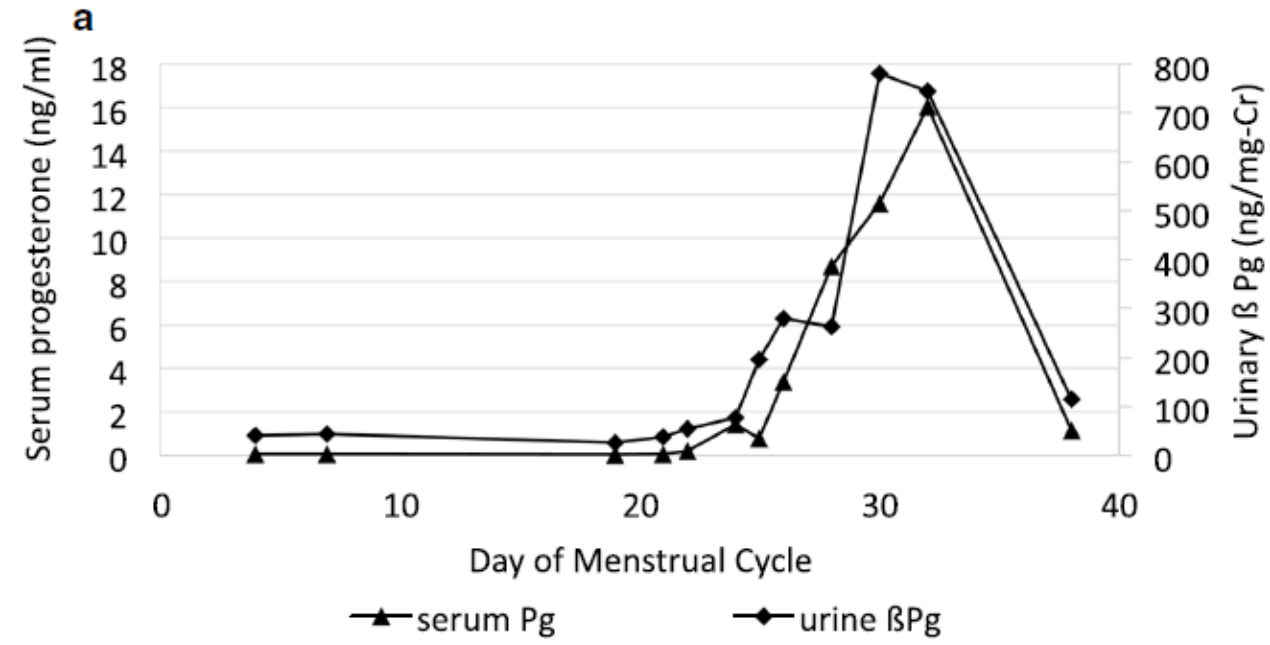
Evaluating urinary estrogen and progesterone metabolites using dried filter paper samples and gas chromatography with tandem mass spectrometry (GC–MS/MS)

Mark Newman[1*], Suzanne M. Pratt[2], Desmond A. Curran[1] and Frank Z. Stanczyk[3]

Abstract

Background: Measuring concentrations of metabolites of estradiol and progesterone in urine, instead of measuring serum concentrations, is common in research and also is used in patient care. The primary aim of this study was to

Conclusions: For estradiol and progesterone, the dried urine assay is a good surrogate for serum testing. The 4-spot method can be used instead of 24-h urine collections and dried urine results are comparable to liquid urine. The dried urine assay is useful for some clinical assessments of hormone disorders and may be useful in large epidemiologic studies due to ease of sample handling.



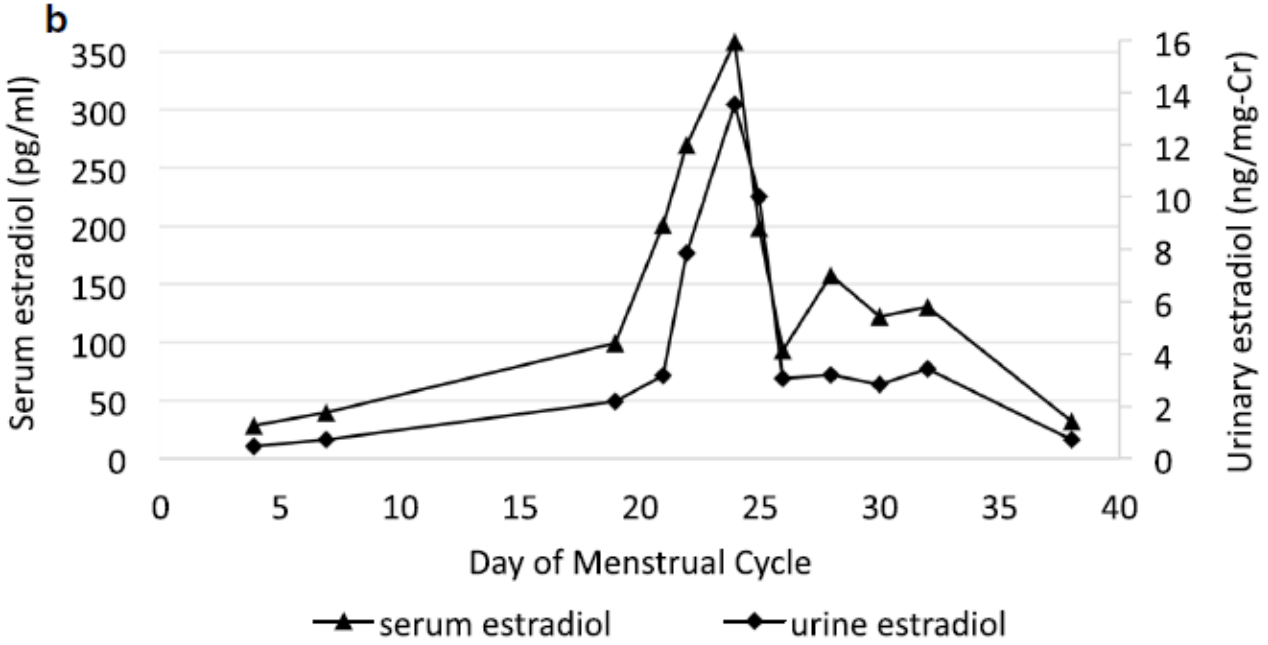


Fig. 2 Hormone profiles of serum progesterone versus urinary β-pregnanediol (**a**) and serum versus urinary estradiol (**b**) in one premenopausal woman's cycle. Metabolites of subject 2. Cr, creatinine; βPg, β-pregnanediol

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METHODOLOGY ARTICLE

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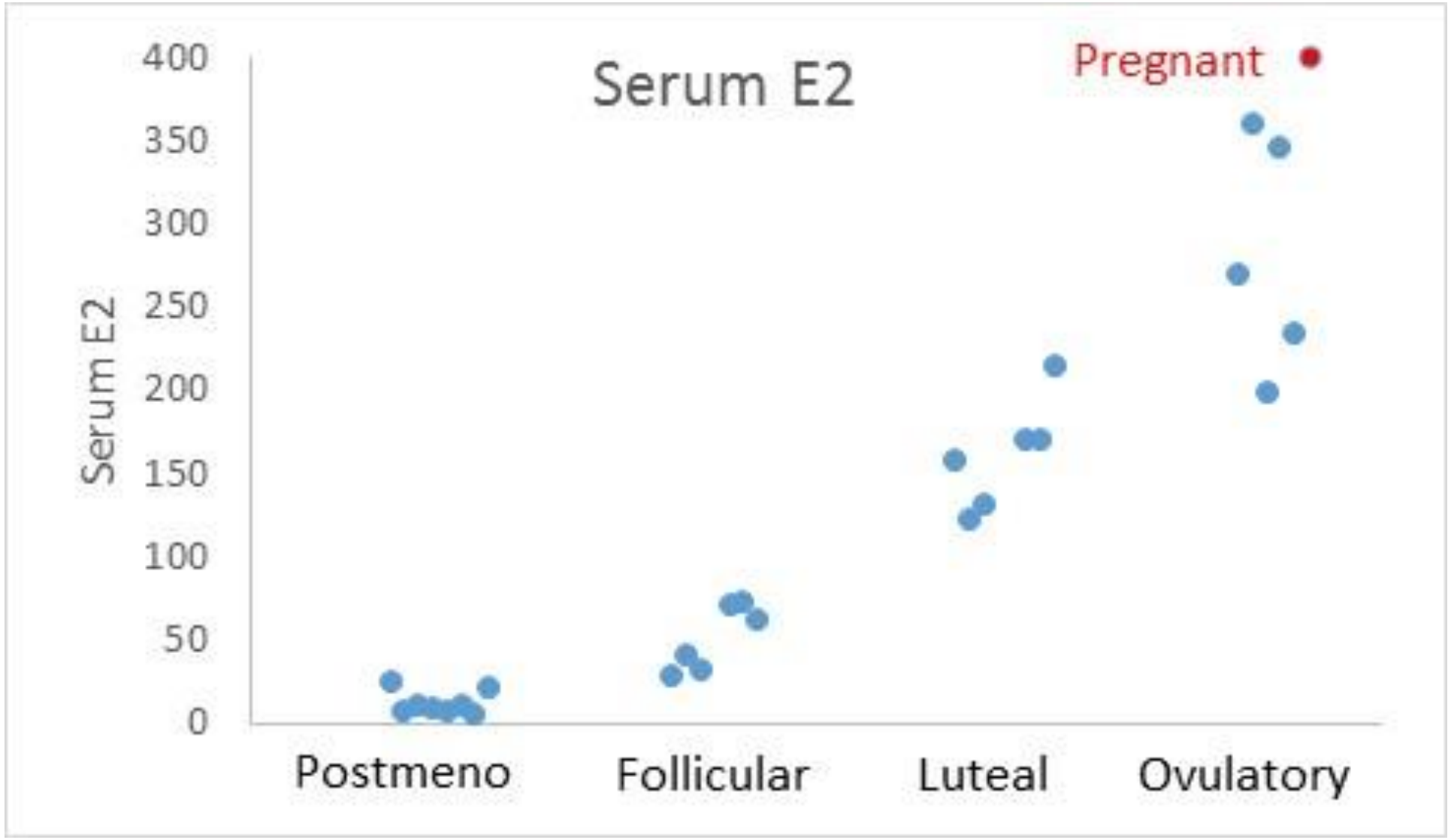
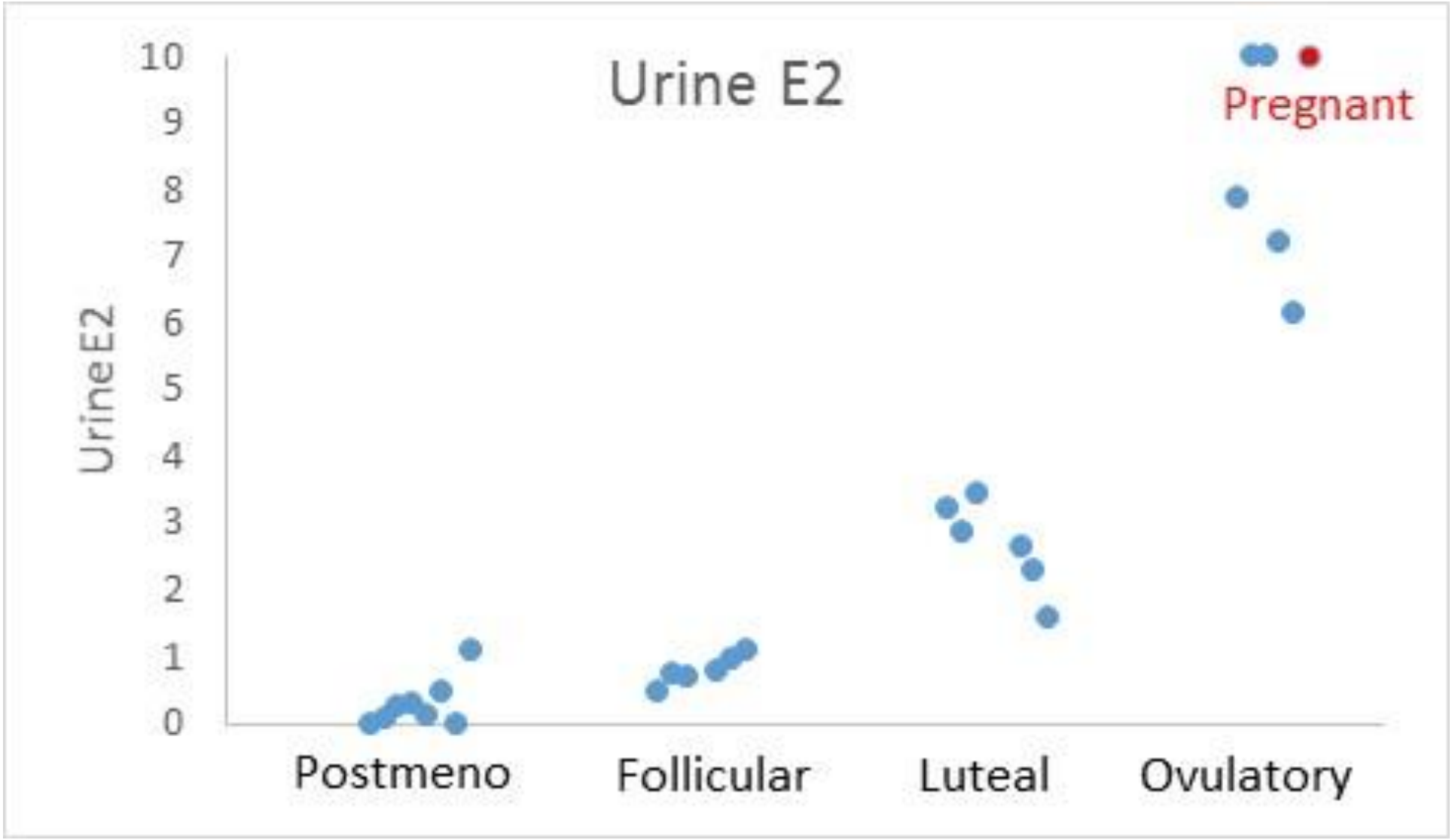
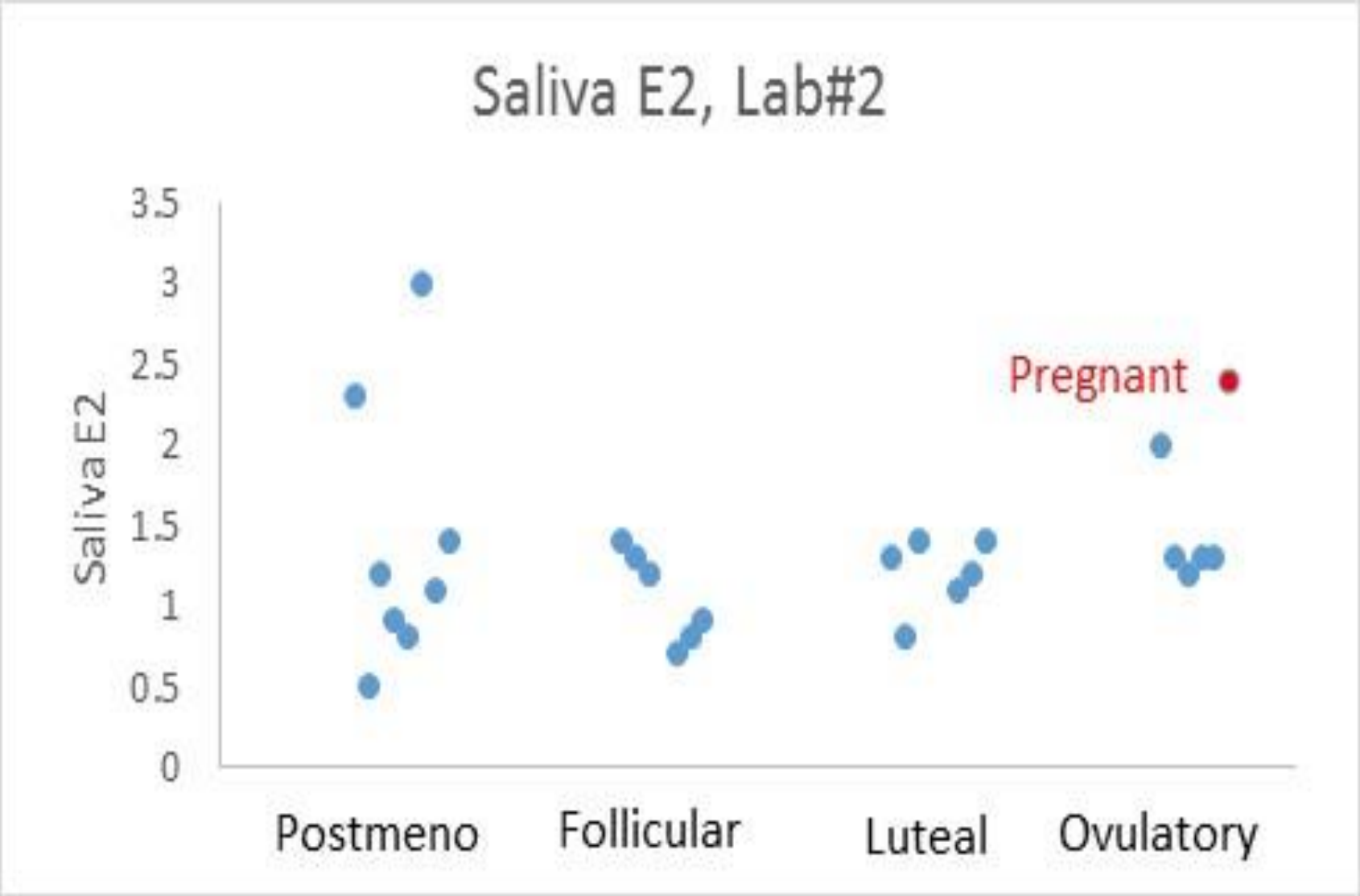
Serum Data


Serum E2
Pregnant
400
350
300
250
200
150
100
50
0
Serum E2
Postmeno
Follicular
Luteal
Ovulatory

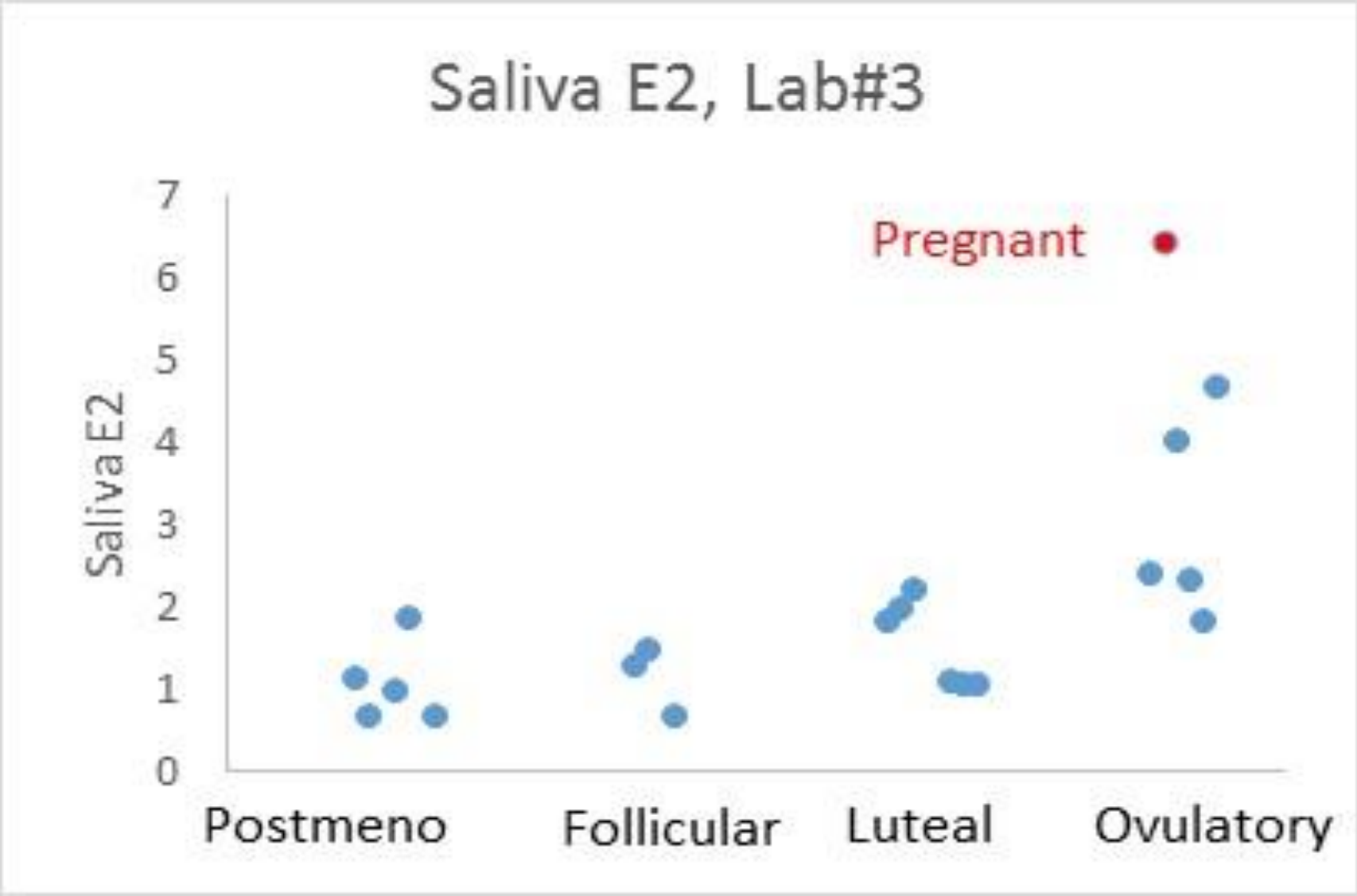
DUTCH Data


Urine E2
Pregnant
UrineE2
10
9
8
7
6
5
4
3
2
1
0
Postmeno
Follicular
Luteal
Ovulatory

Saliva Data (lab#2)

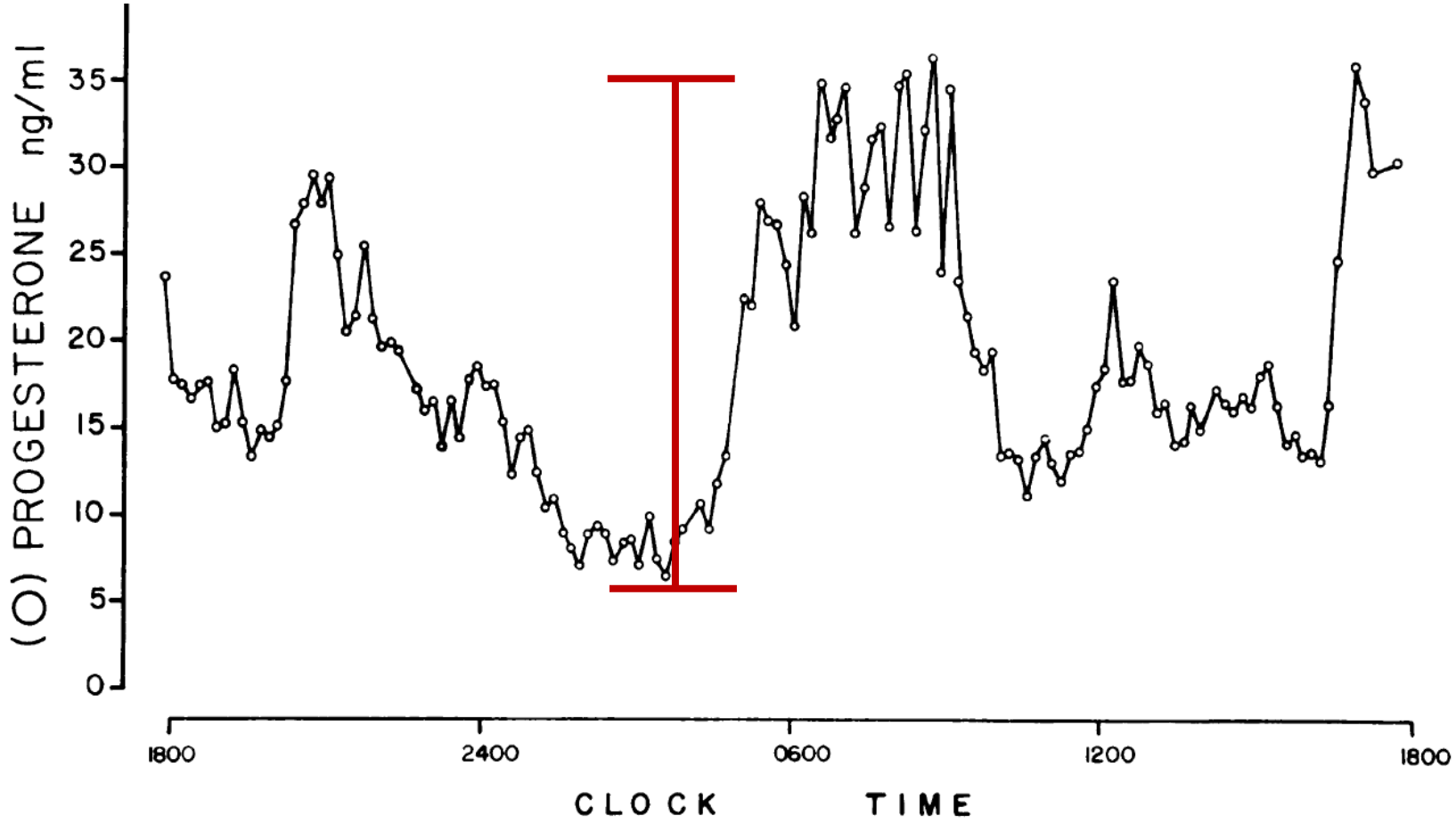


Saliva Data (lab#3)



Why DUTCH?

- Improved averaging of peaks and troughs



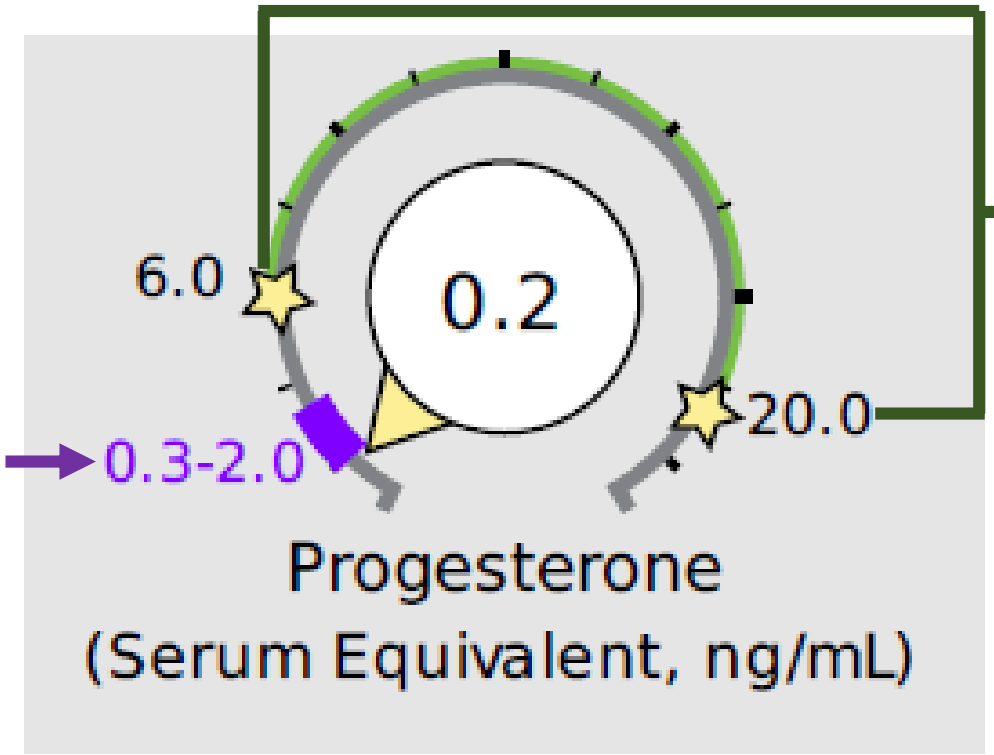
Filicori, J Clin Investigation, 1984

Why DUTCH?

- Improved averaging of peaks and troughs
- Addition of metabolites

Dried
Urine
Test for
Comprehensive
Hormones

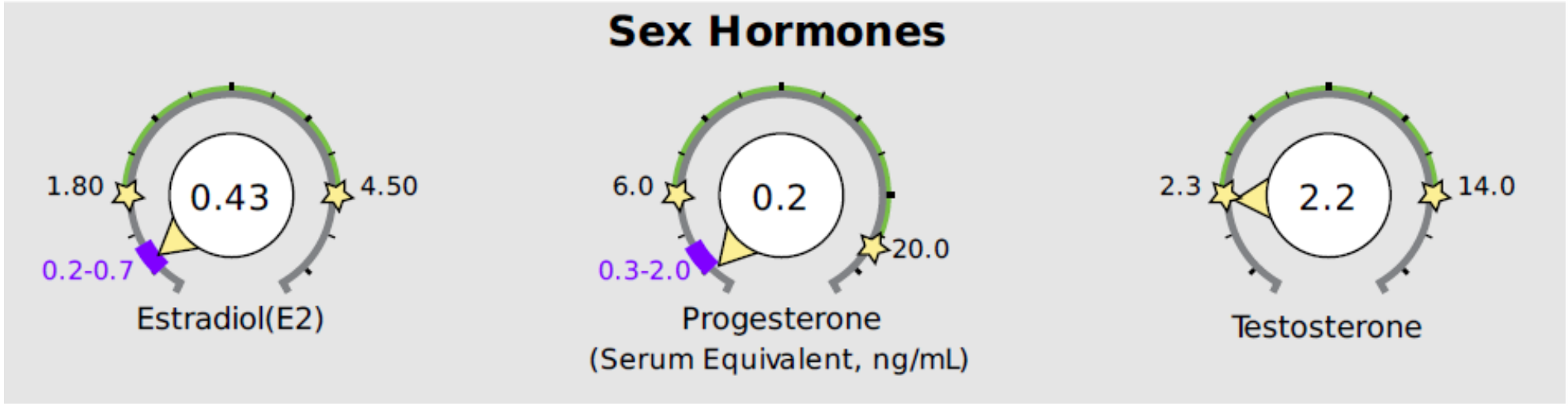
Why DUTCH?

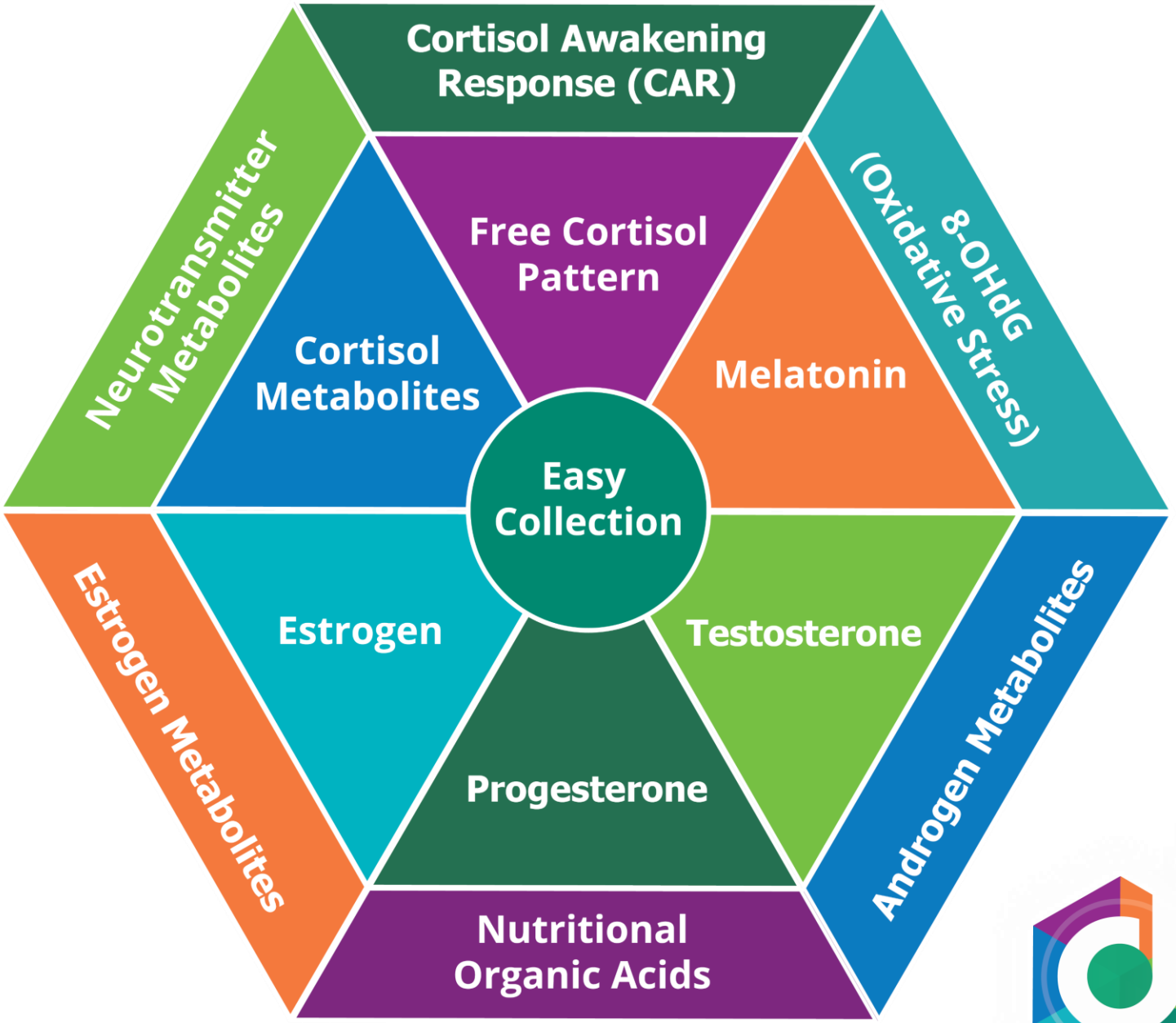


Premenopausal Range

Postmenopausal Range

Why DUTCH?

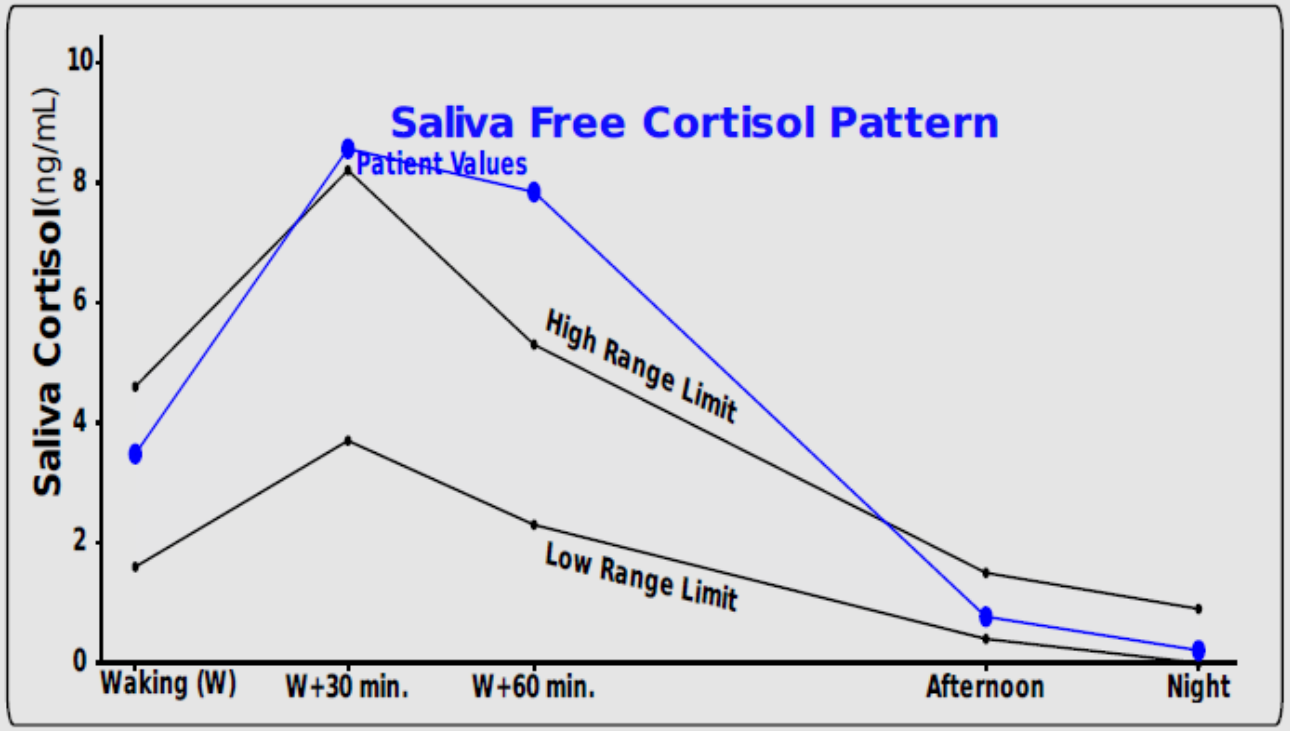

Sex Hormones
1.80
0.43
4.50
0.2-0.7
Estradiol(E2)
6.0
0.2
20.0
0.3-2.0
Progesterone
(Serum Equivalent, ng/mL)
2.3
2.2
14.0
Testosterone

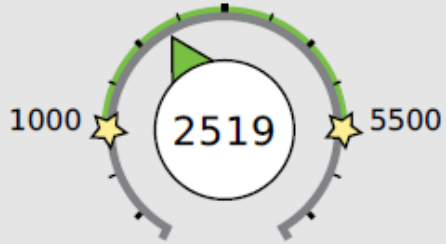


dutchtest

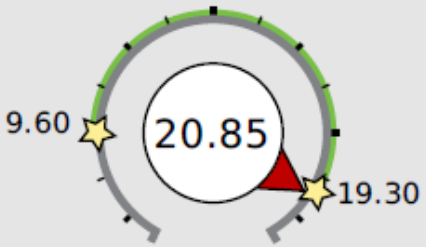
Dried Urine Test for Comprehensive Hormones

“Normal” Cortisol



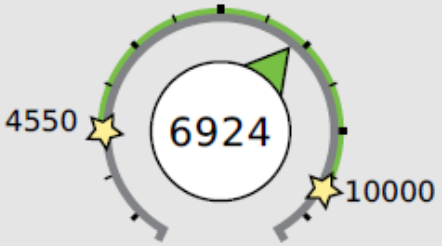


Total DHEA Production
(DHEAS + Etiocholanolone + Androsterone



Saliva Cortisol Total
(Sum of 5 values)

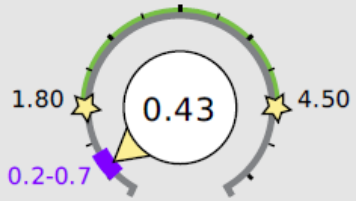
cortisol
metabolism



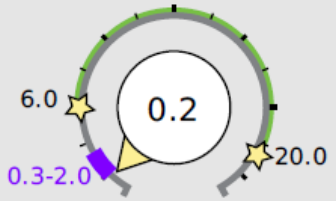
Metabolized Cortisol (THF+THE)
(Total Cortisol Production)

Why DUTCH?

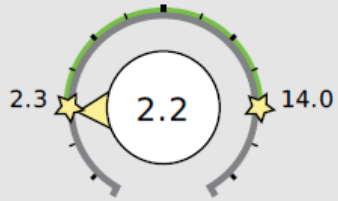
Sex Hormones



Estradiol(E2)

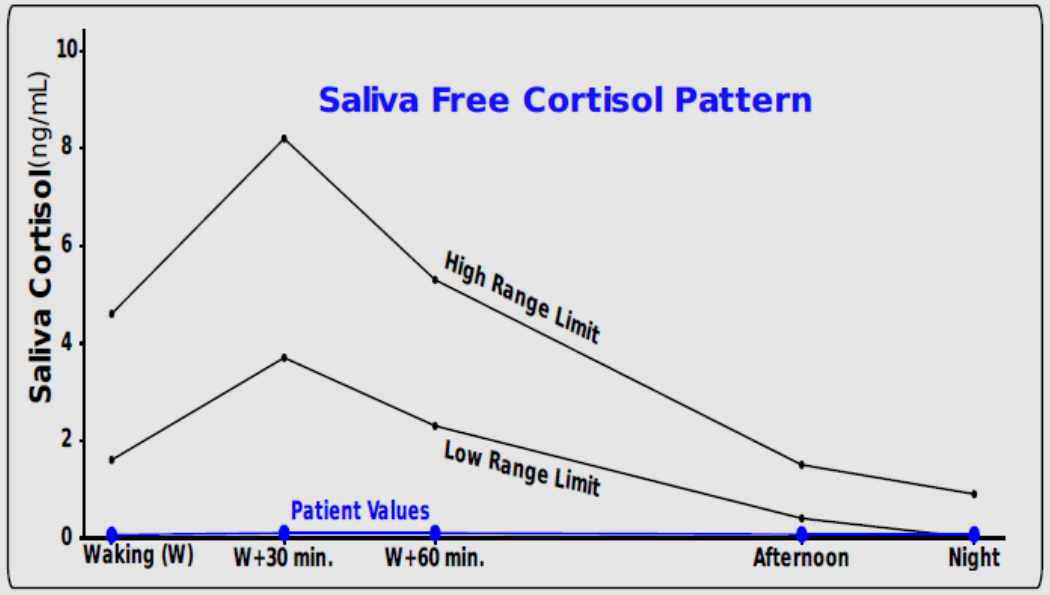


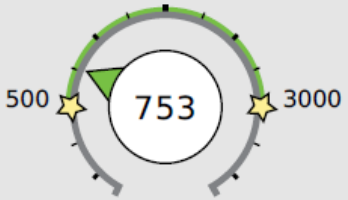
Progesterone
(Serum Equivalent, ng/mL)



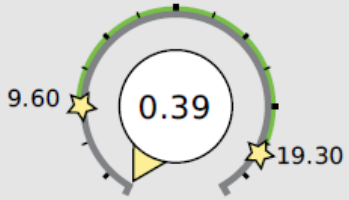
Testosterone

Adrenal Hormones



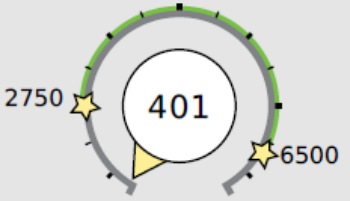


Total DHEA Production
(DHEAS + Etiocholanolone + Androsterone)



Saliva Cortisol Total
(Sum of 5 values)

cortisol metabolism

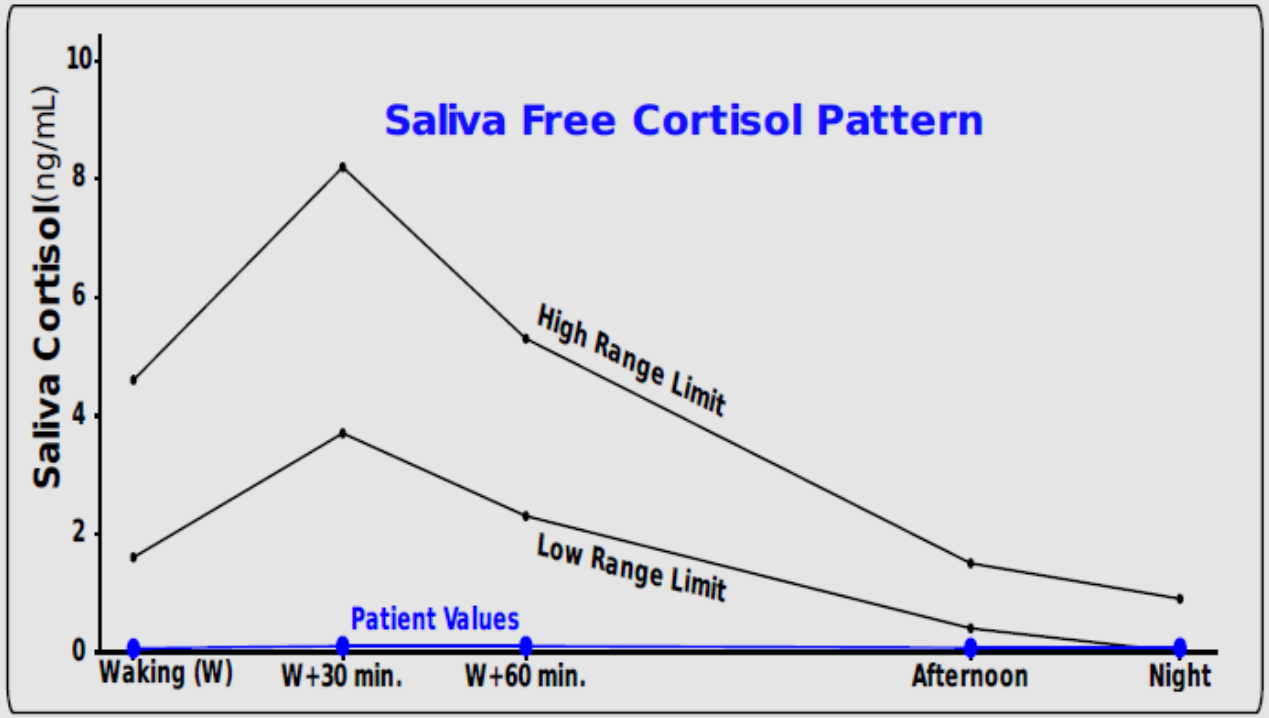


Metabolized Cortisol (THF+THE)
(Total Cortisol Production)

PLEASE INDICATE IF YOU ARE TAKING ANY OF THE FOLLOWING PRESCRIPTIONS.

- ☐ DIM / I-3-C
- ☑ Thyroid (T3, T4)
- ☐ Hydrocortisone Cream
- ☑ Steroid Inhaler or Nasal Spray
- ☐ Glucocorticoid (Prednisone, Dexamethsone, etc.)
- ☐ Creatine
- ☐ Diabetes Medications
- ☑ Opioid (Narcotic) Pain Medications (hydrocodone, fentanyl, codeine, oxycodone, etc.)
- ☐ Blood Pressure Medications

Symbyax
~~Synthroid~~



Total DHEA Production
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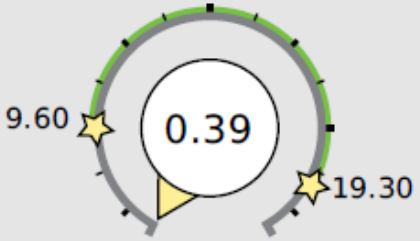
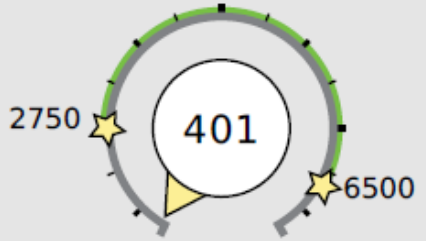


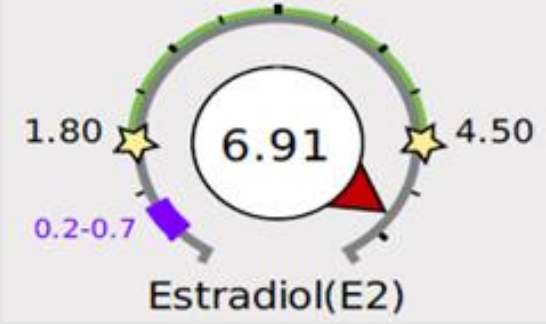
Saliva Cortisol Total
(Sum of 5 values)

cortisol metabolism →

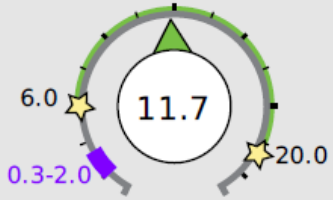


Metabolized Cortisol (THF+THE)
(Total Cortisol Production)

HRT+++E2/Pg/DHEA

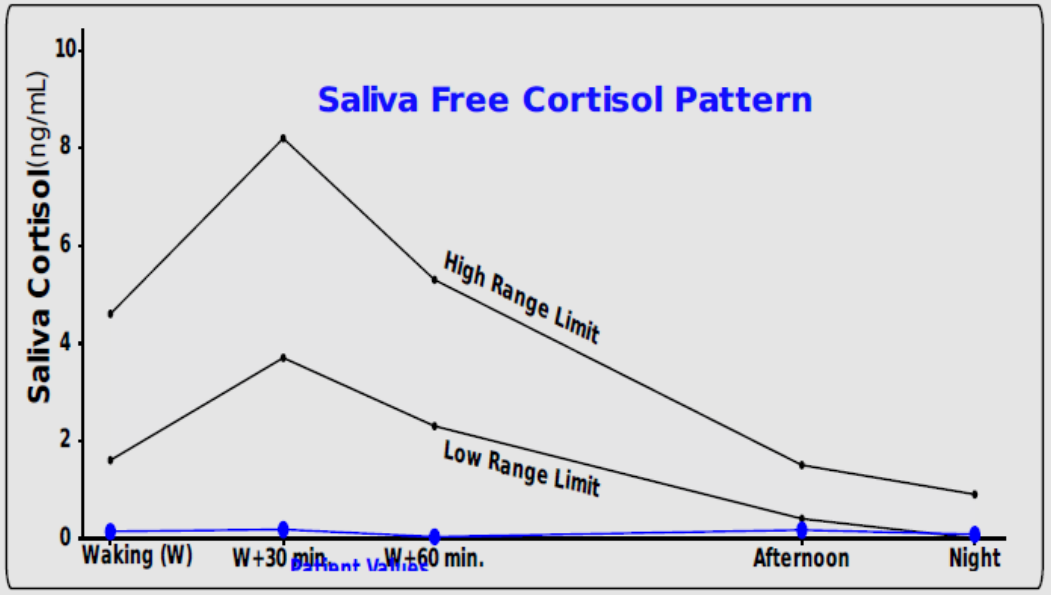


Sex Hormones

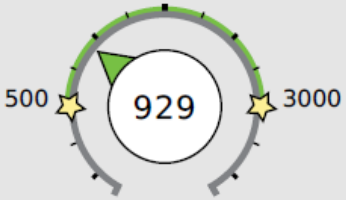


Progesterone
(Serum Equivalent, ng/mL)

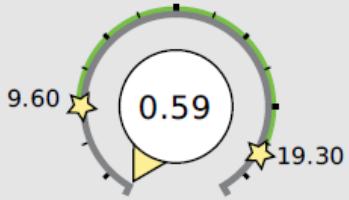
Testosterone



Adrenal Hormones

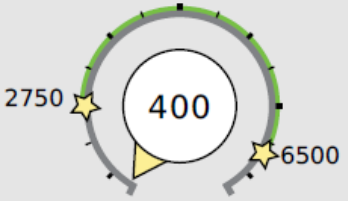


Total DHEA Production
(DHEAS + Etiocholanolone + Androsterone)

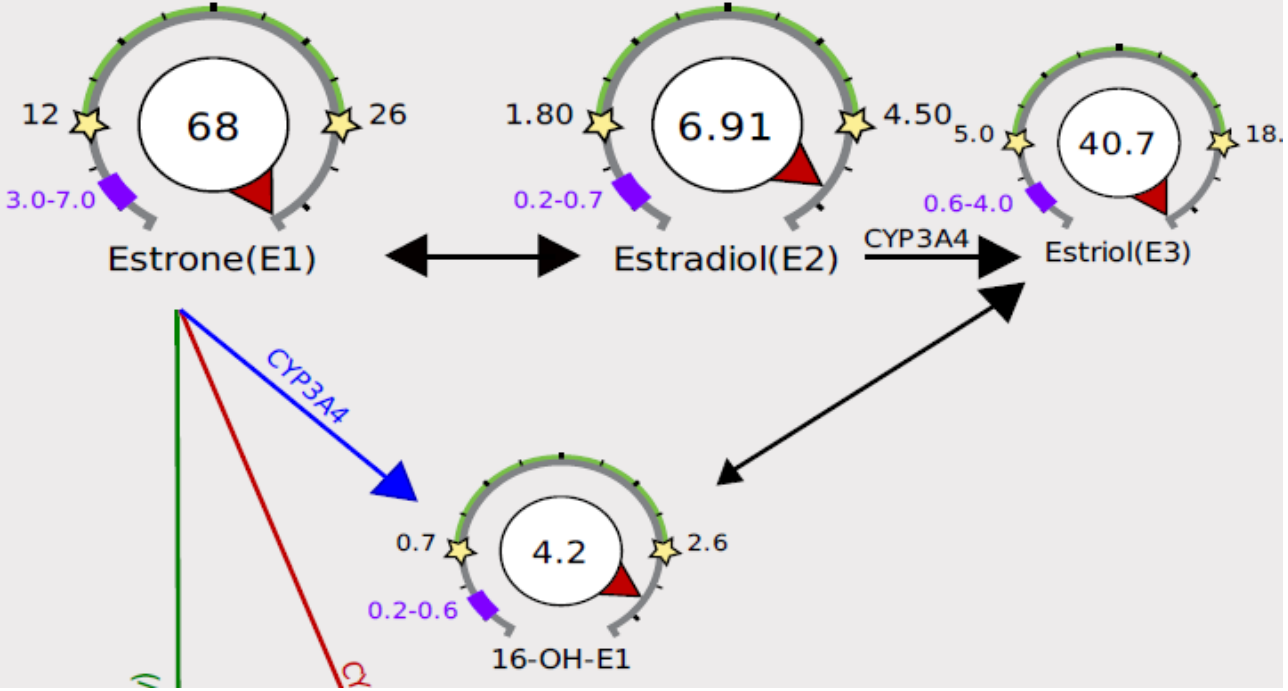


Saliva Cortisol Total
(Sum of 5 values)

cortisol
metabolism

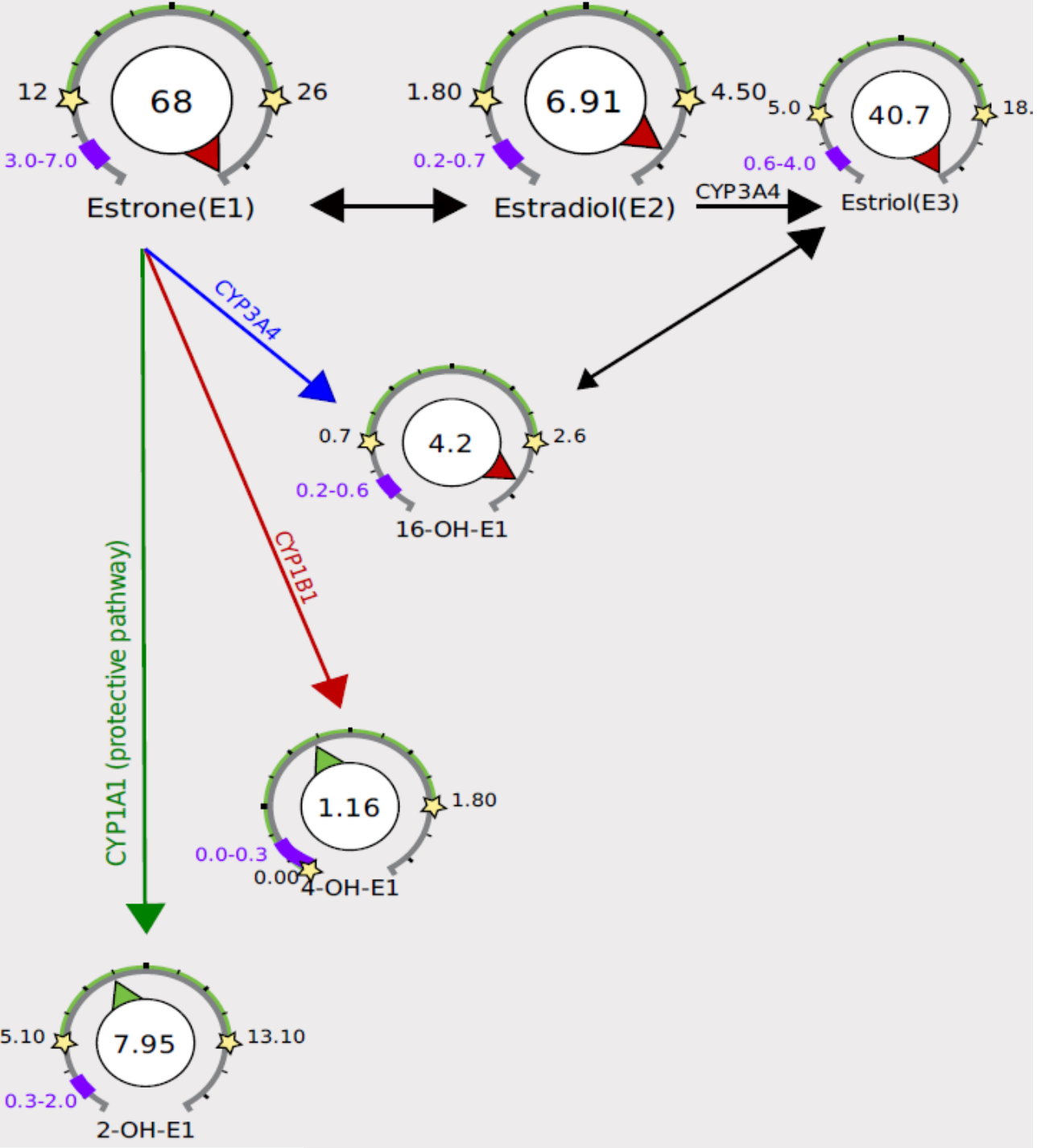


Metabolized Cortisol (THF+THE)
(Total Cortisol Production)



- Too much E2
- Too much 16-OH

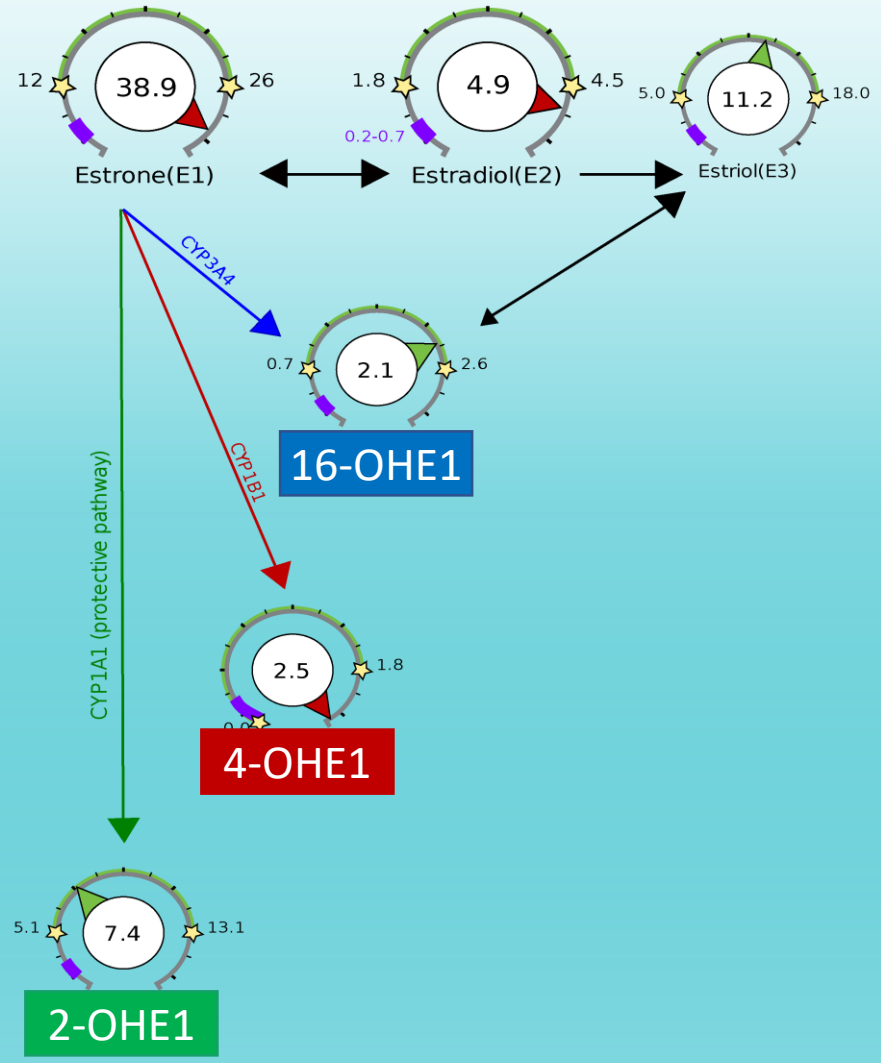
- Lower the dose



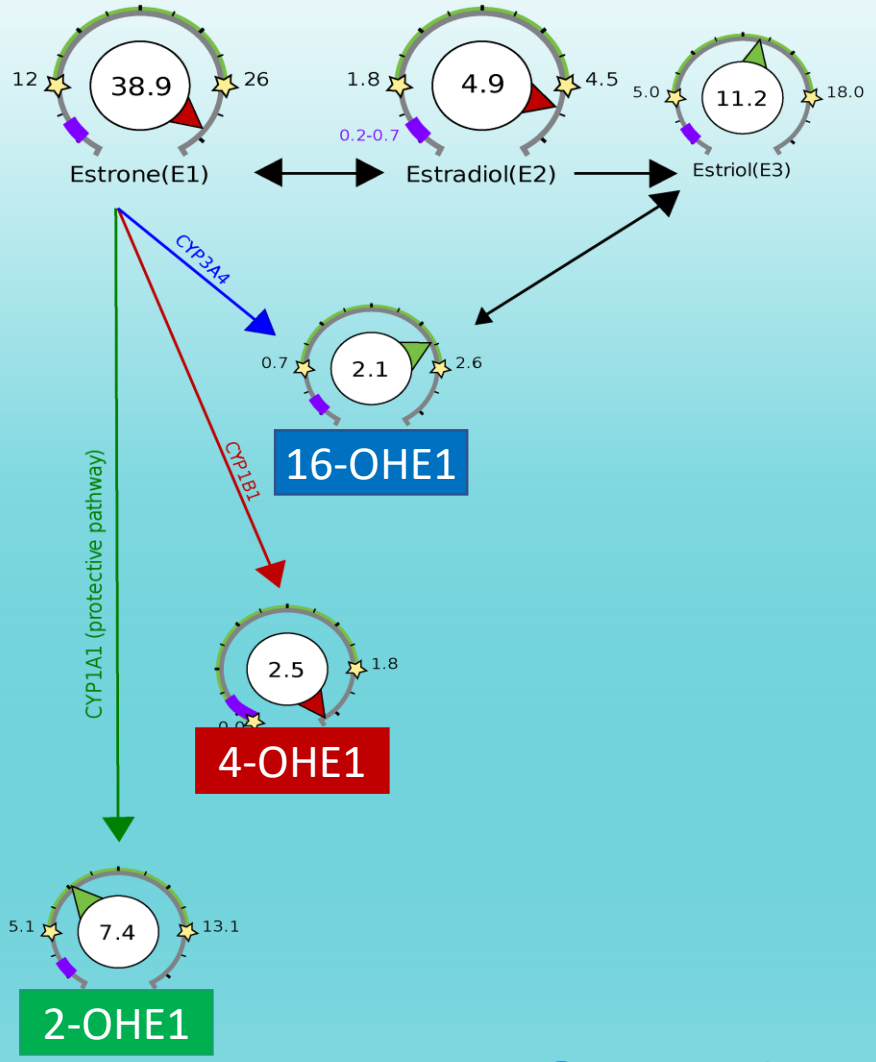
- **Too much E2**
- **Too much 16-OH**

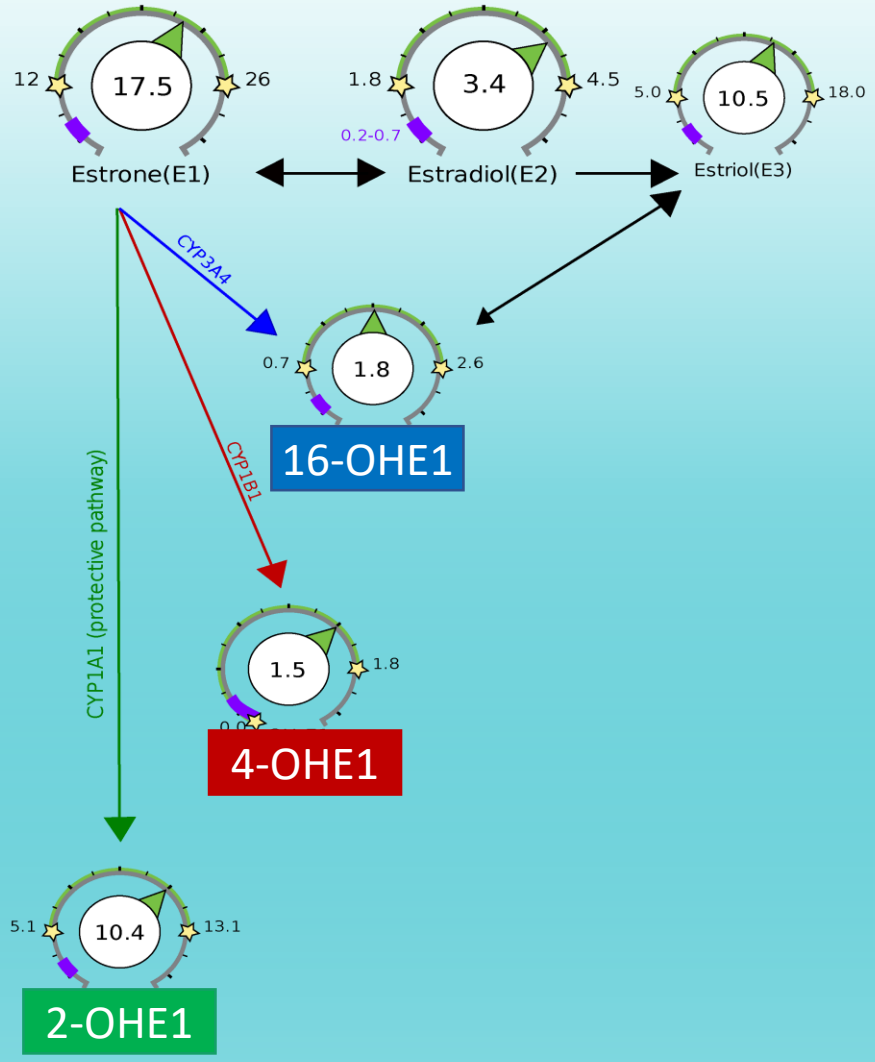
- **Lower the dose**
- **DIM?**

Why We Test Metabolites



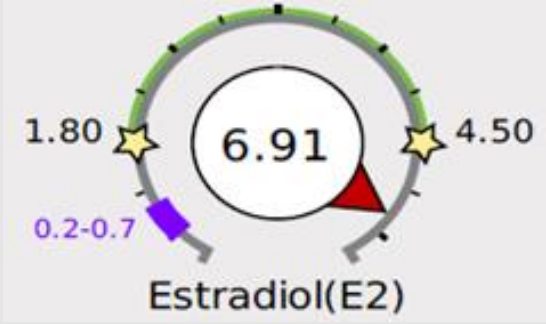
Why We Test Metabolites



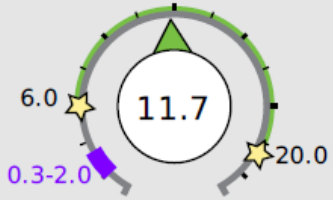


BEFORE & AFTER D.I.M.

HRT+++E2/Pg/DHEA

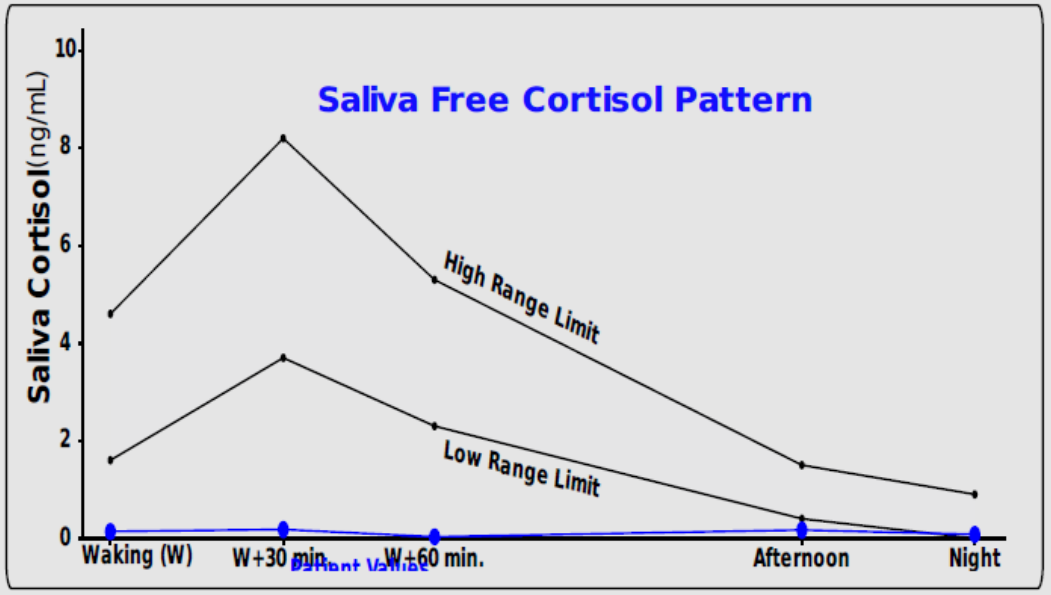


Sex Hormones

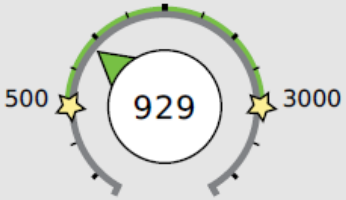


Progesterone
(Serum Equivalent, ng/mL)

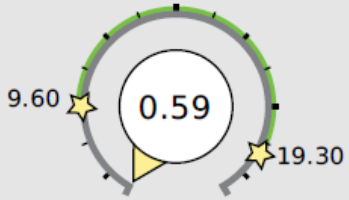
Testosterone



Adrenal Hormones

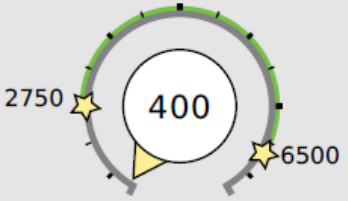


Total DHEA Production
(DHEAS + Etiocholanolone + Androsterone)



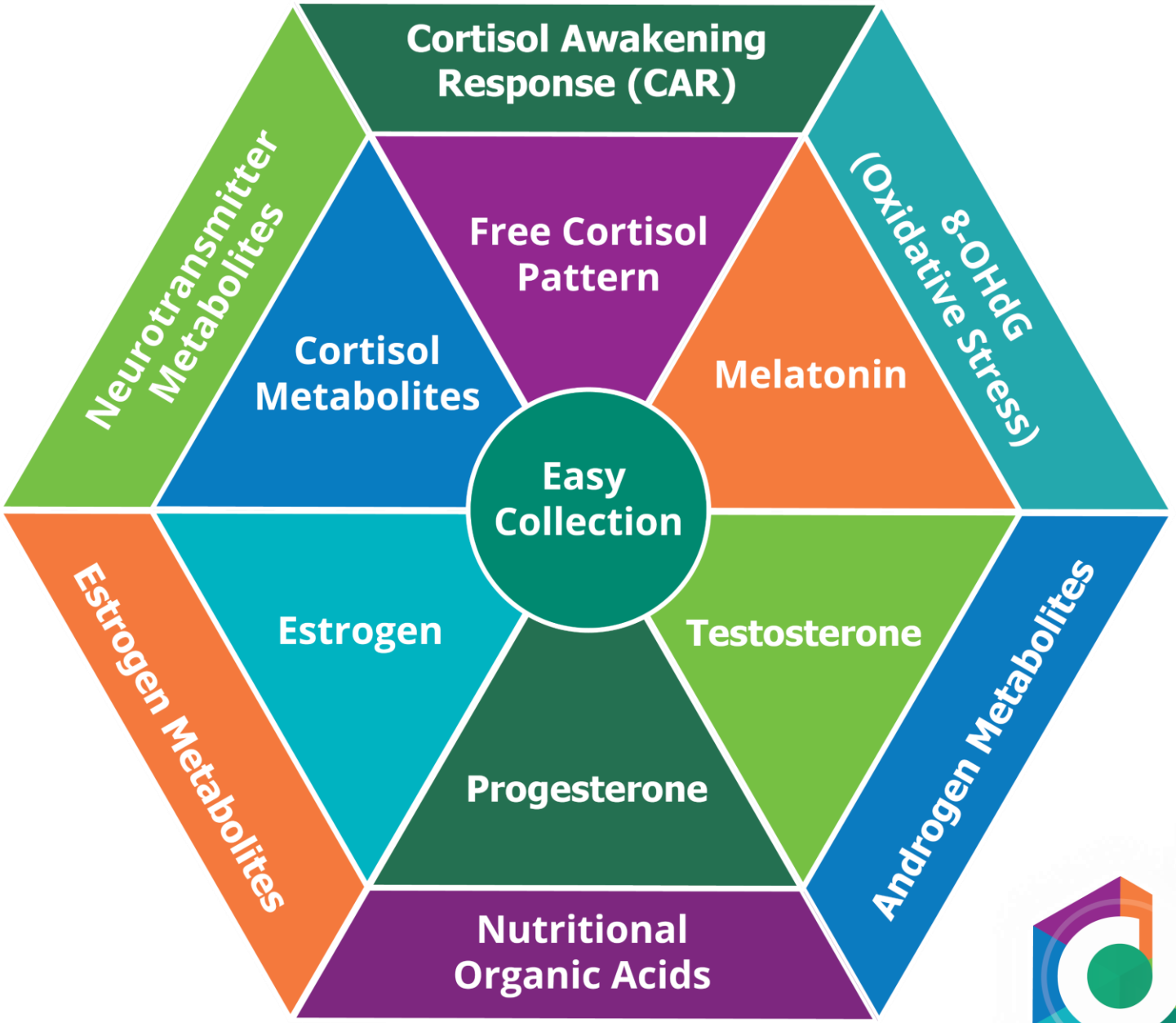
Saliva Cortisol Total
(Sum of 5 values)

cortisol
metabolism



Metabolized Cortisol (THF+THE)
(Total Cortisol Production)

Category	Test		Result	Normal Range
Nutritional Organic Acids				
Vitamin B12 Marker (may be deficient if high) - (Urine)				
	Methylmalonate (MMA)	Within range	0.5	0 - 2.2
Vitamin B6 Markers (may be deficient if high) - (Urine)				
	Xanthurenate	Within range	0.5	0 - 1.4
	Kynurenate	Within range	4.4	0 - 7.3
Glutathione Marker (may be deficient if low or high) - (Urine)				
	Pyroglutamate	Within range	46.6	32 - 60
Neurotransmitter Metabolites				
Dopamine Metabolite - (Urine)				
	Homovanillate (HVA)	Above range	15.8	4 - 13
Norepinephrine/Epinephrine Metabolite - (Urine)				
	Vanilmandelate (VMA)	Within range	3.2	2.4 - 6.4
Melatonin (*measured as 6-OH-Melatonin-Sulfate) - (Urine)				
	Melatonin* (Waking)	Low end of range	23.4	10 - 85
Oxidative Stress / DNA Damage, (8-OHdG) - (Urine)				
	8-OHdG (Waking)	Within range	1.4	0 - 5.2



dutchtest

Dried Urine Test for Comprehensive Hormones

DUTCH and HRT

- Injections
- Pellets (E2, T)
- ~~Oral Progesterone~~
- ~~Vaginal Estrogen, Testosterone~~
- ~~Transdermal Estrogen, Testosterone~~

DUTCH and HRT

- Injections
- Pellets (E2, T)
- Oral Progesterone
- Vaginal Estrogen, Testosterone
- Transdermal Estrogen, Testosterone

DUTCH and HRT

- Injections
- Pellets (E2, T)
- Oral Progesterone
- Vaginal Estrogen, Testosterone
- Transdermal Estrogen, Testosterone

~~Oral Estrogen, Vaginal Pg~~ (serum)

DUTCH and HRT

- Injections
- Pellets (E2, T)
- Oral Progesterone
- Vaginal Estrogen, Testosterone
- Transdermal Estrogen, Testosterone

~~Oral Estrogen, Vaginal Pg~~ (serum)
~~Sublingual E/T/Pg, Transdermal Pg~~

MONITORING (B)HRT WITH LAB TESTING

Tutorials available at www.dutchtest.com/videos/hormone-tutorials

PRECISION ANALYTICAL INC.
CREATORS OF THE DUTCH TEST

Oral Progesterone	Patch, Pellet, Injection	Transdermal Estrogen	Transdermal Testosterone	Transdermal Progesterone	Vaginal or Anal Mucosa	Oral Estrogen	Sublingual
✓ DUTCH	✓ DUTCH	✓ DUTCH	✓ DUTCH	✗ DUTCH	✓ DUTCH (E/T)	✗ DUTCH	✗ DUTCH
The DUTCH test provides useful feedback when using oral progesterone to aid sleep disturbance related to menopause. 5a (more active) and 5b (less active) metabolites are measured to individualize doses of oral progesterone.	Values increase intuitively with dosing. For estrogen patches, see Transdermal Estrogen comments. Pellets and injections also increase levels intuitively, but the increase may exceed what is seen in serum testing. DUTCH allows for monitoring both the proper dosing of hormones as well as metabolic patterns.	Target values between the top of the postmenopausal range and the lower third of the premenopausal range correlate with patient clinical improvement (bone density, hot flash relief, etc.). Doses that push levels to the middle of the premenopausal range and beyond may be excessive. DUTCH is preferred over serum due to the inclusion of metabolites.	Levels generally parallel measurable clinical outcomes (increased lean body mass, decreased LH values in men). Epi-testosterone values can also be used to assess gonadal suppression due to TRT (levels decrease as TRT increases and are <10 ng/mg with complete suppression).	Creams and gels cannot be effectively monitored with any lab testing. Values increase only slightly with dosing. Because of the uncertainty of tissue levels, take caution to use concurrently with estrogen therapy without endometrium surveillance (ultrasound or biopsy).	Special method removes potential contamination. Monitoring testosterone and estrogens is effective and parallels the increases seen in serum testing.	Cannot be used to effectively monitor dosing due to 1st-pass metabolism. Most of the hormone in urine has not been in circulation as "free" hormone.	Lab testing is not effective. DUTCH is confounded by the hormone that is swallowed.
					✗ DUTCH (P)	✓ DUTCH	✓ DUTCH
					Urine metabolites of progesterone underestimate systemic progesterone when taken vaginally.	While dosing is not effectively monitored with DUTCH, metabolite patterns can be effectively assessed.	While dosing is not effectively monitored with DUTCH, metabolite patterns can be effectively assessed.
✗ SERUM	✓ SERUM	✓ SERUM	✓ SERUM	✗ SERUM	✓ SERUM	✓ SERUM	✗ SERUM
Results go up-and-down quickly. If taken at bedtime, levels return to baseline within a few hours. Results can also be inaccurate due to progesterone metabolites cross-reacting with immunoassay tests.	Serum testing is well suited for use with these types of therapies.	Effective for monitoring estrogen creams and gels similarly to patches. Levels may have an up-and-down pattern throughout the day, unlike when using patches.	Results correlate to clinical symptoms. In men, lean body mass increases only when serum (and likely urine) results increase.	Values do not increase significantly with dosing.	While serum levels likely represent systemic uptake of hormone, interpret with care as you may not know if your value represents a peak or a trough.	Serum testing offers the best feedback on monitoring the actual dose of oral estradiol.	Serum testing is not effective. Results rise and fall too rapidly for useful testing. In many cases, results are back to baseline within a few hours.
✗ SALIVA	✓ SALIVA	✗ SALIVA	✗ SALIVA	✗ SALIVA	✗ SALIVA	✓ SALIVA	✗ SALIVA
Ineffective for monitoring oral progesterone for the same reason as serum above.	Testing can conceptually be used, but available testing is less accurate than serum.	Results are exaggerated, do not correlate to clinical symptoms and are highly variable.	Results are exaggerated, do not correlate to clinical symptoms and are highly variable.	Values are exaggerated and highly variable. Levels may remain elevated for months after cessation of therapy.	Testing has not been shown to be effective for monitoring vaginal/anal hormones.	Testing can conceptually be used, but available testing is less accurate than serum.	Saliva is contaminated directly, and testing is not meaningful.

DUTCH TESTING & (B)HRT GUIDE - WOMEN

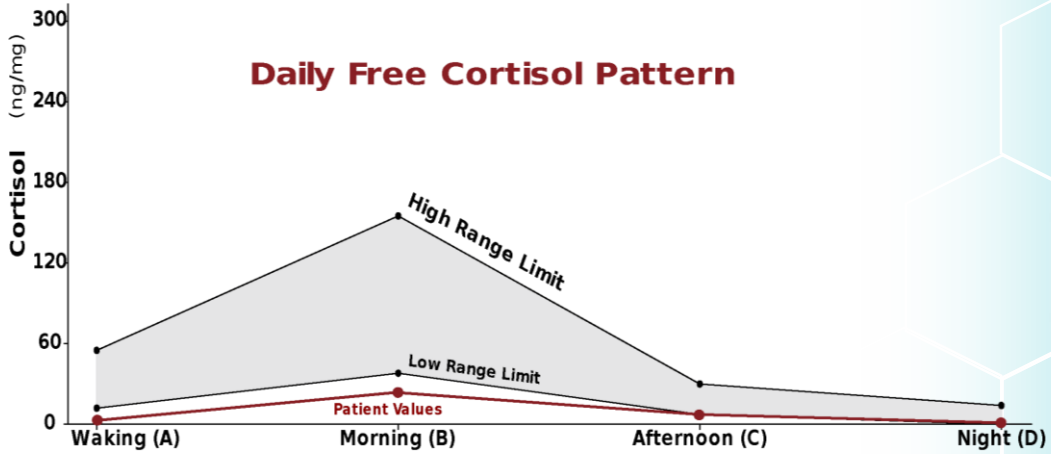
Oral Progestrone	Estradiol Patch	Estradiol Cream/Gel	Testosterone or Estradiol Pellet	Vaginal Estrogen or Testosterone	Testosterone Cream/Gel	DHEA
Why						
Effective at balancing ERT, but clinical effects are due largely to metabolites formed in the gut. A good option when postmenopausal women struggle with sleep. A different ROA may be better for premenopausal women. 100-200mg has been shown to balance con-current ERT.	Patches offer consistent hormone dosing over time and are very effective at managing hot flashes. Even low doses typically increase bone mineral density (BMD).	Proven to increase serum and urine levels as well as improve hot flashes and BMD. Transdermal E2 is attractive because it is easy to use and bypasses first pass metabolism. Estriol often given in does 1 - 4 times higher than estradiol.	Pellets offer consistent hormone dosing over time for testosterone and estradiol. Research is limited on effects on hot flashes and BMD. Because serum/urine E2 levels match or exceed those seen in patches, E2 pellets are likely to help with hot flashes and BMD.	Low doses increase local tissue levels while higher doses also increase systemic levels. Placing in the top 1/3 of the vagina significantly increases uterine levels. Estriol often given in does 1 - 4 times higher than estradiol.	Transdermal testosterone can be used to correct low T and improve sex drive and muscle mass.	Sublingual or oral DHEA will increase systemic levels and also contribute to downstream androgens (testosterone) and estrogens.
	ERT, especially with an intact uterus, should be balanced with adequate progesterone (vaginal or oral preferred).					
Common Dosing Strategies						
Low 25 - 50 mg **High** >200 mg **Most Common** 100 - 200 mg *Consider taking continuously or as an on/off cycle*	**Low** 0.012 - 0.025 mg **High** 0.1 mg **Most Common** 0.05 mg *Consider taking continuously or as an on/off cycle and changed 1 - 2 times per week*	**Low** 0.1 - 0.25 mg Estradiol 0.1 - 1.0 mg Estriol **High** 1.0 - 2.5 mg Estradiol 2.0 - 5.0 mg Estriol **Most Common** 0.25 - 0.5 mg Estradiol 0.25 - 2.5 mg Estriol *Consider taking daily continuously or as an on/off cycle*	**Low** <5 mg Estradiol 20 - 50 mg Testosterone **High** >12 mg Estradiol >125 mg Testosterone **Most Common** 5 mg Estradiol 100 mg Testosterone *Inserted every 3 - 4 months*	**Low** 0.01 mg Estradiol 0.25 mg Testosterone **High** 0.5 mg Estradiol 2 mg Testosterone **Most Common** 0.1 mg Estradiol 0.25 - 1.0 mg Estriol 0.25 - 1.0 mg Testosterone *Taken daily, possibly with cycling*	**Low** 0.5 - 2.0 mg **High** 10 - 20 mg **Most Common** 1 - 5 mg *Taken daily, at waking or bedtime*	**Low** 1 - 5 mg **High** 25 - 50 mg **Most Common** 5 - 10 mg *Usually taken daily*
How to Monitor with DUTCH						
DUTCH results only show which metabolites are preferred. Evaluate which pathway is dominant (alpha or	**Monitoring Estrogen Replacement Therapy (ERT)** Target values between the top of the postmenopausal range (0.7ng/mg for estradiol) and within the first third of the premenopausal range (about 2.5ng/mg). The specific target for a patient depends on the patient's history and symptoms as			Levels above the postmenopausal range imply systemic uptake. For localized (vaginal) effects only, results	It is optimal if levels of T (as well as metabolites) are in range. Less is needed if metabolites are 5a favored.	Monitor conversion to testosterone, E2 and metabolites of both. DHEA and testosterone metabolites may be

DUTCH Resources

- Education
  - HRT Matrix
  - Treatment Matrix
  - Video Tutorials
- Personal Education
  - Consultations with Experts

DUTCH Resources

- **Education**
  - HRT Matrix
  - Treatment Matrix
  - Video Tutorials
- **Personal Education**
  - Consultations with Experts
- **The DUTCH Test!**
  - DUTCH Complete



DUTCH Resources

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- **Personal Education**
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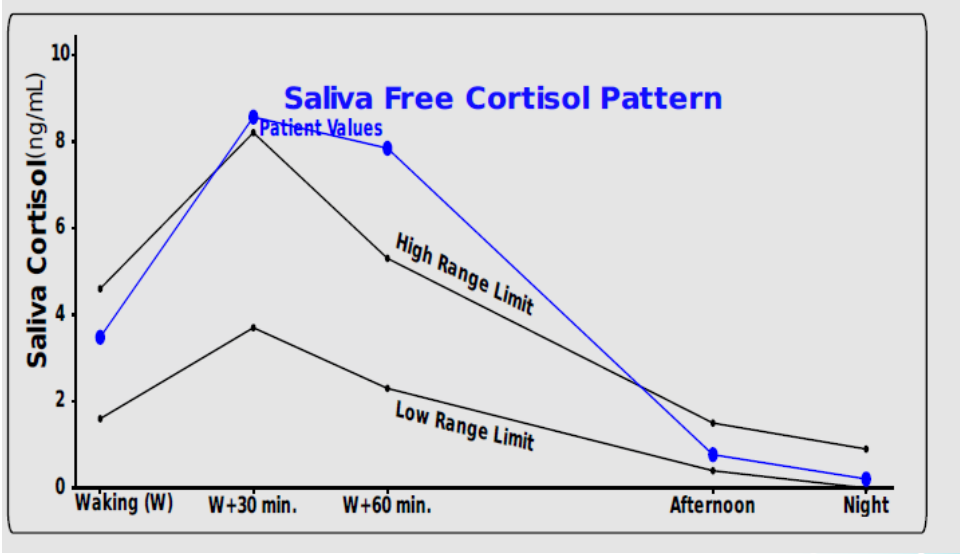
  - DUTCH Complete
  - DUTCH Plus



DUTCH Resources

- **Education**
  - HRT Matrix
  - Treatment Matrix
  - Video Tutorials
- **Personal Education**
  - Consultations with Experts
- **The DUTCH Test!**
  - DUTCH Complete
  - DUTCH Plus
  - ½ Price Offer

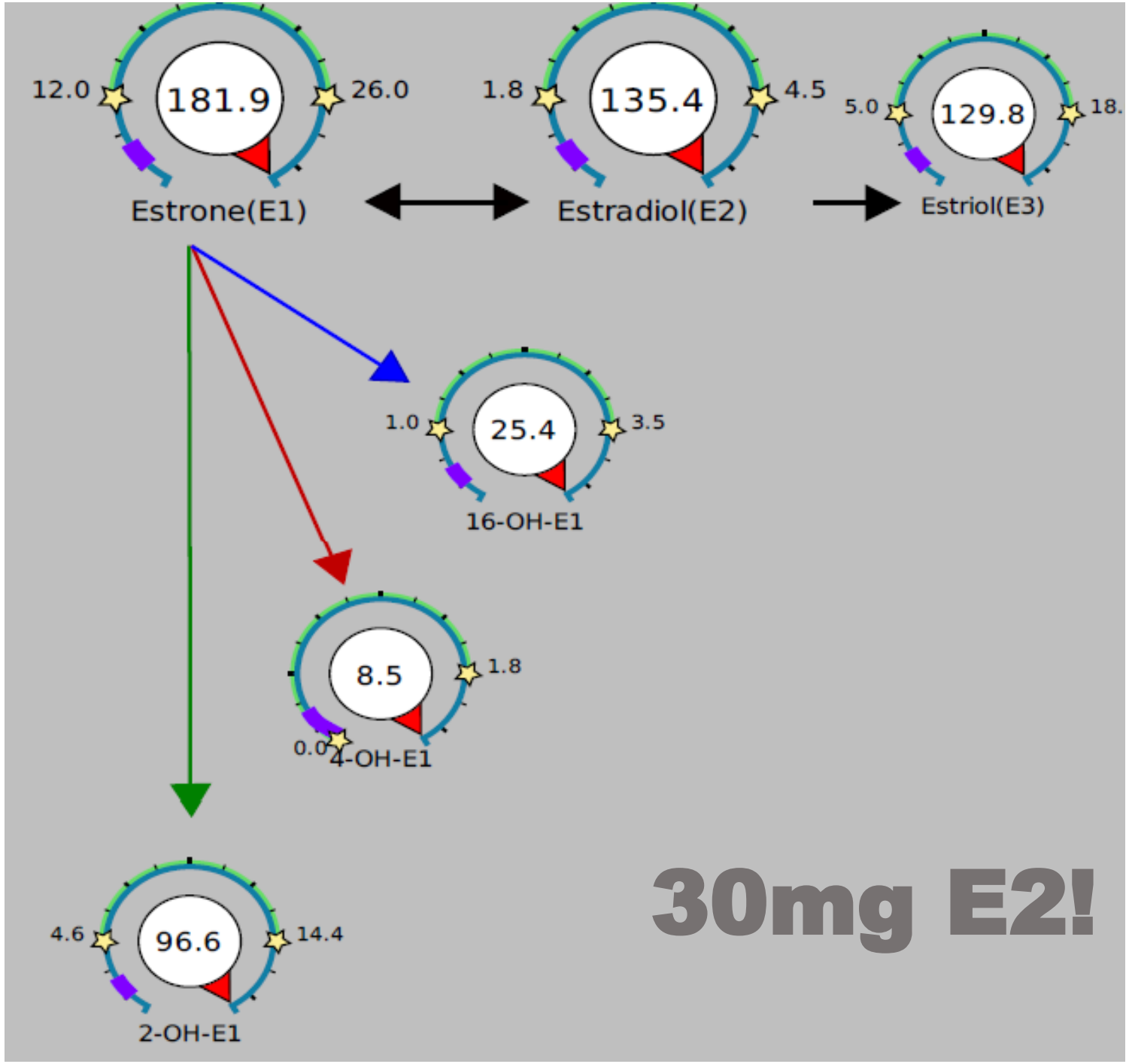
HRT Confusion

- Transdermal Estrogen (E2) Creams/Gels

HRT Confusion

- **Transdermal Estrogen (E2) Creams/Gels**
  - Different philosophies (how much E2 do we want?)
  - Different lab tests (saliva vs serum/urine)
  - VERY different dosages (0.025mg E2 vs. >8mg E2)

Hormone	Brand	ROA (1-10)	Dose (mg)	Date Last Used	Times per Day	Length of use
Estradiol (WE) Compounded	Wiley Protocol	3	10 mg/mL 1.5 mL	4/1/16 pm	2	6 yrs 7 mos
Testosterone (WE) Compounded	Wiley protocol	3	10 mg/mL .2-.5 mL	4/1/16 pm	2	6 yrs 7 mos
Progesterone (WE) Compounded	Wiley protocol	3	200 mg/mL .4-1.4 mL	4/1/16 p.m.	2 Day 14-28	6 yrs 7 mos


12.0
181.9
26.0
Estrone(E1)
1.8
135.4
4.5
Estradiol(E2)
5.0
129.8
18.
Estriol(E3)
1.0
25.4
3.5
16-OH-E1
8.5
1.8
0.0
4-OH-E1
4.6
96.6
14.4
2-OH-E1
30mg E2!

Transdermal Creams/Gels

Which one is right?

- Serum and urine tell similar stories for gels
  - Serum data for creams limited
- Saliva gives MUCH higher responses for creams and gels (not true of patches)

What I believe to be true

Transdermal Progesterone

- Saliva values are very elevated, highly variable and NOT clinically relevant
- Serum/Urine values do NOT increase significantly with dosing
- No lab test offers useful feedback for monitoring a transdermal dose of progesterone

What I believe to be true

Transdermal Testosterone

- Saliva values are very elevated and NOT clinically relevant
- Serum/Urine values DO increase significantly with dosing and parallel clinical changes
  - Related symptoms in females
  - LH suppression in males
  - Muscle mass increase in males

What I believe to be true

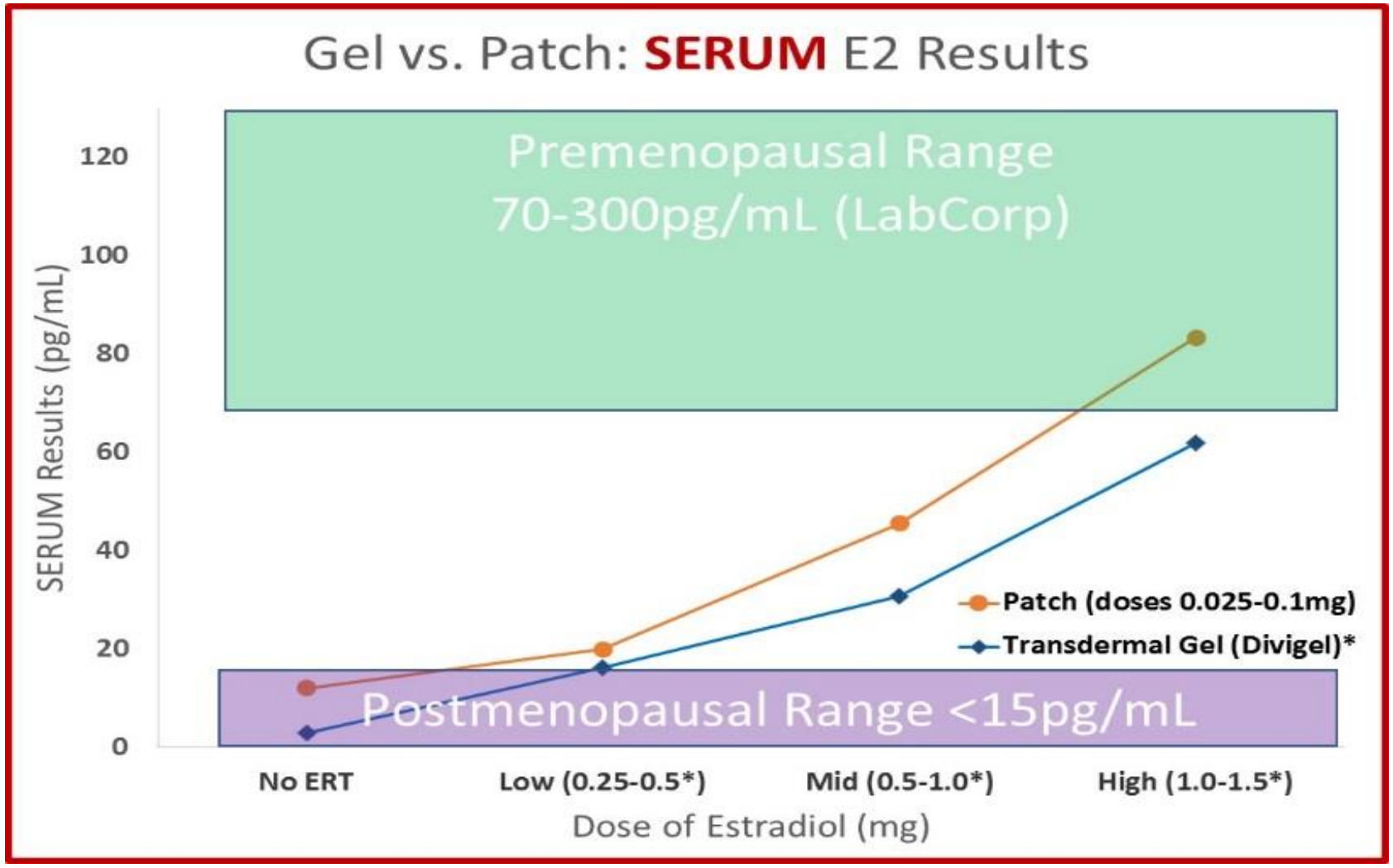
Transdermal Estradiol

- Saliva values are very elevated and NOT clinically relevant
- Serum/Urine values DO increase significantly with dosing and parallel clinical changes
  - Changes in hot flashes
  - Increases in bone mineral density
  - Decreases in FSH

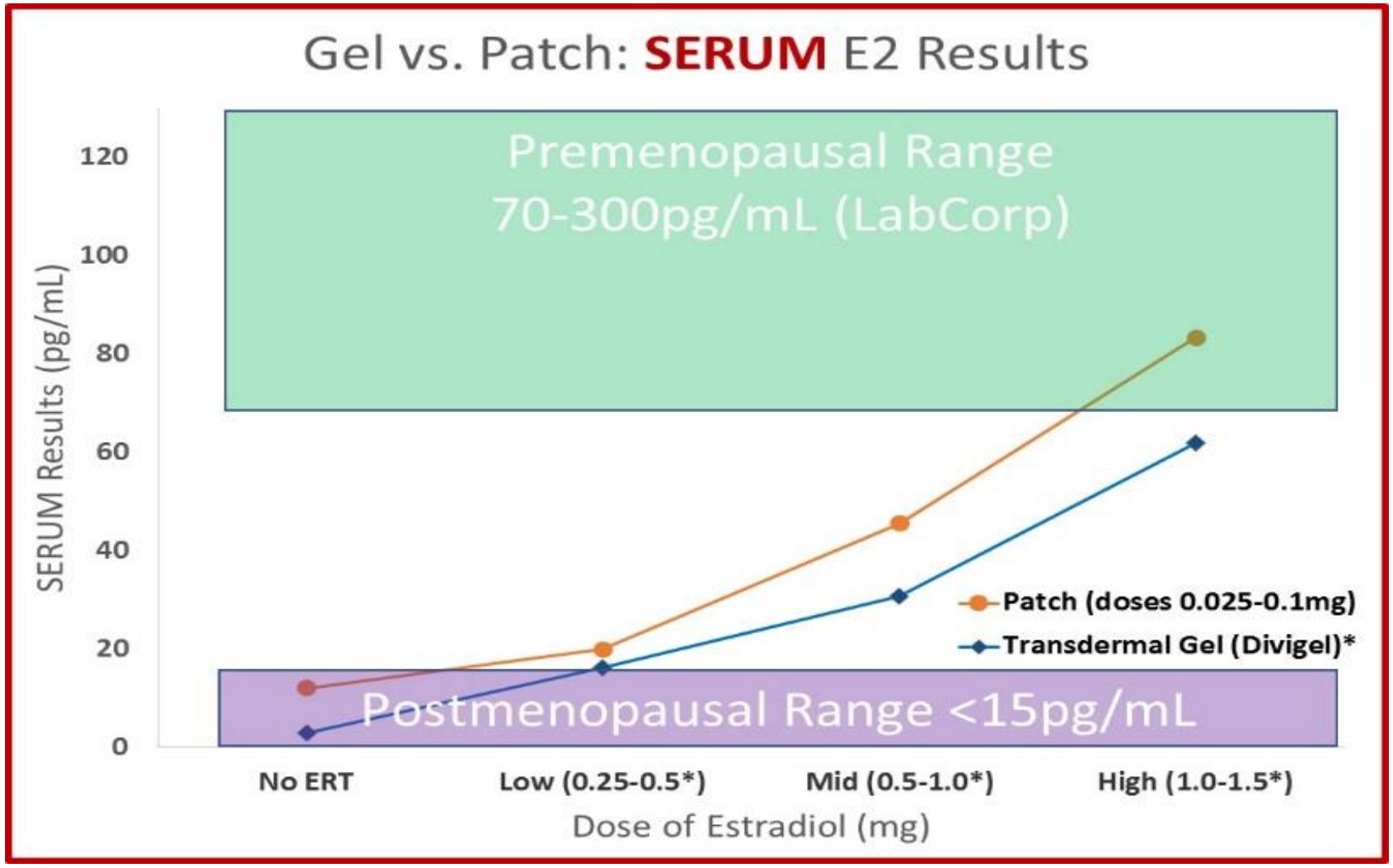
How did we get here? The saliva story

- Without HRT, saliva results may represent tissue exposure…commonly accepted w/cortisol
- Saliva values increase with TD hormone dose
- Values go up when serum does not (especially Pg)
- Patients seem to feel better

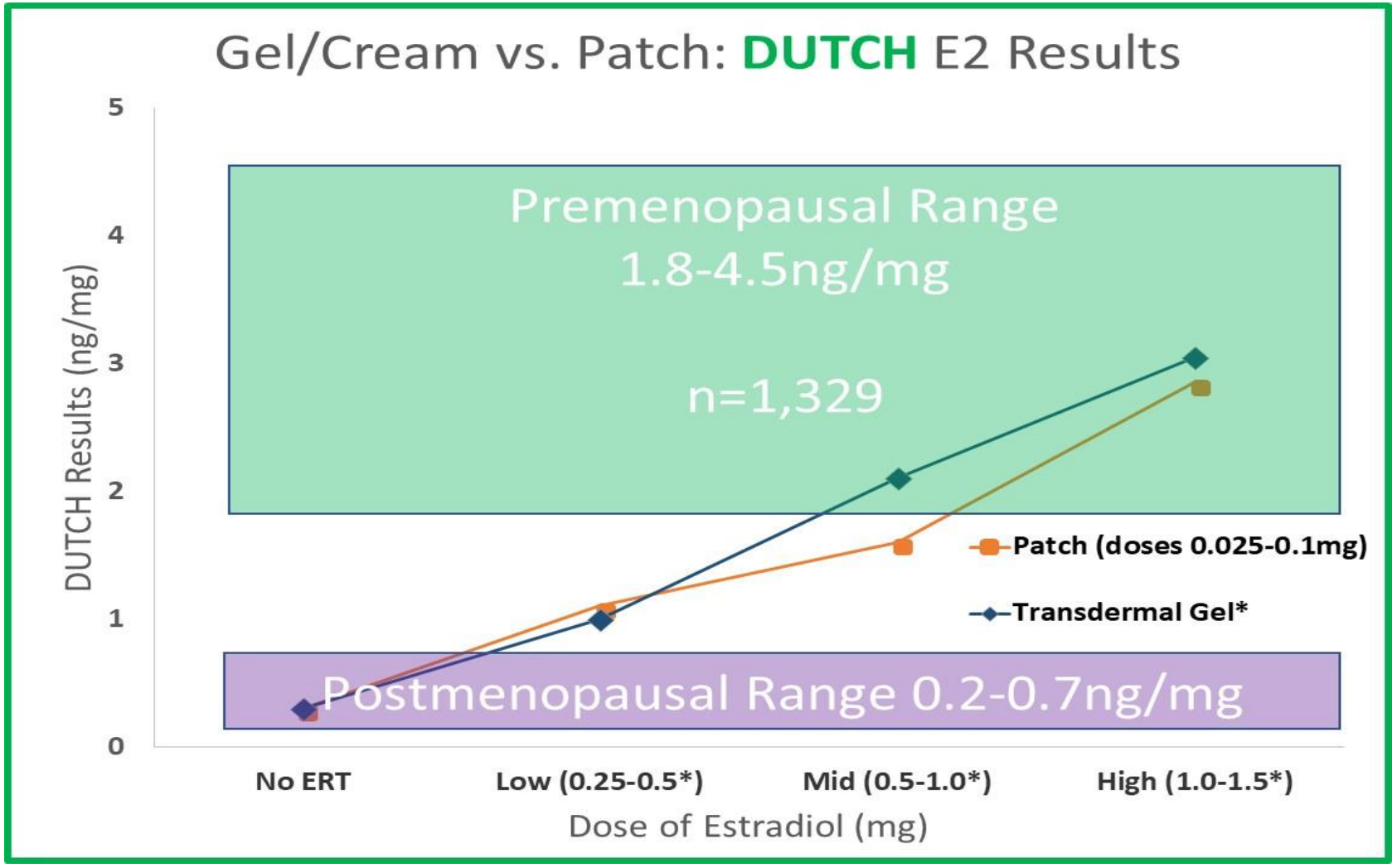
Serum Results



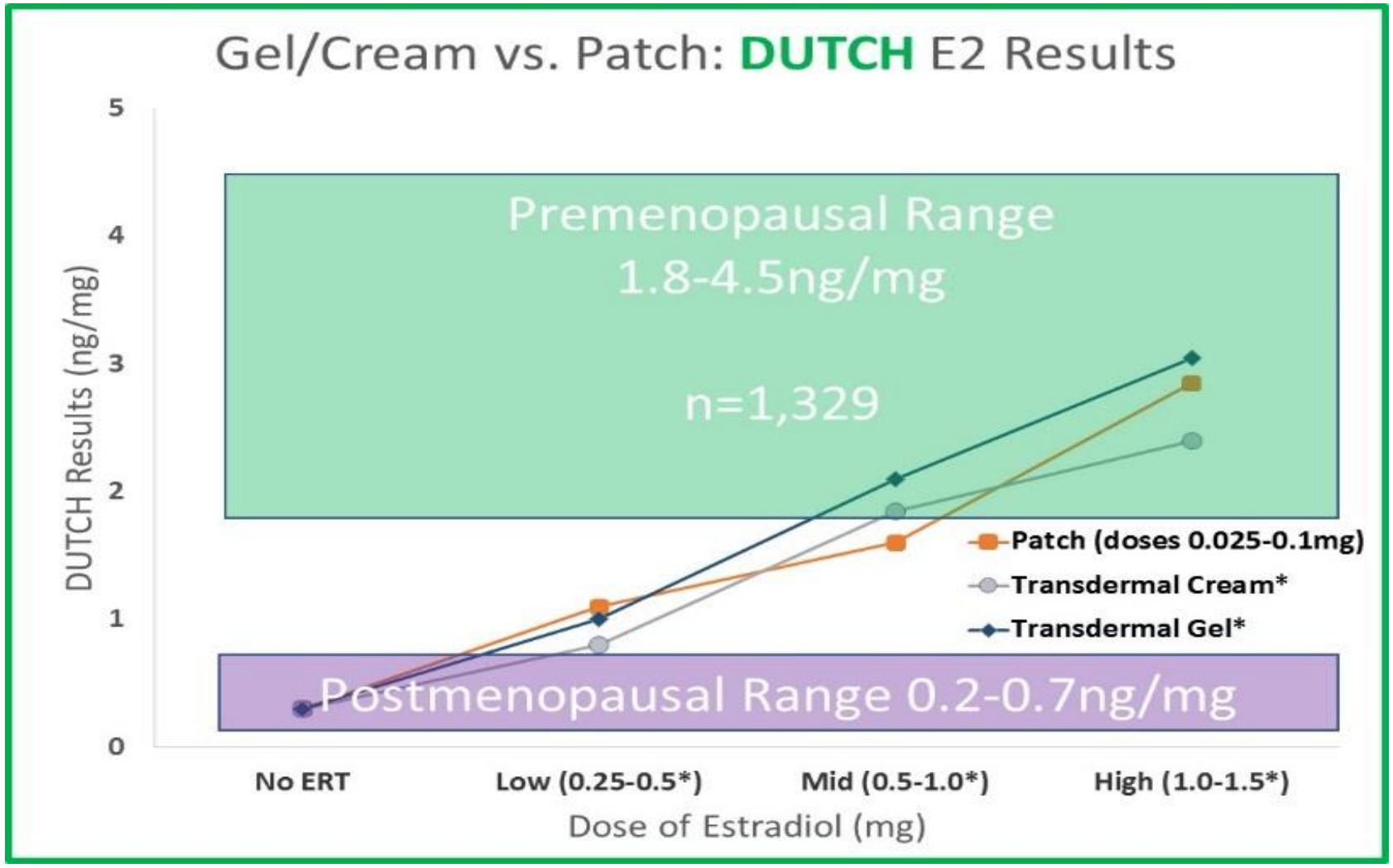
Patches = Gel


Gel vs. Patch: SERUM E2 Results
Premenopausal Range
70-300pg/mL (LabCorp)
Postmenopausal Range <15pg/mL
SERUM Results (pg/mL)
120
100
80
60
40
20
0
No ERT
Low (0.25-0.5*)
Mid (0.5-1.0*)
High (1.0-1.5*)
Dose of Estradiol (mg)
Patch (doses 0.025-0.1mg)
Transdermal Gel (Divigel)*

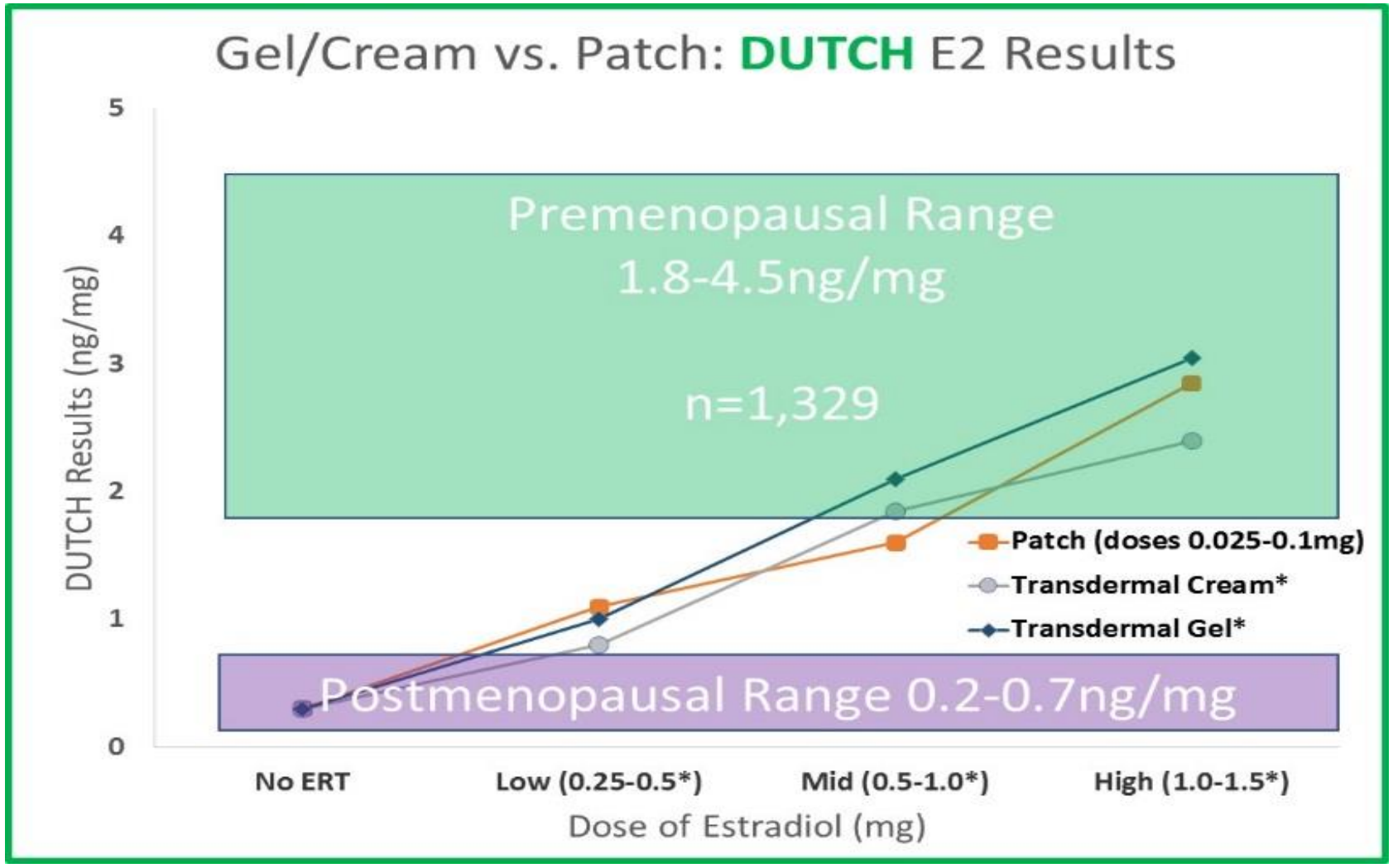
DUTCH (urine) Results



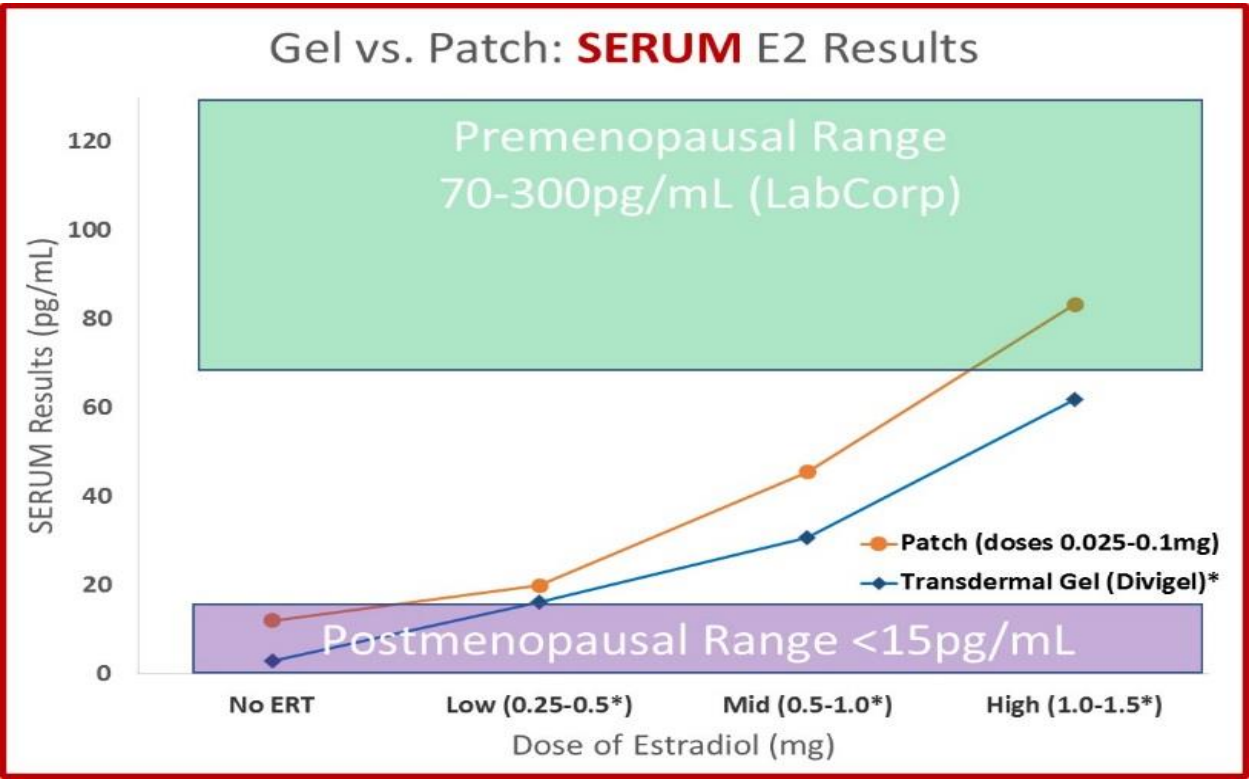
DUTCH (urine) Results

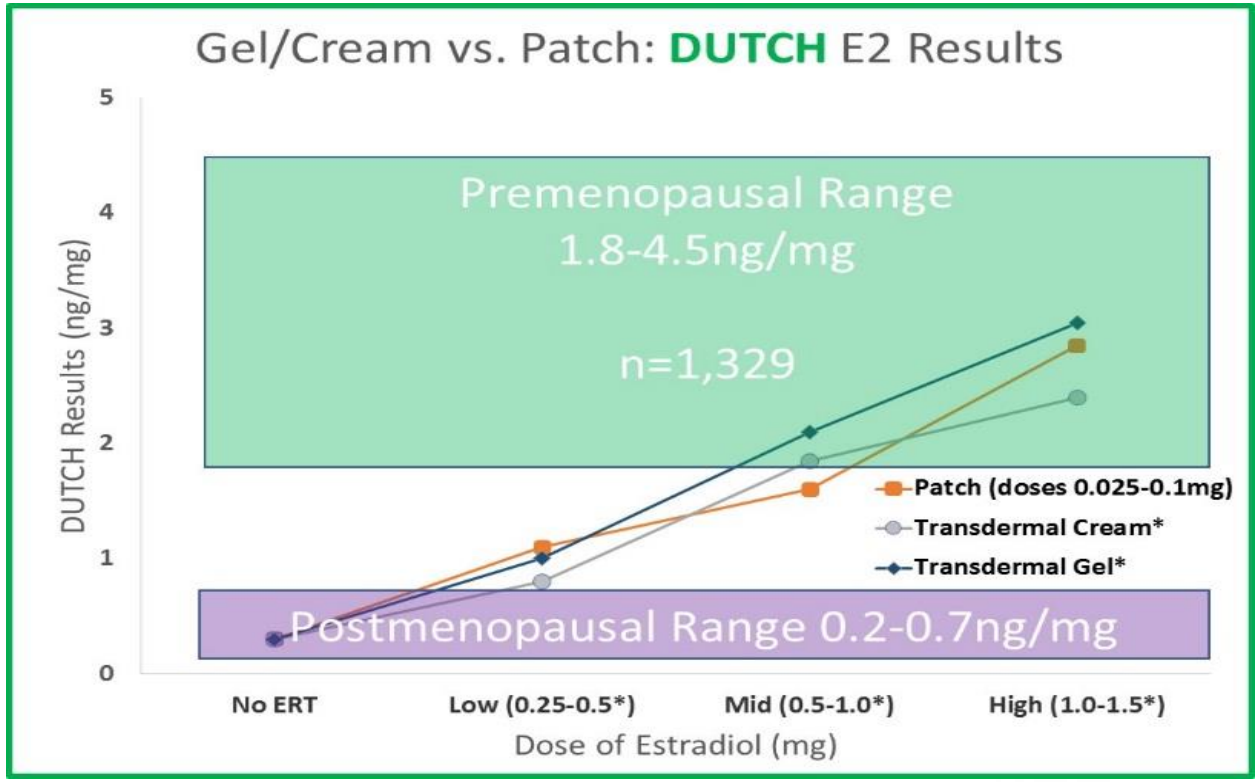


Patches = Gel = Cream



Serum, Urine: Similar Responses
Patches, Gels: Similar Responses





Serum data from published FDA studies. Urine data from Precision Analytical (2018)

Saliva: A very Different Response

Saliva: A very Different Response

Extraction Prior to Enzyme Immunoassay Gives Reliable Salivary Estradiol Monitoring during Estrogen Therapy

Mark Newman[1], Frank Stanczyk[2] and David Zava[1]

[1]ZRT Laboratory, Beaverton, OR, United States [2]University of Southern California, Los Angeles, CA, United States

Abstract

Saliva analysis is a convenient, non-invasive and rapid method for assessing estradiol (E2) levels. However, particularly in postmenopausal women, the low salivary E2 levels are often near or below the sensitivity of available assays, compromising both accuracy and precision. We present results using an extraction step prior to E2 assay, which concentrates the sample to increase sensitivity and removes potentially interfering substances.

Morning saliva samples were obtained from premenopausal (mid-luteal phase, n=4,651) and postmenopausal women (n=1,770) not taking hormones, and from postmenopausal women receiving oral conjugated equine estrogens (Cenestin, n=119; Premarin, n=439), oral micronized E2 (Estrace, n=145; compounded E2, n=1618), transdermal E2 patches (Climara, n=623; Vivelle, n=1619); or topical E2 cream (compounded E2, n=107). E2 levels were determined by an automated enzyme immunoassay (EIA) after solid phase extraction.

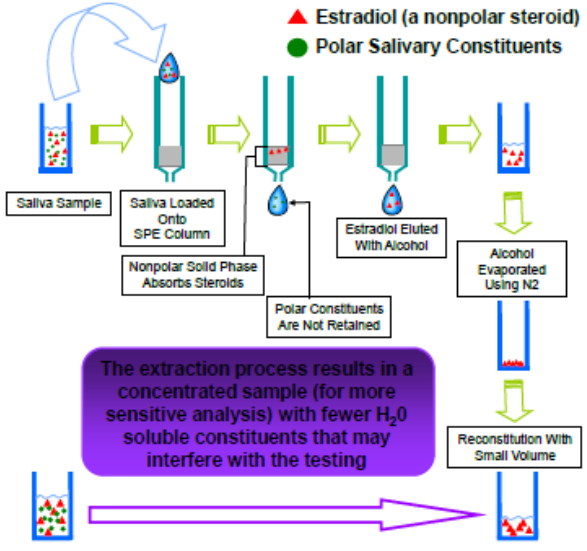
The functional sensitivity of the assay was determined to be 0.8 pg/ml, compared with >2 pg/ml without extraction.

Results are shown in the tables below:

Salivary E2 levels corresponded with the hormone dosage, suggesting a reliable assessment of unbound E2 levels with each formulation, dosage and type of estrogen therapy.

Extraction prior to EIA in an automated assay dramatically increased precision and accuracy at low concentrations. Omitting the extraction step may have contributed to poor serum versus saliva correlations in other studies. This method may therefore allow reliable monitoring of estrogen therapy without the need for expensive and inconvenient blood tests.

Solid Phase Extraction (SPE)



Median (Mean) Salivary E2 without Estrogen Therapy

Premenopausal 1.9 (2.3) pg/mL

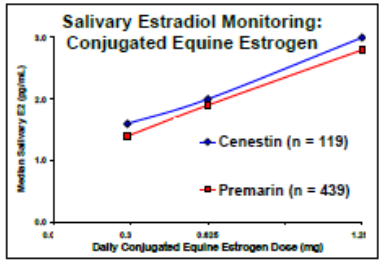
Postmenopausal 1.0 (1.3) pg/mL

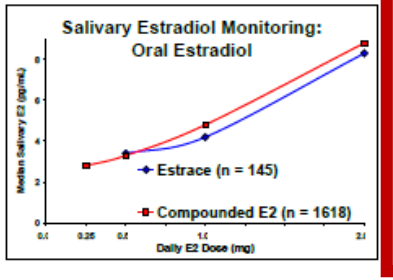
Median Salivary E2 During Estrogen Therapy

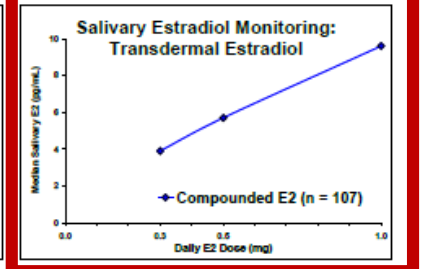
Therapy	Dosage (mg/day)	Median Salivary E2 (pg/mL)	Therapy	Dosage (mg/day)	Median Salivary E2 (pg/mL)
Cenestin[1]	0.3	1.8	Premarin[1]	0.3	1.4
	0.625	2.0		0.625	1.9
	1.25	3.0		1.25	2.8
Estrace[2]	n/a	n/a	Compounded E2[2]	0.25	2.8
	0.5	3.4		0.5	3.3
	1.0	4.2		1.0	4.8
	2.0	8.3		2.0	8.8
Climara[3]	0.025	1.2	Vivelle[3]	0.025	1.2
	0.05	1.4		0.05	1.2
	0.1	1.7		0.1	1.8
Compounded E2[4]	0.3	3.9			
	0.6	6.7			
	1.0	9.6			

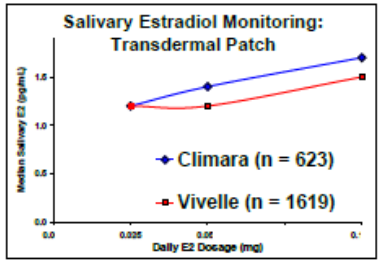
[1]Oral Conjugated Equine Estrogens, [2]Oral E2, [3]E2 Patch, [4]Topical Cream

Results: Linear, Dose-Dependent Relationships with Therapy

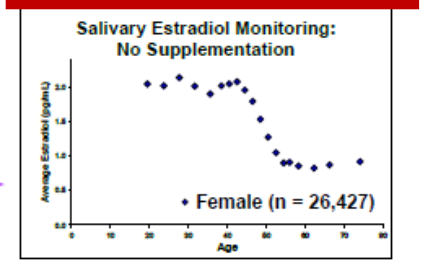








Data from ZRT's database of test results accumulated over the past four years illustrate the sensitivity of the estradiol assay to clearly differentiate premenopausal and postmenopausal estradiol levels. This differentiating power is enhanced by the use of an extracted method.



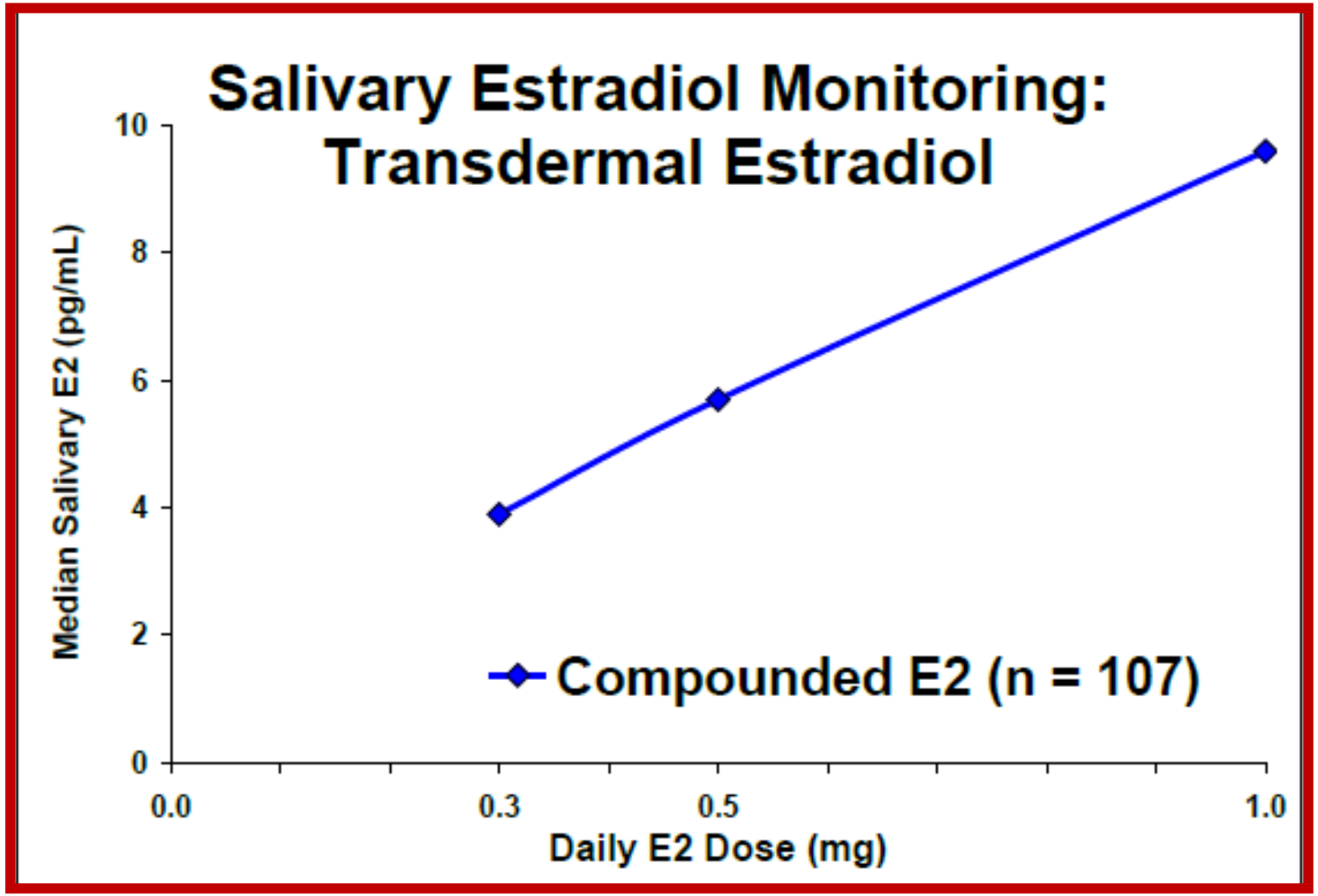
Conclusions

Our results demonstrate the clinical utility of an extracted EIA method for analysis of salivary estradiol. Monitoring of estrogen therapy ensures that levels do not exceed the normal physiological levels seen in premenopausal women, since excessive circulating estrogen has potential cardiovascular and cancer risks. This method allows clinicians to take advantage of the convenience of saliva testing, with the sensitivity required to assess the extremely low estradiol levels found in the patient population most likely to require monitoring of estrogen therapy.

References

1. Toniolo P, Lukanova A. The challenge of measuring circulating estradiol at low concentrations. Breast Cancer Res. 2005;7:45-7.
2. Wong YF, Mao K, Panesar NS, Loong EP, Chang AM, Mi ZJ. Salivary estradiol and progesterone during the normal ovulatory menstrual cycle in Chinese women. Eur J Obstet Gynecol Reprod Biol. 1990;34:129-35.
3. Stanczyk FZ, Cho MM, Endres DB, Morrison JL, Patel S, Paulson RJ. Limitations of direct estradiol and testosterone immunoassay kits. Steroids. 2003;68:1173-8.

Saliva: A very Different Response



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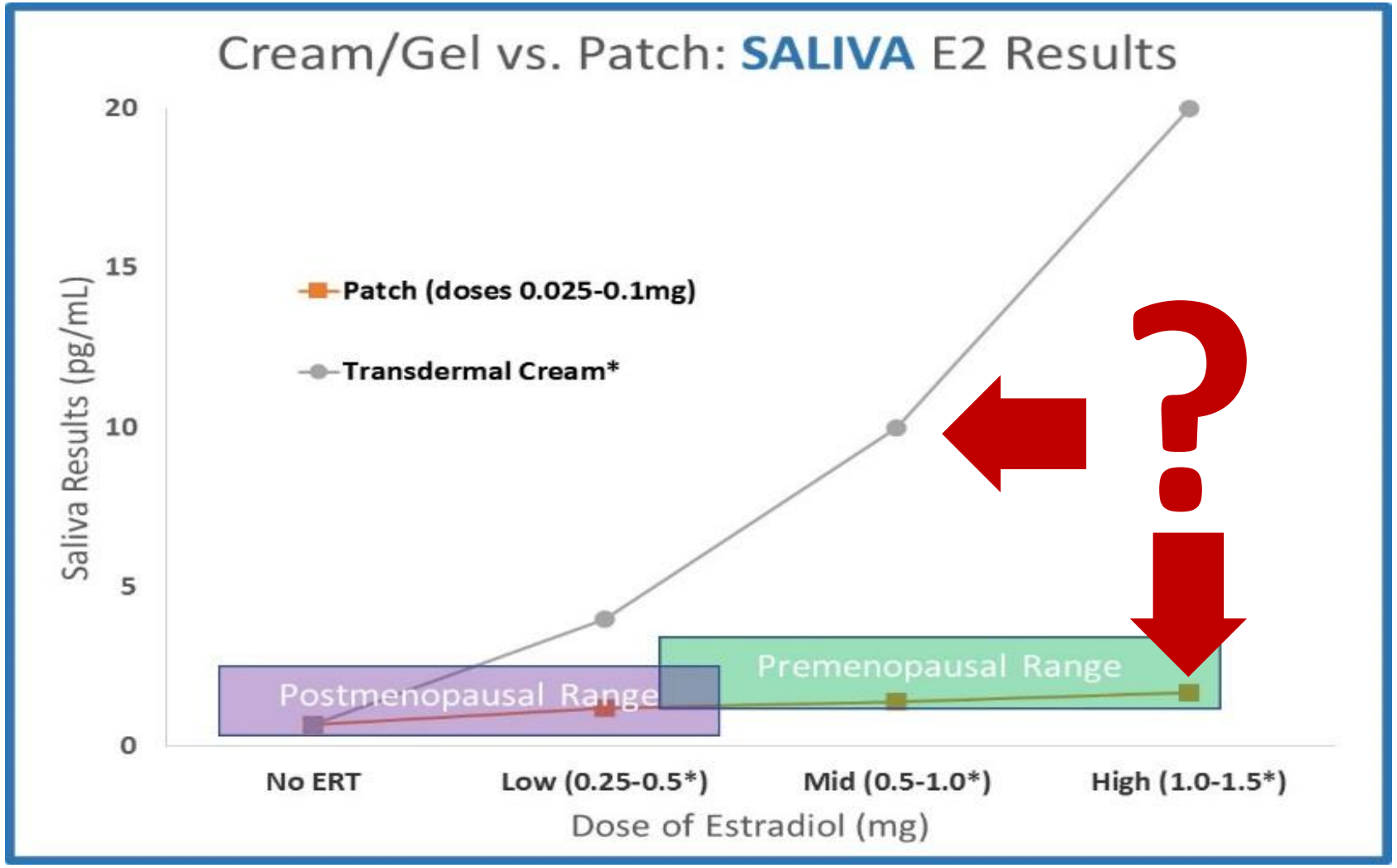
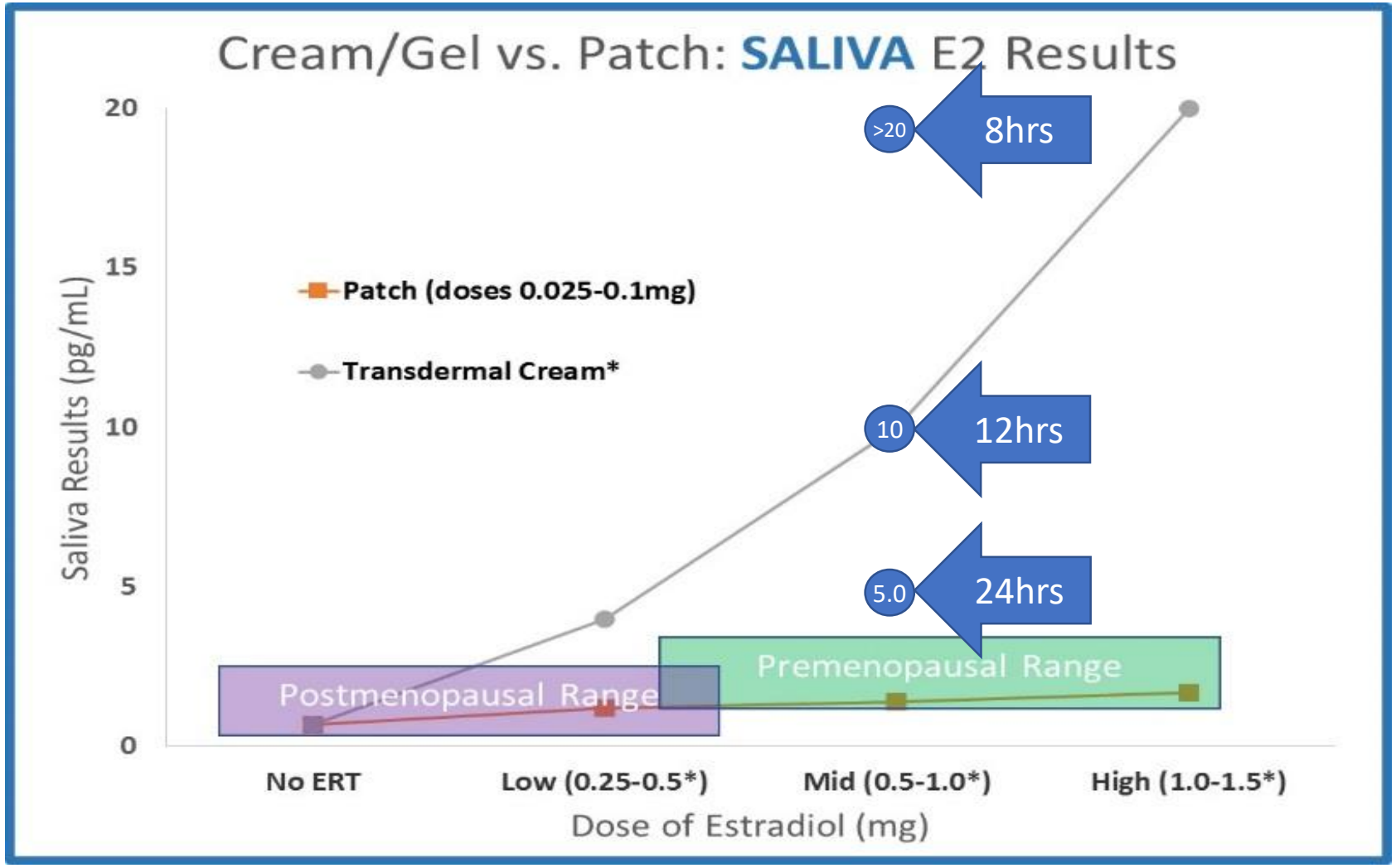
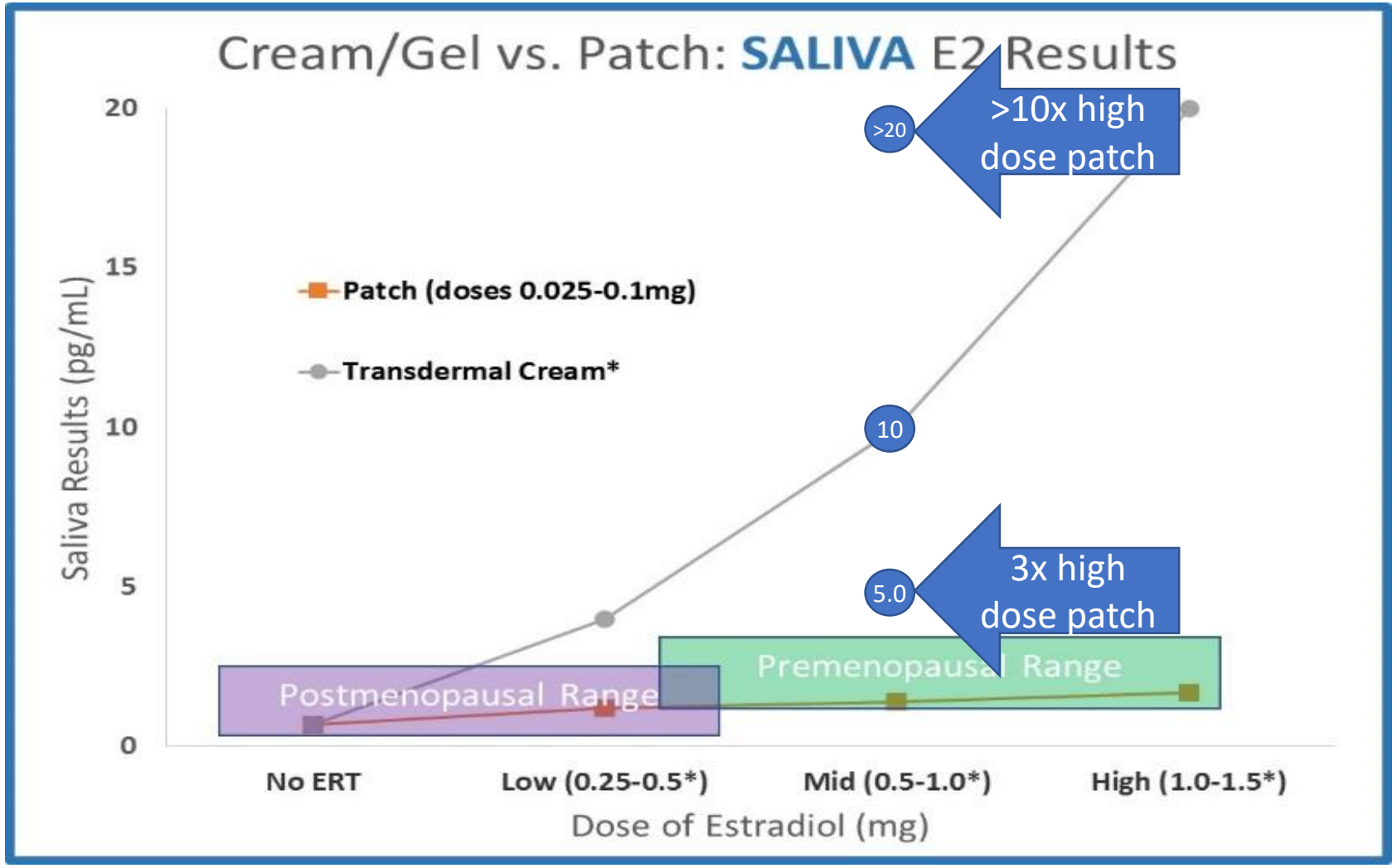


Supplementation expected values published online by ZRT Laboratory (2012)

Saliva says 0.5mg is a high dose



Saliva says 0.5mg is a very high dose



Transdermal Estrogen

Which one is right?

- Serum and urine tell similar stories
- Saliva gives MUCH higher responses

This is a unique issue for transdermal. 0.5mg E2 taken vaginally gives supraphysiological levels in serum, urine and saliva.

Transdermal Estrogen

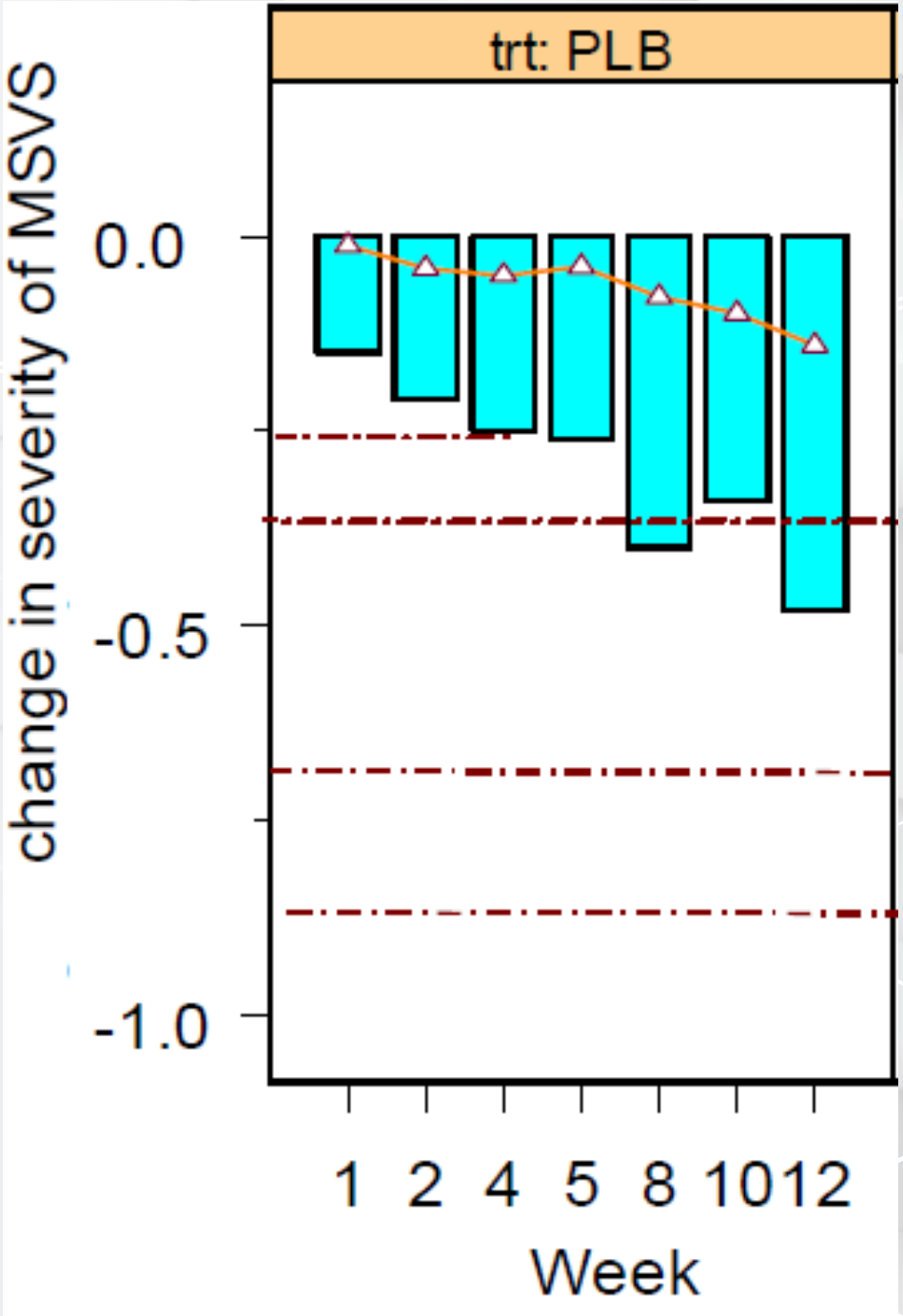
Which one is right?

- Serum and urine tell similar stories
- Saliva gives MUCH higher responses

ASK THE CLINICAL DATA!

Estrogen therapy and hot flashes (MSVS)

Data from Divigel Clinical Pharmacology Review



Missing pieces for saliva when on HRT

- Do values correlate inversely with LH/FSH?
- Do values correlate with bone(E2), muscle(T)?
- Do values correlate with decreased hot flashes compared to placebo?
- Do females get high T symptoms when concentrations are elevated?

Hormone Gel Clinical Evidence

- **Testosterone** (females on 1-2mg exceed male range)
  - Facial hair growth (female)

Hormone Gel Clinical Evidence

- **Testosterone** (females on 1-2mg exceed male range)
  - Facial hair growth (female)

CLIMACTERIC Published 27 February 2019

Efficacy of oral estrogen plus testosterone gel to improve sexual function inpostmenopausal women

S. Chaikittisilpa[a], K. Soimongkol[b] and U. Jaisamrarn[a]

Table 3. Serum testosterone at baseline and 8 weeks after treatment.

	Testosterone group (n = 32)	
	Baseline	*8 weeks*
Total testosterone (nmol/l)	0.32 (0.18, 0.51)	0.40 (0.20, 0.70)
Free testosterone (pmol/l)	4.25 (1.76, 6.34)	5.63 (2.44, 7.19)
Bioavailable testosterone (nmol/l)	0.11 (0.04, 0.16)	0.10 (0.03, 0.03)
SHBG (nmol/l)	63.3 (47.2, 83.9)	81.8 (63.2, 124.2)

Hormone Gel Clinical Evidence

- **Testosterone** (females on 1-2mg exceed male range)
  - Facial hair growth (female)

J Sex Med 2014;11:3002–3011

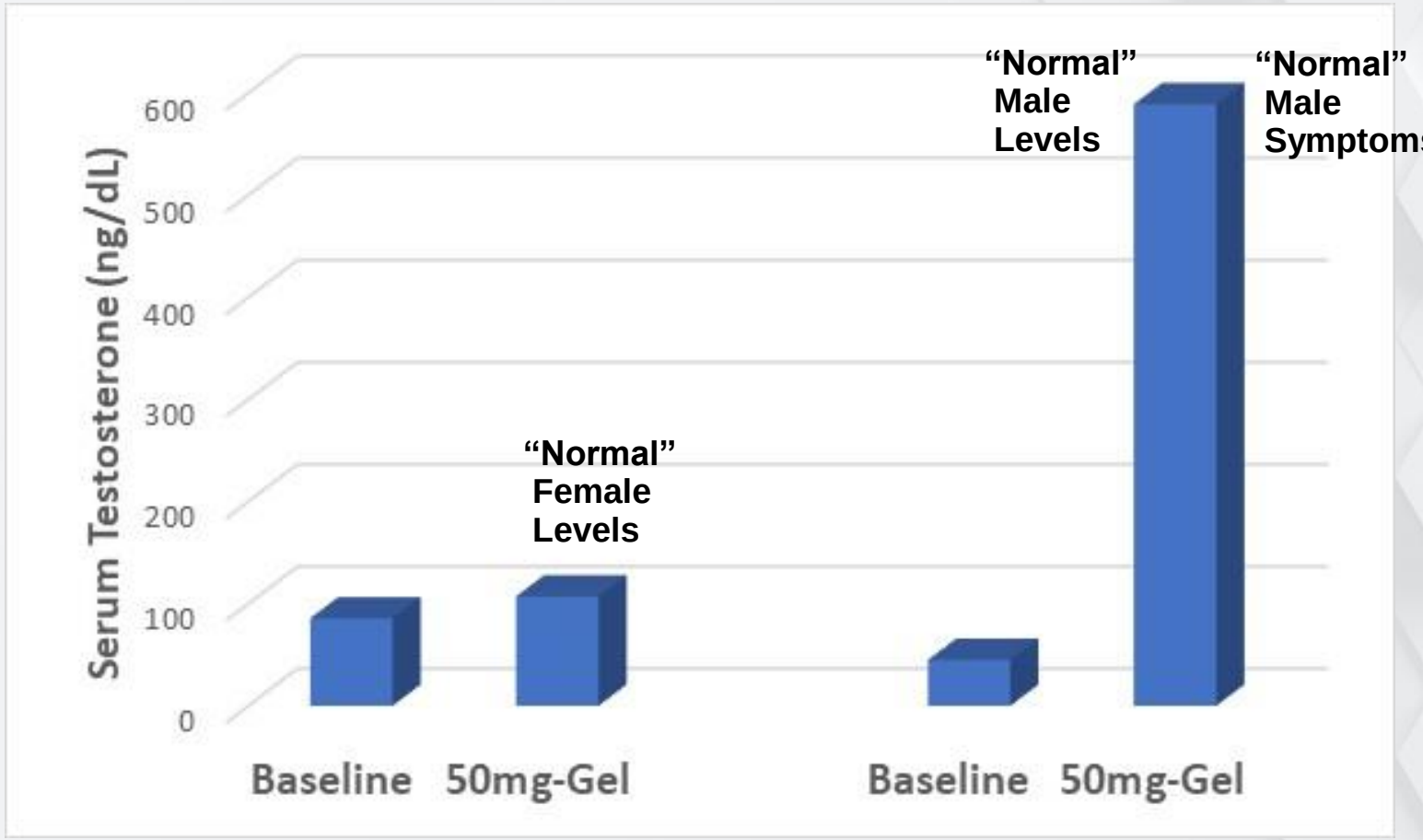
Table 2 Hormone values at baseline and at week 54 of T administration in the three subject groups

	Groups	Baseline	Week 54	GLM analysis	
			Posttreatment	*P* value vs. posttreatment	*P* value vs. groups
T (ng/mL)	TD	0.54 (0.43–0.65)	7.39 (5.33–9.46)	$P < 0.0005$	n.s.
	T-gel	0.45 (0.35–0.55)	5.89 (4.01–7.78)		
	TU	0.44 (0.35–0.54)	6.14 (4.39–7.89)		
SHBG (nmol/L)	TD	65.4 (48.4–82.4)	31.8 (22.3–41.4)	$P = 0.000$	$P = 0.587$
	T-gel	65.2 (48.9–81.4)	31.6 (22.5–40.7)		
	TU	60.3 (44.0–76.5)	34.3 (25.2–43.4)		
cFT (nmol/L)	TD	0.01 (0.01–0.02)	0.31 (0.09–0.54)	$P = 0.000$	$P = 0.910$
	T-gel	0.01 (0.00–0.01)	0.34 (0.12–0.57)		
	TU	0.01 (0.00–0.01)	0.28 (0.06–0.49)		

Data are expressed as mean (95% CI)
cFT = calculated free testosterone; CI = confidence interval; E = estradiol; FSH = follicle-stimulating hormone; GLM = general linear model; LH = luteinizing hormone; n.s. = not significant; PRL = prolactin; SHBG = sex hormone-binding globulin; T = testosterone; TD = testoviron depot; T-gel = testosterone gel; TU = testosterone undecanoate

Hormone Gel Clinical Evidence

- Testosterone
  - Facial hair growth (female)



Hormone Gel Clinical Evidence

- Testosterone
  - Facial hair growth (female)

dutchtest™ BY PRECISION ANALYTICAL INC.

Accession # 003510
Transmale Patient

PRECISION ANALYTICAL INC. SIMPLY · BETTER · TESTING

Ordering Physician:
Precision Analytical

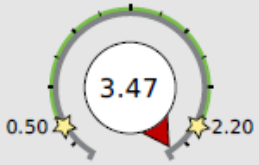
DOB: 1980-
Age: 37
Gender: Male

Collection Times:

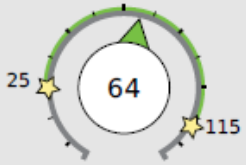
Hormone Testing Summary

Key (how to read the results):

Sex Hormones



Estradiol(E2)



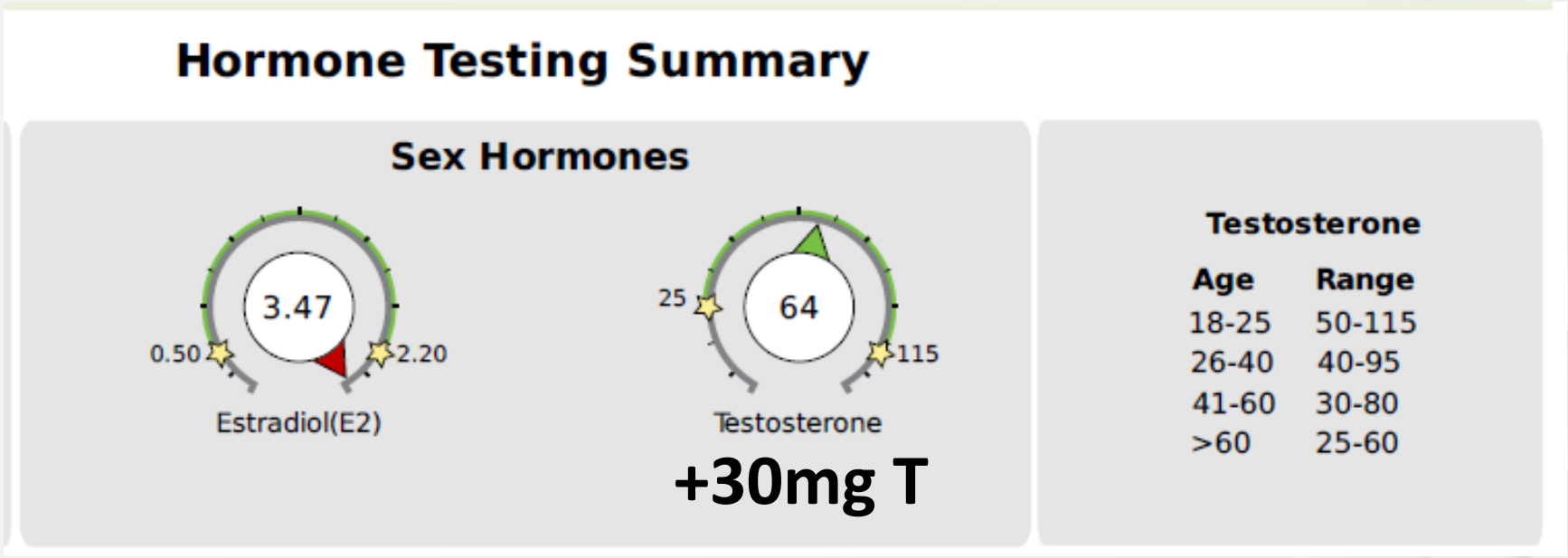
Testosterone

Testosterone

Age	Range
18-25	50-115
26-40	40-95
41-60	30-80
>60	25-60

Hormone Gel Clinical Evidence

- Testosterone
  - Facial hair growth (female)



Hormone Gel Clinical Evidence

- **Testosterone** (50mg, saliva 7-300x normal male)
  - Facial hair growth (female)
  - LH suppression
  - Muscle mass increase

Journal of Gerontology: MEDICAL SCIENCES

Testosterone Threshold Levels and Lean Tissue Mass Targets Needed to Enhance Skeletal Muscle Strength and Function: The HORMA Trial

Fred Sattler,[1,2] Shalender Bhasin,[3] Jiaxiu He,[4] Chih-Ping Chou,[4] Carmen Castaneda-Sceppa,[5]

Methods. One hundred and twelve men aged 65–90 years received testosterone gel (5 g/d vs 10 g/d via Leydig cell clamp) and rhGH (0 vs 3 vs 5 µg/kg/d) in a double-masked 2 × 3 factorial design for 16 weeks. Outcomes included lean

Results. Increases in total testosterone of 1046 ng/dL (95% confidence interval = 1040–1051) and 898 ng/dL (95% confidence interval = 892–904) were necessary to achieve median increases in lean body mass of 1.5 kg and appendicular skeletal muscle mass of 0.8 kg, respectively, which were required to significantly enhance one-repetition maximum

Conclusions. To enhance muscle strength and physical function, threshold improvements in lean body mass and appendicular skeletal muscle mass are necessary and these can be achieved by targeting changes in testosterone levels. rhGH augments the effects of testosterone. To maximize functional improvements, the doses of anabolic hormones should be titrated to achieve target blood levels.

Hormone Cream/Gel Clinical Evidence

- **Testosterone – LH suppression**
  Swerdloff R S et al. JCEM 2000, Kornmann, J Andrology, 2009
- **Testosterone – Muscle mass improvement**
  Sattler, J Gerontol A Biol Sci Med Sci. 2011, Frederiksen, AGE, 2012
- **Estradiol – Hot flash improvement**
  FDA Data, Climara, Vivelle, Esclim, Divigel, Elestrin, Estrasorb; Sattler, J Gerontol A Biol Sci Med Sci. 2011
- **Estradiol – Bone mineral density improvement**
  Climara/Vivelle FDA Data; Yang, J Chin Med Ass, 2007; Gonnelli, J Bone & Min Resrch; Alexandersen, JCE & M 1999; Abdi F, IJPR, 2017
- **Estradiol – FSH suppression**
  Sattler, J Gerontol A Biol Sci Med Sci. 2011; Andersson, Maturitas, 2000; Gupta, Menopause, 2008; Osório-Wender, Braz J Med Biol Res, 1997; Callejon, Arq Bras Cardiol 2009

Hormone Gel Clinical Evidence

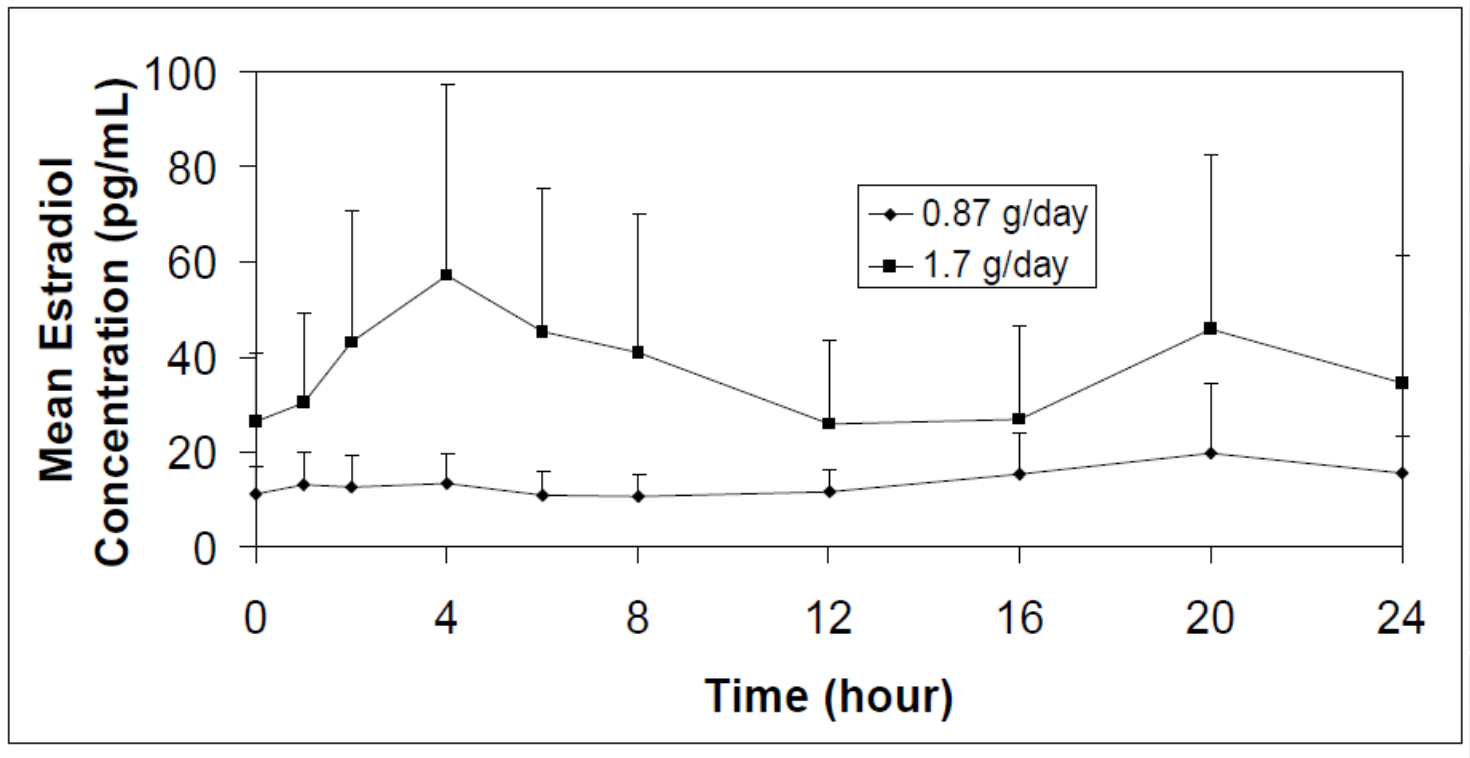
- Estradiol
  - Hot flash improvement
  - Bone mineral density improvement

Estradiol Gels vs. 0.025mg Patch

- **Patch (Vivelle/Climara) 0.025mg**
  - **Drives serum to ~20pg/mL**
  - **Improved hot flash severity at 4 and 12 weeks**
  - **Improved bone mineral density (BMD) at 6 and 12 months**
  - **Higher doses increase serum linearly and add to BMD**

Estradiol Gels vs. 0.025mg Patch

- **Divigel 0.25mg – transdermal gel**
  - Drives serum to similar levels (~16pg/mL, 0.5mg dose ~ 32pg/mL)
  - Does NOT match patch effectiveness for relieving hot flashes
  - The higher dose (0.5mg) DOES match the patches effectiveness



Estradiol Gels vs. 0.025mg Patch

- **Divigel 0.25mg – transdermal gel**
  - Drives serum to similar levels (~16pg/mL, 0.5mg dose ~ 32pg/mL)
  - Does NOT match patch effectiveness for relieving hot flashes
  - The higher dose (0.5mg) DOES match the patches effectiveness
- **Elestrin 0.52mg – transdermal gel**
  - Drives serum to similar values (~15pg/mL, 1.0mg dose ~ 39pg/mL)
  - Does NOT match patch effectiveness for relieving hot flashes
  - The higher dose (1.0mg) DOES match the patches effectiveness
- **Evamist 1.5mg – transdermal gel**
  - Drives serum to ~20pg/mL, 3.0mg dose ~ 31pg/mL)
  - Does match patch effectiveness for relieving hot flashes

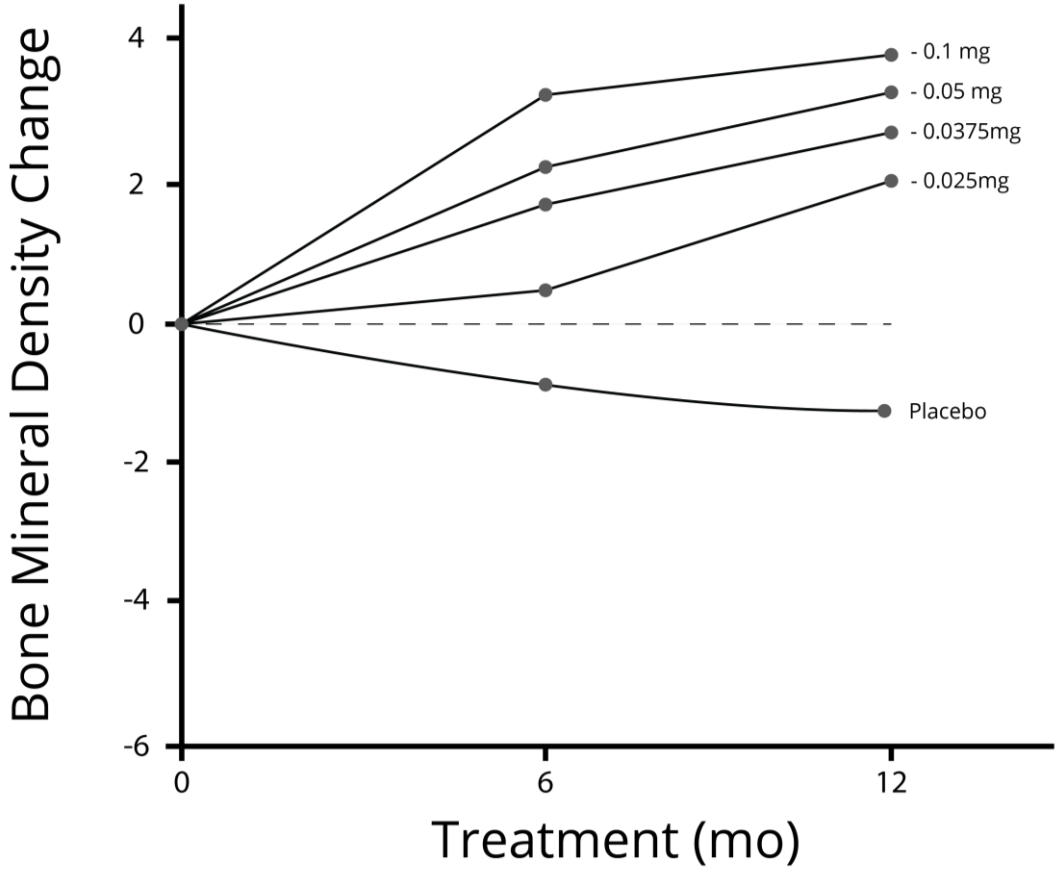
Estradiol Gels vs. 0.025mg Patch

- **Estrasorb 8.625mg** (yes, really)
  - Drives serum to about 60pg/mL (2.9mg, serum~30pg/mL)
  - Does match patch effectiveness for relieving hot flashes
  - Hot flash data for “low” 2.9mg dose not given
  - This formula is an emulsion
  - Serum data implies its absorption is 6x less than Divigel

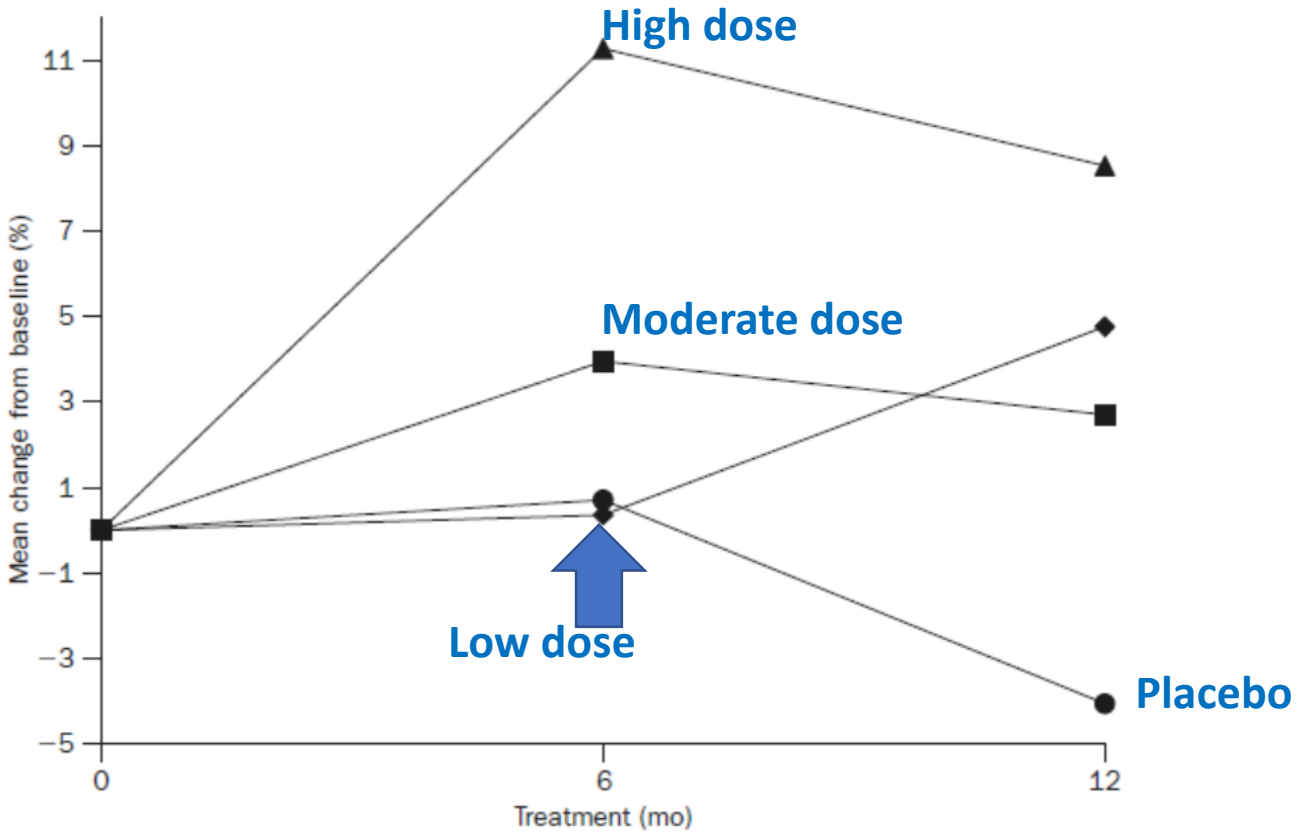
Estradiol Gels vs. 0.025mg Patch

- **Estrogel 0.75mg**
  - Drives serum to about 28pg/mL
  - Does match patch effectiveness for relieving hot flashes
  - Does NOT match patch effectiveness for increasing BMD
  - The higher dose (1.5mg) DOES match the patch for BMD

E2 Patches vs. Gel: BMD

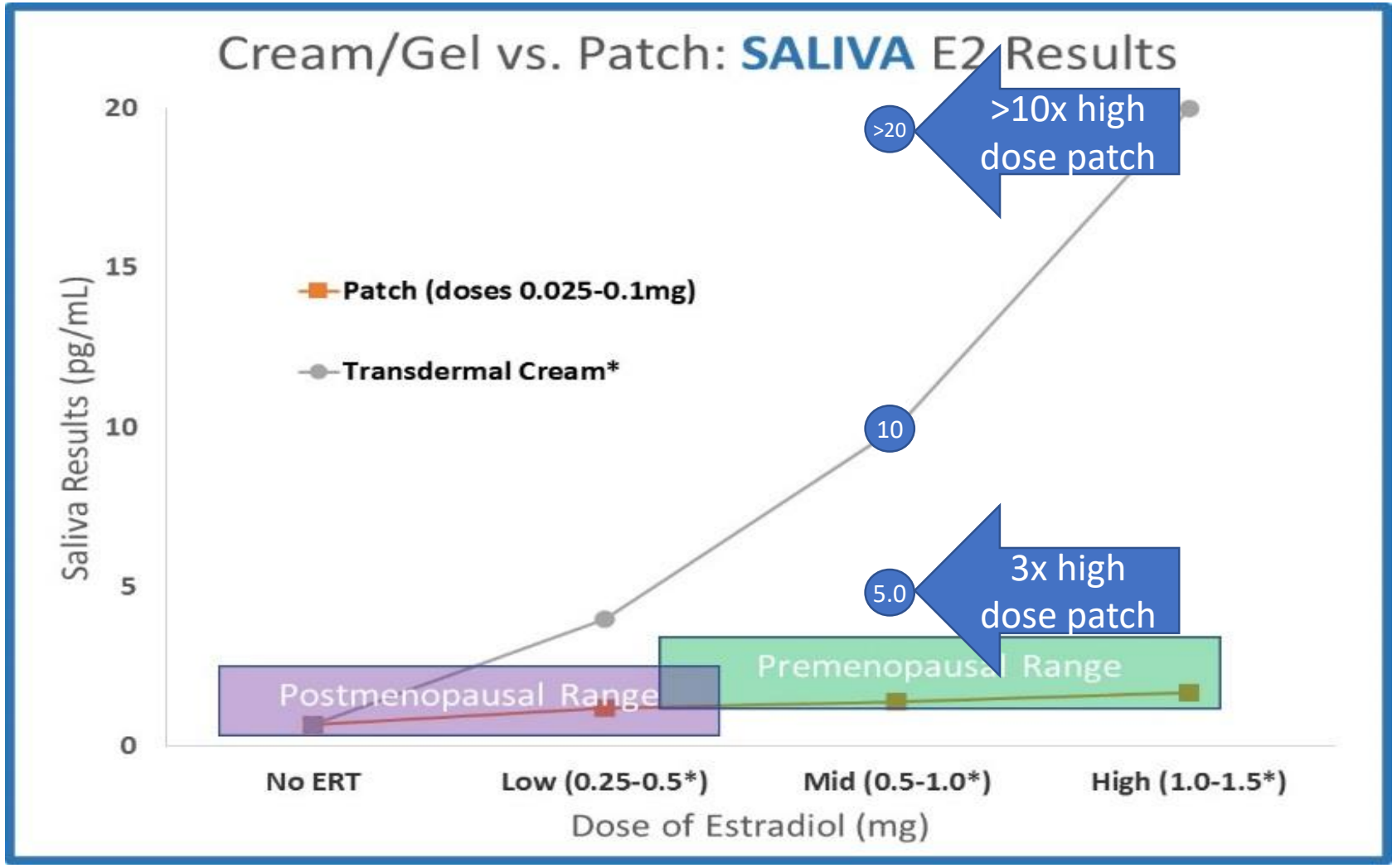


Patch



Estrogel

Saliva says 0.5mg is a very high dose



Estradiol Gels vs. 0.025mg Patch

- No literature evidence found to confirm that systemic exposure from 0.25-0.5 mg doses is “high” as saliva implies

Estradiol Gels vs. 0.025mg Patch

- Chang study showed breast tissue levels of E2 increased ~100-fold with 1.5mg E2 gel
  - Serum moved from ~60 → ~200pg/mL
  - These were premenopausal women
  - **BUT the E2 was put directly on the breast!**

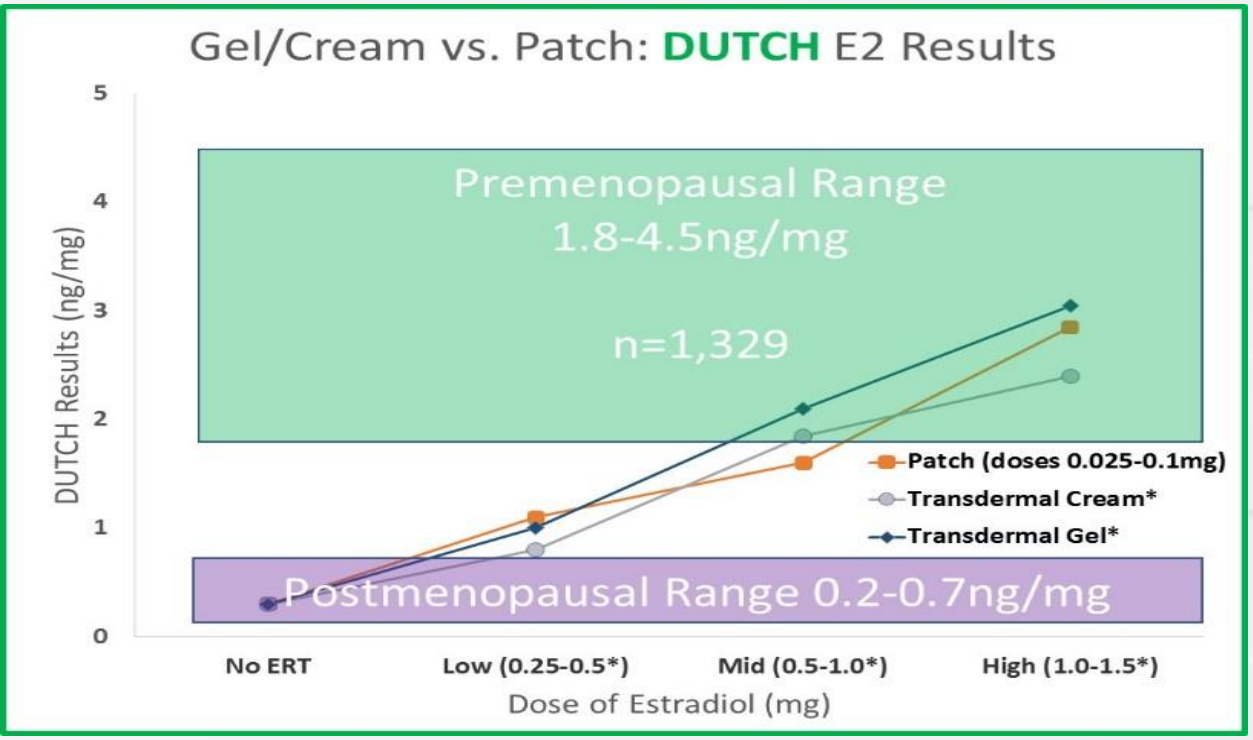
Chang, Fertility & Sterility, 1995

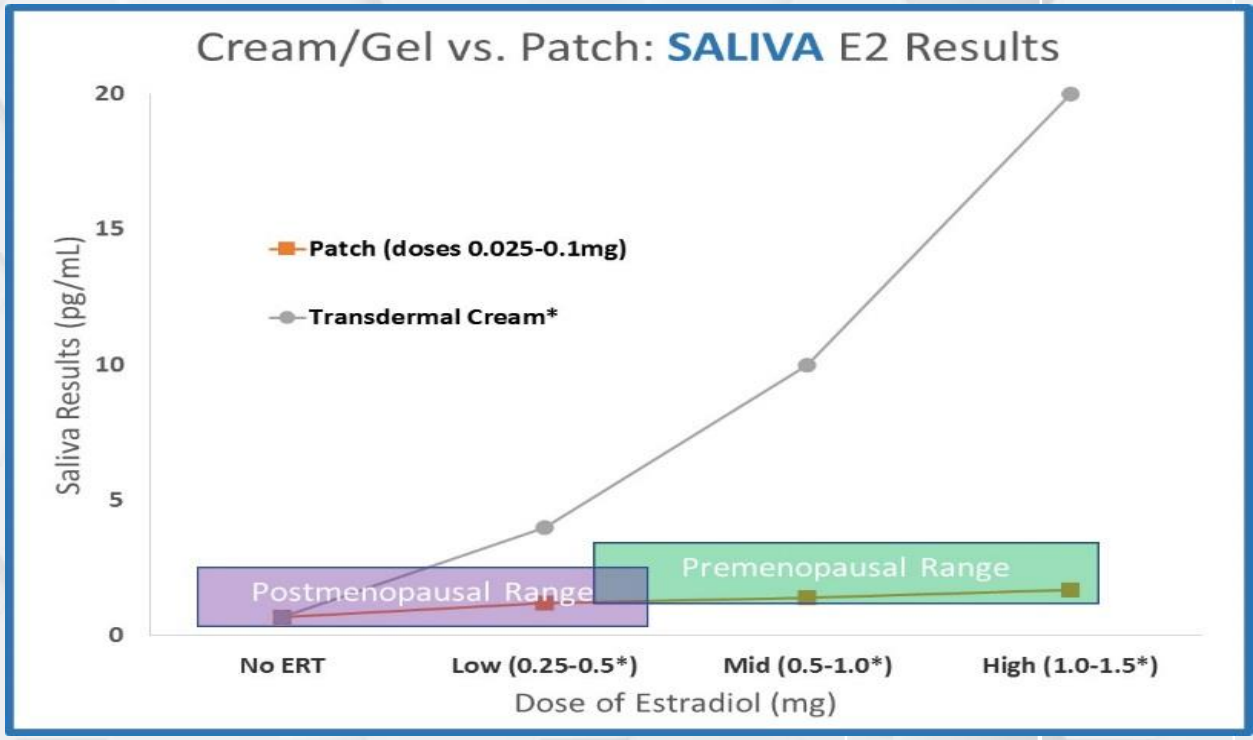
Estradiol Gels vs. 0.025mg Patch

- Leonetti[(1)] showed TD Pg antiproliferative
  - Other studies show a lack of antiproliferative effects[(2)]
  - No lab measurements in Leonetti study
  - Saliva values remain elevated for months after cessation of therapy so not likely reflective of endometrium[(3)]

(1)Leonetti, Fertility & Sterility, 2003
(2)Wren, Lancet, 1999
(3)Ilyia, Int J Pharm Compounding, 1998

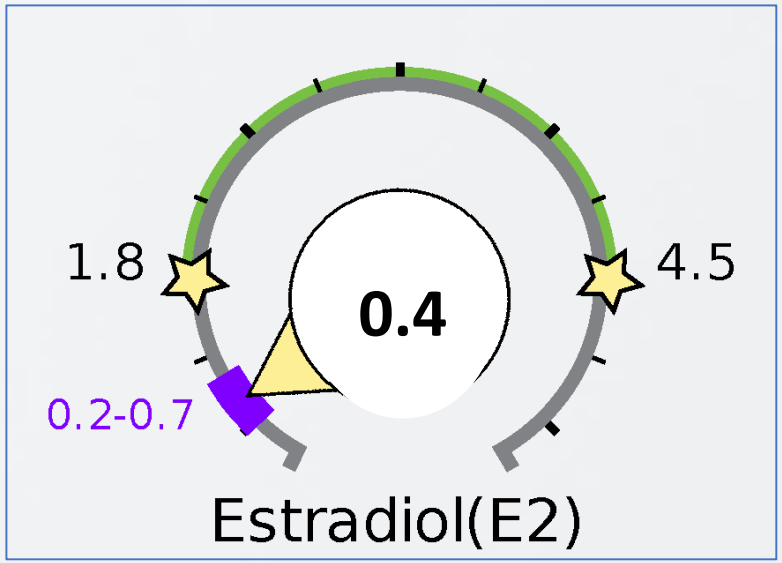
Urine Matches Clinical Picture, Saliva does NOT



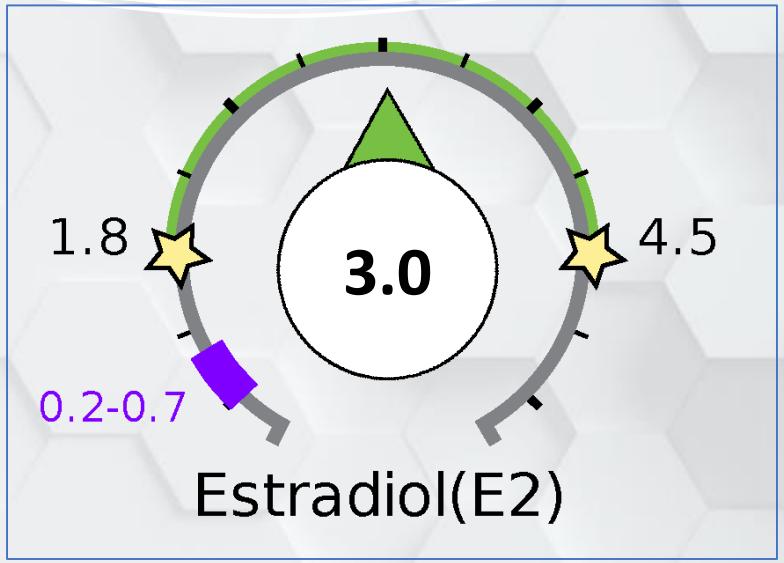


The above statement is regarding transdermal estradiol

ERT with DUTCH



Postmenopausal



Premenopausal

MONITORING (B)HRT WITH LAB TESTING

Tutorials available at www.dutchtest.com/videos/hormone-tutorials

PRECISION ANALYTICAL INC.
CREATORS OF THE DUTCH TEST

Oral Progesterone	Patch, Pellet, Injection	Transdermal Estrogen	Transdermal Testosterone	Transdermal Progesterone	Vaginal or Anal Mucosa	Oral Estrogen	Sublingual
✓ DUTCH	✓ DUTCH	✓ DUTCH	✓ DUTCH	✗ DUTCH	✓ DUTCH (E/T)	✗ DUTCH	✗ DUTCH
The DUTCH test provides useful feedback when using oral progesterone to aid sleep disturbance related to menopause. 5a (more active) and 5b (less active) metabolites are measured to individualize doses of oral progesterone.	Values increase intuitively with dosing. For estrogen patches, see Transdermal Estrogen comments. Pellets and injections also increase levels intuitively, but the increase may exceed what is seen in serum testing. DUTCH allows for monitoring both the proper dosing of hormones as well as metabolic patterns.	Target values between the top of the postmenopausal range and the lower third of the premenopausal range correlate with patient clinical improvement (bone density, hot flash relief, etc.). Doses that push levels to the middle of the premenopausal range and beyond may be excessive. DUTCH is preferred over serum due to the inclusion of metabolites.	Levels generally parallel measurable clinical outcomes (increased lean body mass, decreased LH values in men). Epi-testosterone values can also be used to assess gonadal suppression due to TRT (levels decrease as TRT increases and are <10 ng/mg with complete suppression).	Creams and gels cannot be effectively monitored with any lab testing. Values increase only slightly with dosing. Because of the uncertainty of tissue levels, take caution to use concurrently with estrogen therapy without endometrium surveillance (ultrasound or biopsy).	Special method removes potential contamination. Monitoring testosterone and estrogens is effective and parallels the increases seen in serum testing.	Cannot be used to effectively monitor dosing due to 1st-pass metabolism. Most of the hormone in urine has not been in circulation as "free" hormone.	Lab testing is not effective. DUTCH is confounded by the hormone that is swallowed.
					✗ DUTCH (P)	✓ DUTCH	✓ DUTCH
					Urine metabolites of progesterone underestimate systemic progesterone when taken vaginally.	While dosing is not effectively monitored with DUTCH, metabolite patterns can be effectively assessed.	While dosing is not effectively monitored with DUTCH, metabolite patterns can be effectively assessed.
✗ SERUM	✓ SERUM	✓ SERUM	✓ SERUM	✗ SERUM	✓ SERUM	✓ SERUM	✗ SERUM
Results go up-and-down quickly. If taken at bedtime, levels return to baseline within a few hours. Results can also be inaccurate due to progesterone metabolites cross-reacting with immunoassay tests.	Serum testing is well suited for use with these types of therapies.	Effective for monitoring estrogen creams and gels similarly to patches. Levels may have an up-and-down pattern throughout the day, unlike when using patches.	Results correlate to clinical symptoms. In men, lean body mass increases only when serum (and likely urine) results increase.	Values do not increase significantly with dosing.	While serum levels likely represent systemic uptake of hormone, interpret with care as you may not know if your value represents a peak or a trough.	Serum testing offers the best feedback on monitoring the actual dose of oral estradiol.	Serum testing is not effective. Results rise and fall too rapidly for useful testing. In many cases, results are back to baseline within a few hours.
✗ SALIVA	✓ SALIVA	✗ SALIVA	✗ SALIVA	✗ SALIVA	✗ SALIVA	✓ SALIVA	✗ SALIVA
Ineffective for monitoring oral progesterone for the same reason as serum above.	Testing can conceptually be used, but available testing is less accurate than serum.	Results are exaggerated, do not correlate to clinical symptoms and are highly variable.	Results are exaggerated, do not correlate to clinical symptoms and are highly variable.	Values are exaggerated and highly variable. Levels may remain elevated for months after cessation of therapy.	Testing has not been shown to be effective for monitoring vaginal/anal hormones.	Testing can conceptually be used, but available testing is less accurate than serum.	Saliva is contaminated directly, and testing is not meaningful.

DUTCH TESTING & (B)HRT GUIDE - WOMEN

Oral Progestrone	Estradiol Patch	Estradiol Cream/Gel	Testosterone or Estradiol Pellet	Vaginal Estrogen or Testosterone	Testosterone Cream/Gel	DHEA
Why						
Effective at balancing ERT, but clinical effects are due largely to metabolites formed in the gut. A good option when postmenopausal women struggle with sleep. A different ROA may be better for premenopausal women. 100-200mg has been shown to balance con-current ERT.	Patches offer consistent hormone dosing over time and are very effective at managing hot flashes. Even low doses typically increase bone mineral density (BMD).	Proven to increase serum and urine levels as well as improve hot flashes and BMD. Transdermal E2 is attractive because it is easy to use and bypasses first pass metabolism. Estriol often given in does 1 - 4 times higher than estradiol.	Pellets offer consistent hormone dosing over time for testosterone and estradiol. Research is limited on effects on hot flashes and BMD. Because serum/urine E2 levels match or exceed those seen in patches, E2 pellets are likely to help with hot flashes and BMD.	Low doses increase local tissue levels while higher doses also increase systemic levels. Placing in the top 1/3 of the vagina significantly increases uterine levels. Estriol often given in does 1 - 4 times higher than estradiol.	Transdermal testosterone can be used to correct low T and improve sex drive and muscle mass.	Sublingual or oral DHEA will increase systemic levels and also contribute to downstream androgens (testosterone) and estrogens.
	ERT, especially with an intact uterus, should be balanced with adequate progesterone (vaginal or oral preferred).					
Common Dosing Strategies						
Low 25 - 50 mg **High** >200 mg **Most Common** 100 - 200 mg *Consider taking continuously or as an on/off cycle*	**Low** 0.012 - 0.025 mg **High** 0.1 mg **Most Common** 0.05 mg *Consider taking continuously or as an on/off cycle and changed 1 - 2 times per week*	**Low** 0.1 - 0.25 mg Estradiol 0.1 - 1.0 mg Estriol **High** 1.0 - 2.5 mg Estradiol 2.0 - 5.0 mg Estriol **Most Common** 0.25 - 0.5 mg Estradiol 0.25 - 2.5 mg Estriol *Consider taking daily continuously or as an on/off cycle*	**Low** <5 mg Estradiol 20 - 50 mg Testosterone **High** >12 mg Estradiol >125 mg Testosterone **Most Common** 5 mg Estradiol 100 mg Testosterone *Inserted every 3 - 4 months*	**Low** 0.01 mg Estradiol 0.25 mg Testosterone **High** 0.5 mg Estradiol 2 mg Testosterone **Most Common** 0.1 mg Estradiol 0.25 - 1.0 mg Estriol 0.25 - 1.0 mg Testosterone *Taken daily, possibly with cycling*	**Low** 0.5 - 2.0 mg **High** 10 - 20 mg **Most Common** 1 - 5 mg *Taken daily, at waking or bedtime*	**Low** 1 - 5 mg **High** 25 - 50 mg **Most Common** 5 - 10 mg *Usually taken daily*
How to Monitor with DUTCH						
DUTCH results only show which metabolites are preferred. Evaluate which pathway is dominant (alpha or	**Monitoring Estrogen Replacement Therapy (ERT)** Target values between the top of the postmenopausal range (0.7ng/mg for estradiol) and within the first third of the premenopausal range (about 2.5ng/mg). The specific target for a patient depends on the patient's history and symptoms as			Levels above the postmenopausal range imply systemic uptake. For localized (vaginal) effects only, results	It is optimal if levels of T (as well as metabolites) are in range. Less is needed if metabolites are 5a favored.	Monitor conversion to testosterone, E2 and metabolites of both. DHEA and testosterone metabolites may be

DUTCH Resources

- Education
  - HRT Matrix
  - Treatment Matrix
  - Video Tutorials
- Personal Education
  - Consultations with Experts
- The DUTCH Test!
  - DUTCH Complete
  - DUTCH Plus
  - ½ Price Offer

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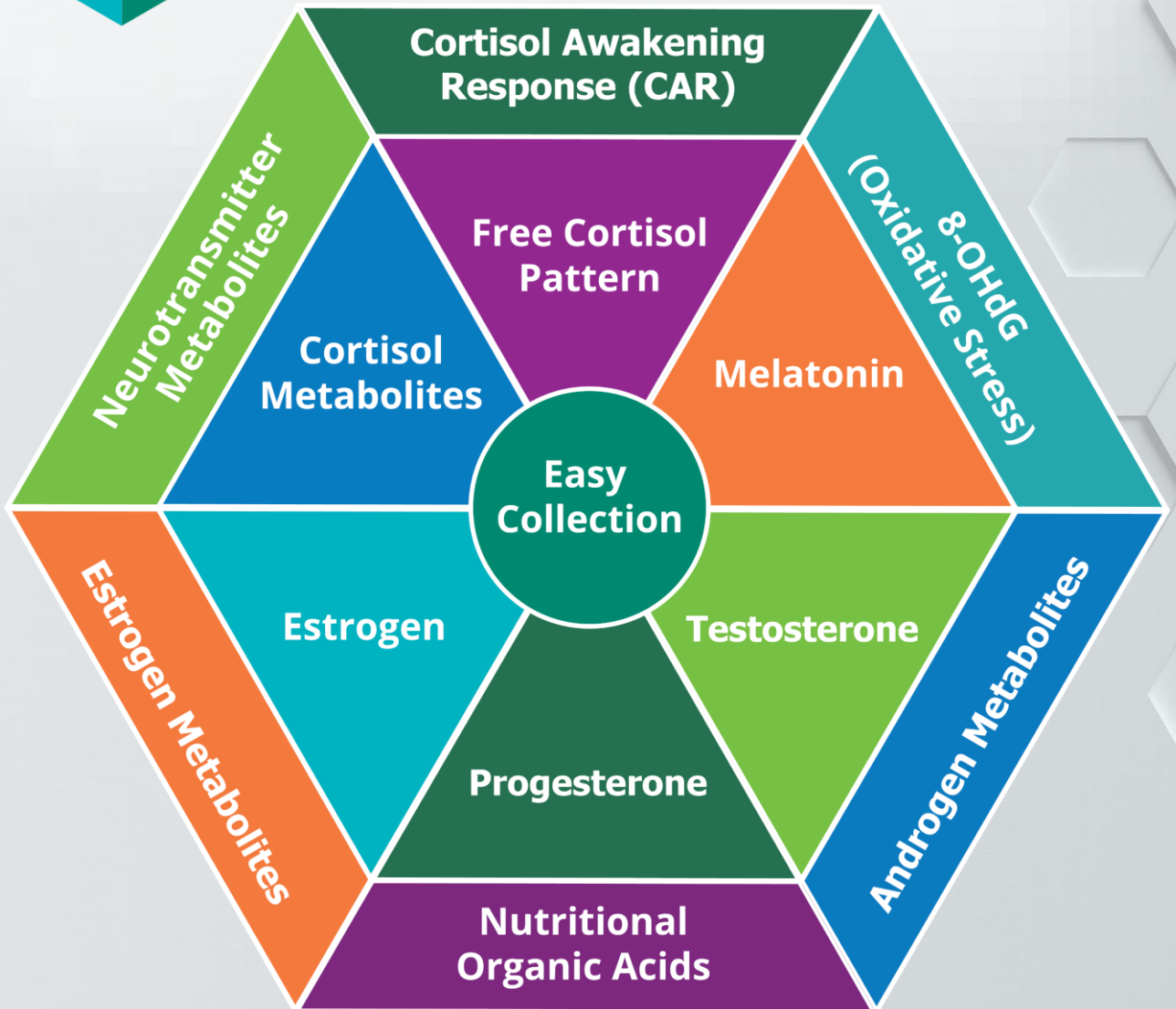

dutchtest

Dried Urine Test for Comprehensive Hormones