
dutch®

Choosing the Right Hormone Test – Serum, Saliva, or Dried Urine?

Lindsey Szczepanski, MSN, APRN, WHNP-BC

August 25, 2026

American College of Apothecaries Group Training

Remember

1. The information in this presentation is provided for informational and educational purposes only and is not medical or treatment advice.

2. Any information and statements regarding dietary or herbal supplements have not been evaluated by the Food and Drug Administration and are not intended to diagnose, treat, cure, or prevent any disease.

3. The use of any information provided in this presentation is solely at your own risk.

Learning Objectives

- **Compare** the clinical utility, advantages, and limitations of serum, saliva, and dried urine hormone testing methods in assessing baseline hormone status.
- **Apply** evidence-based strategies to select the most appropriate laboratory testing modality for assessing baseline hormone patterns in clinical practice.
- **Understand** why it might be beneficial to monitor menopausal HRT.
- **Recognize** which laboratory methods and tests are most ideal for monitoring transdermal (TD) estradiol (E2) and oral micronized progesterone (OMP).
- **Gain insights** on the additional value that can come from the DUTCH Test.

Why is Baseline Hormone Testing Important?

Why is baseline hormone testing important?

- Establish correct diagnosis
  - Essential for distinguishing among conditions with overlapping symptoms
  - Ex: Hair loss – is it high androgens or hypothyroid?
- Differentiates the source of hormonal abnormalities
  - Ex: total and free testosterone reflect mixed ovarian and adrenal production, while DHEA-S is almost entirely adrenal origin
- Guides treatment decisions
  - Ex: Treat amenorrhea differently if hypothyroid origin vs functional hypothalamic amenorrhea origin
- Safety and Monitoring of treatment
  - Ex: Estradiol levels on low-end of targeted range, however patient still symptomatic and worried about bone health. Would you increase the dosage?

Who is a good candidate for baseline testing?

Anyone with any of the following complaints may benefit from hormone testing:

- Acne
- Amenorrhea
- Anxiety
- Brain fog
- Breast cancer risk
- Breast tenderness
- Chronic pain
- Cramping
- Cycle irregularities
- Cysts
- Depression
- Dysmenorrhea
- Dyspareunia
- Endometriosis
- Erectile issues
- Excessive body hair
- Excessive facial hair
- Exercise endurance issues
- Exercise intolerance
- Fatigue
- Fibrocystic breasts
- Fibroids
- Hair loss
- Heavy bleeding
- Hirsutism
- Hot flashes
- Impotence
- Insomnia
- Irritability
- Joint pain
- Long cycles
- Low motivation
- Luteal phase defect
- Mastalgia
- Menorrhagia
- Moodiness
- Obesity
- Overweight
- Panic attacks
- Pain with sex
- PCOS
- Pelvic pain
- PMDD
- PMS
- Poor cognition
- Poor muscle mass
- Short cycles
- Osteoporosis
- Vaginal dryness
- Weight gain

dutch

What are the Goals of Testing?

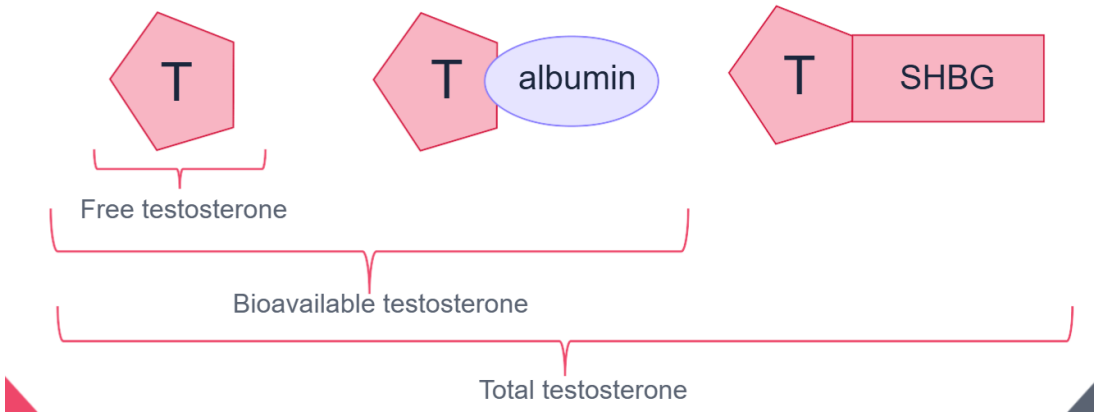
What are the goals for selecting this test?

Do you want to know:

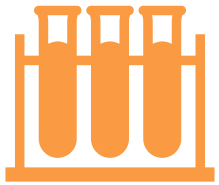
- Free vs. Bioavailable vs. Total hormone levels
- Hormone metabolism pathways
  - Helpful in determining if favoring problematic pathways could contribute to symptoms
- Assessment of cortisol levels
  - diurnal rhythm, insomnia sample, cortisol awakening response, etc.
- For cycling females – do you want to see estrogen and progesterone fluctuations throughout the month? Requiring multiple data points throughout the month/cycle
  - This approach is often helpful for patients with:
    - Fertility concerns, irregular cycles, PCOS, a luteal phase that shifts from month-to-month, long or short cycles, hormonal symptoms that tend to fluctuate throughout the cycle -- PMS, mid-cycle spotting, migraines, etc.
    - Patients with cycling hormones and no menstrual bleeding due to: Partial hysterectomy (ovaries intact but no uterus), uterine ablations, Mirena IUD or other progestin IUDs

Free vs. Bioavailable vs. Total Hormones

- Free
  - Hormone not bound to any protein. Considered the biologically **active** form
- Bioavailable
  - Free hormone + hormone loosely bound to albumin
  - Hormone bound to albumin is considered more “available” because it can easily separate from albumin
- Total
  - The sum of all circulating hormone = hormone tightly bound to SHBG + bioavailable (hormone loosely bound to albumin) + free hormone



Free vs. Bioavailable vs. Total Hormones



Free

Serum

Urine

Saliva

Bioavailable

Urine

Total Hormone

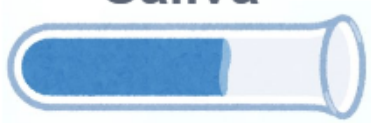
Serum

Serum vs. Saliva vs. Urine – What Are We Testing?

Serum

- Hormones (**mostly non-bioavailable**) that are in transit TO tissues. Collection represents blood levels at that single point in time.
- Production
  ↓
  Blood Stream 

Saliva



- **Free hormones** that have been taken up by *salivary* tissues. Collection represents salivary levels at that single point in time.
- Blood stream
  ↓
  Salivary gland tissue uptake 

Urine (4-Spot)

- **Free & bioavailable hormones** that have been taken up by tissues *throughout the body* AND metabolized. Collection represents average levels over hours of time.
- Blood stream
  ↓
  Whole body tissue uptake
  ↓
  Utilization metabolism
  ↓
  Blood stream → Kidneys ↗

Urine x 4 samples

Common Sex Hormones and Cortisol test options

Common Serum (Blood) Tests

- Total testosterone
- Free testosterone
- DHEA-S
- Estradiol (E2)
- Progesterone
- AM Cortisol

Common Serum (Blood) Tests – non sex hormones

- SHBG
- FSH
- LH
- CBC/CMP
- Iron/Ferritin
- HbA1c
- CRP
- TSH
- Free T4
- Free T3

Common Urine Markers

- Testosterone
- 5a-DHT
- 5a-Androstanediol
- 5b-Androstanediol
- DHEA-S
- Total DHEA
- Etiocholanolone
- Androsterone
- Epi-testosterone
- a-Pregnanediol
- b-Pregnanediol
- Estrone (E1)
- Estradiol (E2)
- Estriol (E3)
- 2-OH-E1
- 2-OH-E2
- 4-OH-E1
- 4-OH-E2
- 16-OH-E1
- 2-Methoxy-E1
- Free Cortisol/Cortisone
- Free Cortisol pattern
- Metabolized Cortisol

Common Saliva Markers

- Free Cortisol/Cortisone
- Free cortisol pattern
- Cortisol Awakening response (CAR)
- Estradiol
- Progesterone
- DHEA-S
- Testosterone

dutch

Why do we care about hormone metabolites?

Why do we care about hormone metabolites?

- What are urine hormone metabolites?
  - **Water-soluble end products** of hormone metabolism
  - Reflects the **body's processing and clearance** of circulating hormones
  - They are **formed when hormones undergo metabolic conversions in the liver** (hydroxylation, glucuronidation, sulfation, methylation, etc.) **and peripheral tissues**, transforming them into conjugated, water-soluble forms suitable for renal excretion
  - **Gives insights** into how the **tissues are utilizing** the hormones

If there is an **issue in the tissue** → hormone metabolism may be valuable in helping **identifying hormone dysfunction** or nuanced cases

4-spot dried urine vs. Serum

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

Mark Newman, Suzanne M. Pratt, Desmond A. Curran & Frank Z. Stanczyk

BMC Chemistry **13**, Article number: 20 (2019) | Cite this article

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.

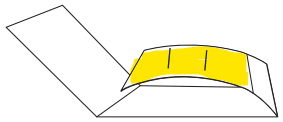
Newman M, et. al. BMC Chem. 2019 Feb 4;13(1):20.

- Urine measures hormones after tissue uptake and metabolism, while serum measures hormones circulating to tissues, baseline urinary estrogen and progesterone metabolites correlate well with serum levels.

dutch®

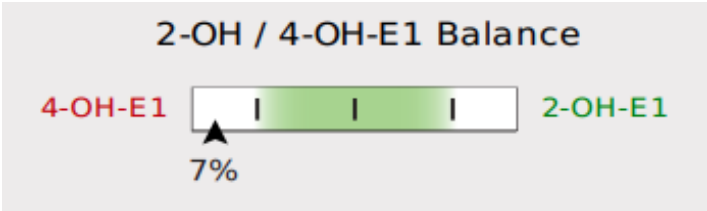
Why is hormone metabolism important: DUTCH testing

Bonus!

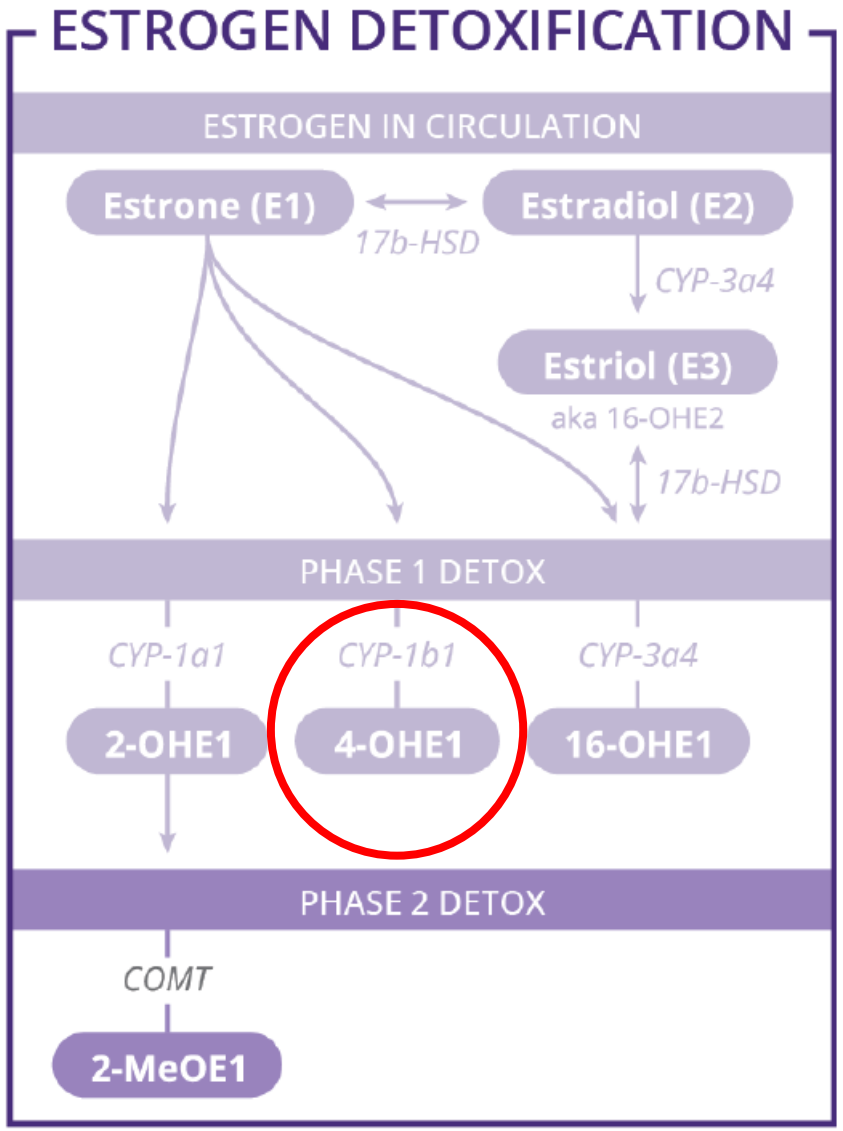


- For example:

  Favoring the **4-OHE1** (genotoxic/DNA damage) pathway in phase 1 may result in an increased risk of estrogen-associated cancers, such as breast and uterine cancers.
  Remember that in addition to assessing the 2OH/4OH balance, 4-OH levels also need to be taken into consideration.



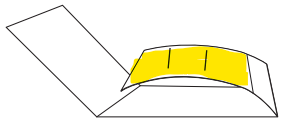
The **2OH/4OH Balance slider** above shows that this patient favors the "carcinogenic" 4-OH pathway.



dutch

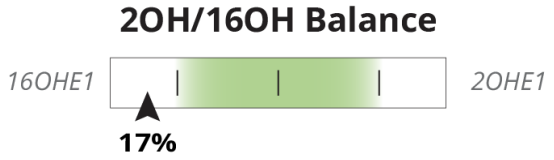
Why is hormone metabolism important: DUTCH testing

Bonus!

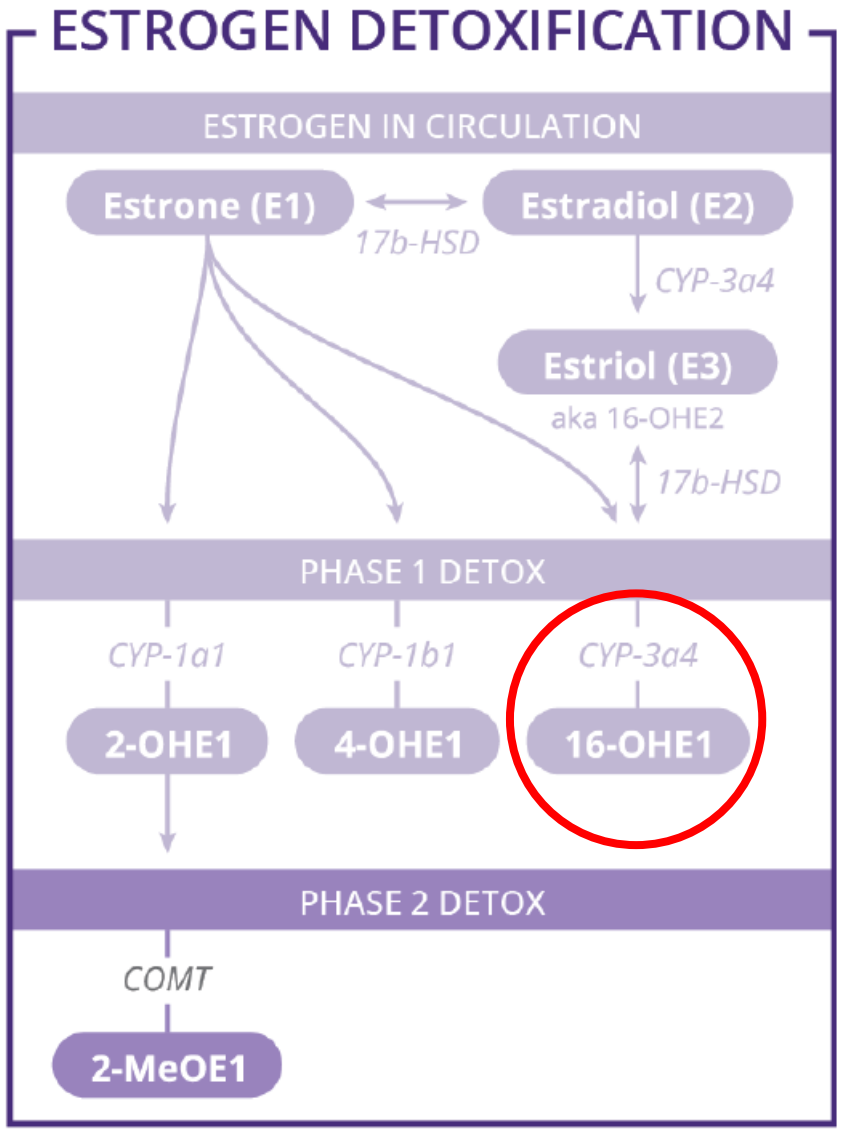


- For example:

  Favoring the **16-OH-E1** (estrogenic/proliferative) pathway may contribute to breast tenderness, heavy bleeding, fibroids and polyp growth, PMS, migraines.
  Also, favoring this pathway may increase the growth of an estrogen receptor positive (ER+) breast cancer that is already present in the body.



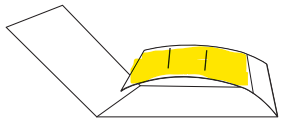
The **2OH/16OH Balance slider** above shows that this patient favors the proliferative 16-OH pathway.



dutch

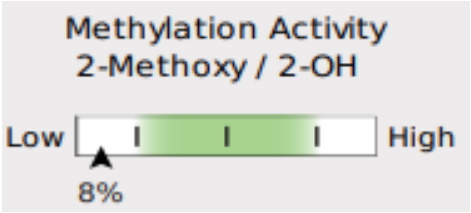
Why is hormone metabolism important: DUTCH testing

Bonus!

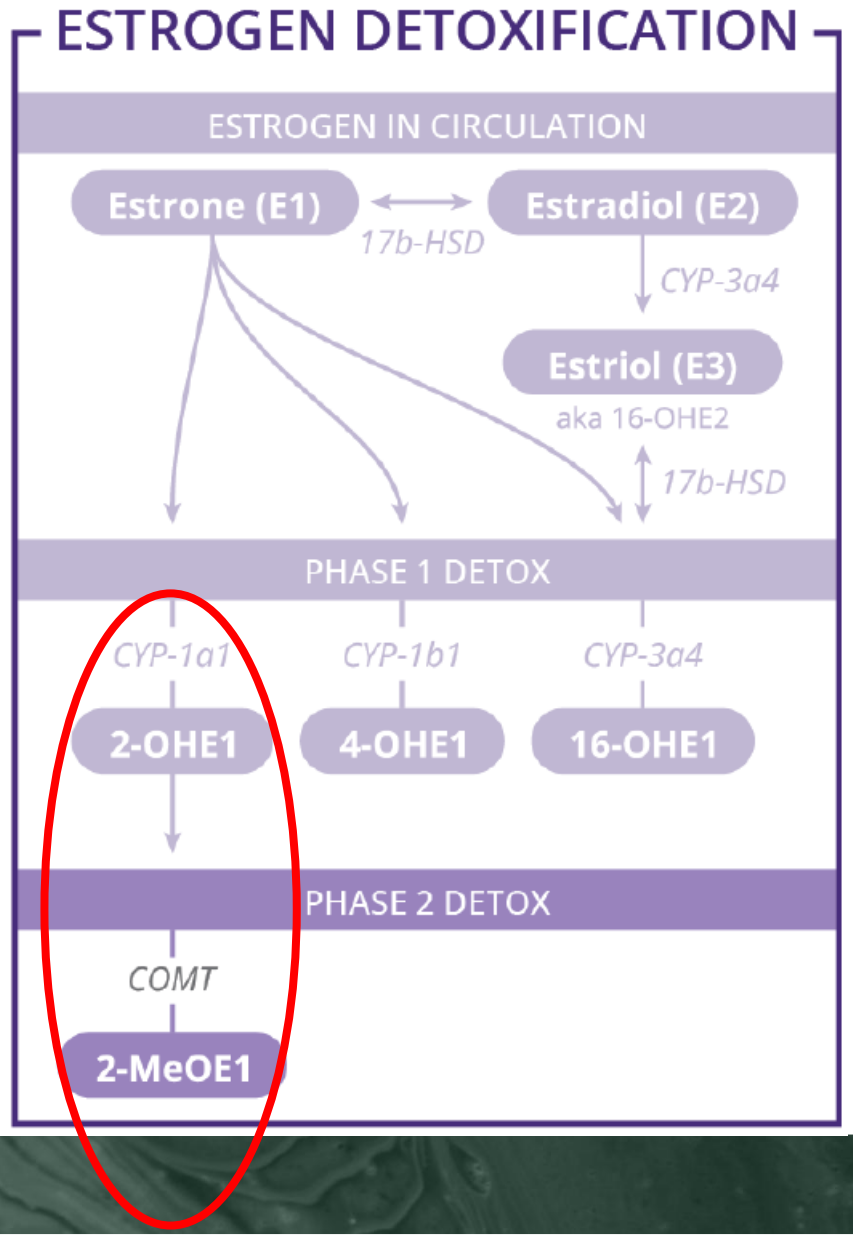


- For example:

  Slow methylation can cause the **4-OH** (and 2-OH) estrogen metabolites to build up in phase 1.
  This can increase a patient's risk of estrogen-associated risk factors or symptoms.



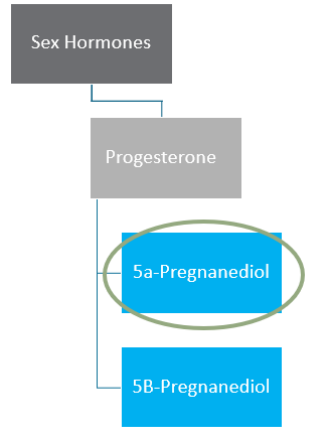
The **Methylation Activity slider** above shows that this patient has slow methylation.

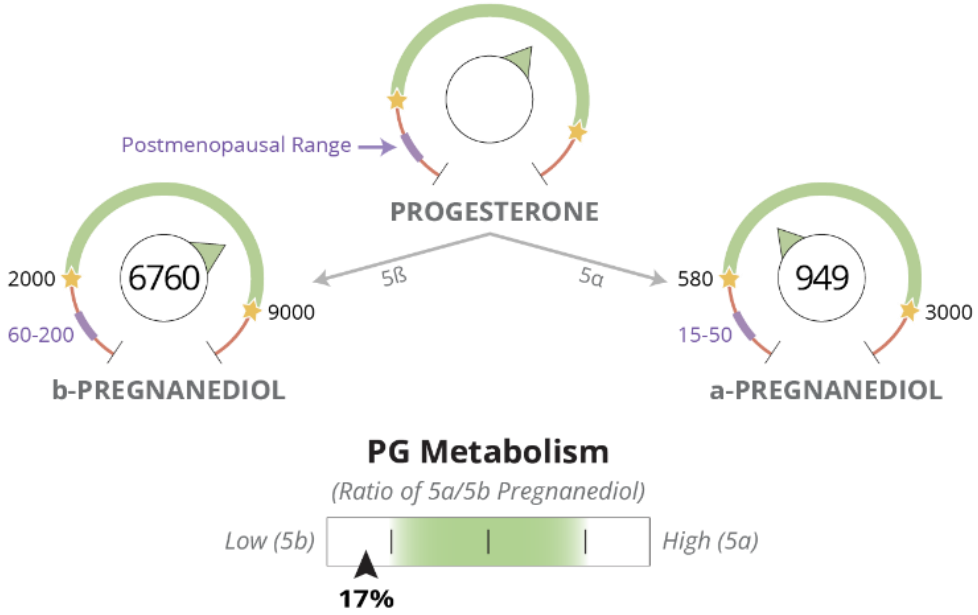


dutch

Why is hormone metabolism important: DUTCH testing

- Progesterone metabolism patterns may be helpful to assess, as the **alpha progesterone metabolites** modulate GABA(A) receptors and generally support mood and sleep.
- For example, when OMP is supplemented, women favoring the beta pathway (as shown below) ***may*** need a higher dose than women favoring the alpha pathway to see similar improvements in mood and sleep.

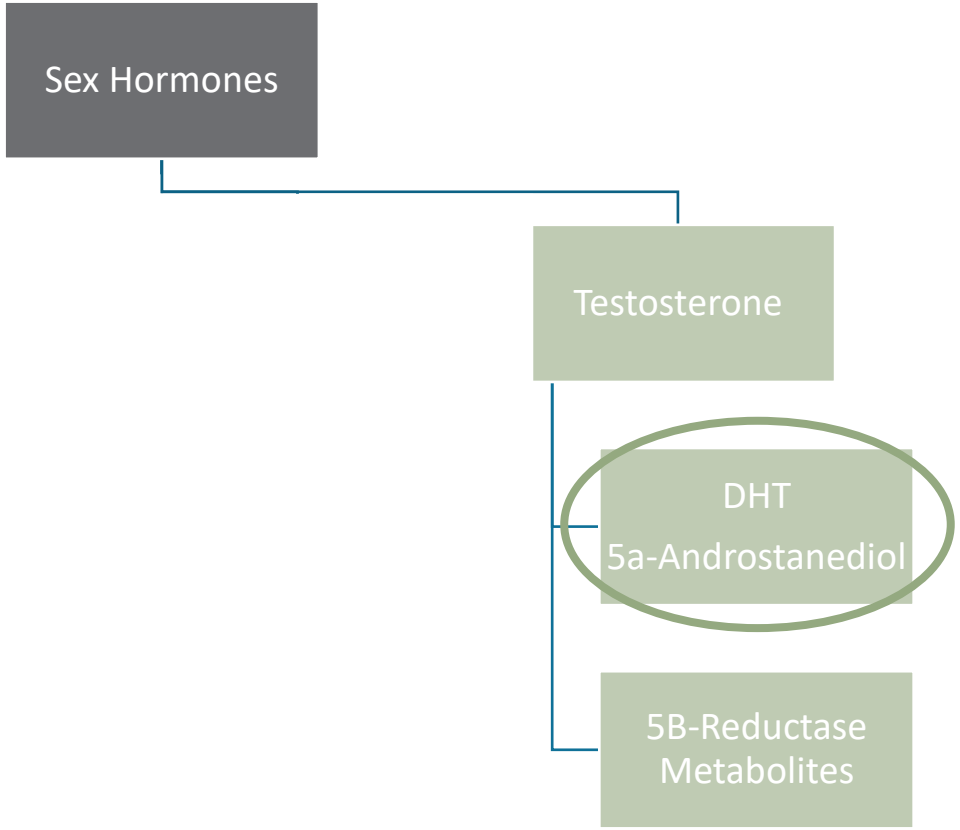




Why is hormone metabolism important: DUTCH testing

Why is hormone metabolism important?

Favoring this **5a androgen** pathway may contribute to androgen excess symptoms, such as acne, scalp hair loss, and body hair growth.



Assessment of Cortisol

Assessment of Cortisol levels

Serum Tests

- Total Cortisol - AM

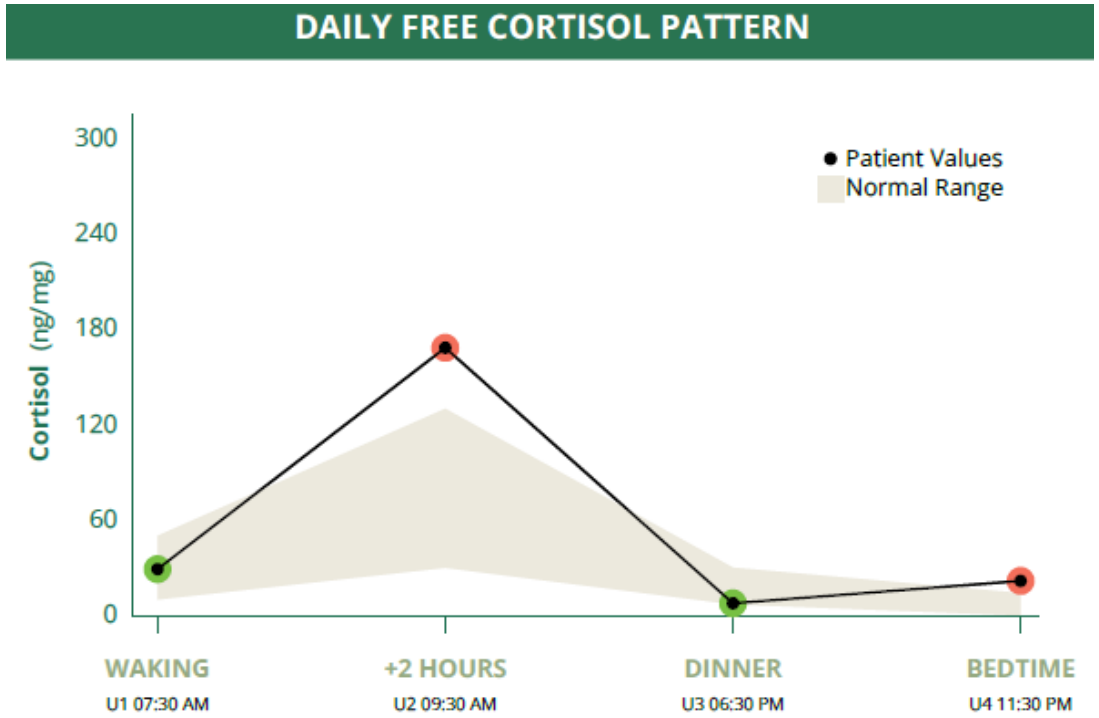
Urine and Saliva

- Diurnal pattern of cortisol
- Diurnal pattern of cortisone
- Metabolized Cortisol (urine only)
- Cortisol awakening response (CAR) (saliva only)
- Insomnia sample

Adrenal Hormone Markers: Serum vs. Urine vs. Saliva

Why is urine and saliva cortisol testing preferred?

- It's impractical to measure the diurnal pattern of cortisol in the serum (5 blood draws in one day??)
- Urine and saliva can be comfortably collected at home and can yield valuable data about free cortisol levels throughout the day.



4-spot dried urine vs. Saliva for Cortisol Assessments

Highlights:

- 4-spot dried urine provides a practical alternative to 24-hour urine collection
- Four dried urine samples accurately reflect the daily (diurnal) cortisol rhythm
- Urinary free cortisol shows a diurnal pattern comparable to salivary cortisol
- Combining saliva and dried urine enables a more comprehensive assessment of cortisol production and metabolism

ELSEVIER

Journal of Clinical & Translational Endocrinology

Volume 22, December 2020, 100243

Dried urine and salivary profiling for complete assessment of cortisol and cortisol metabolites

Mark Newman [a], Desmond A. Curran [a], Bryan P. Mayfield [a b]

Show more

Add to Mendeley Share Cite

https://doi.org/10.1016/j.jcte.2020.100243

Get rights and content

Under a Creative Commons license

Open access

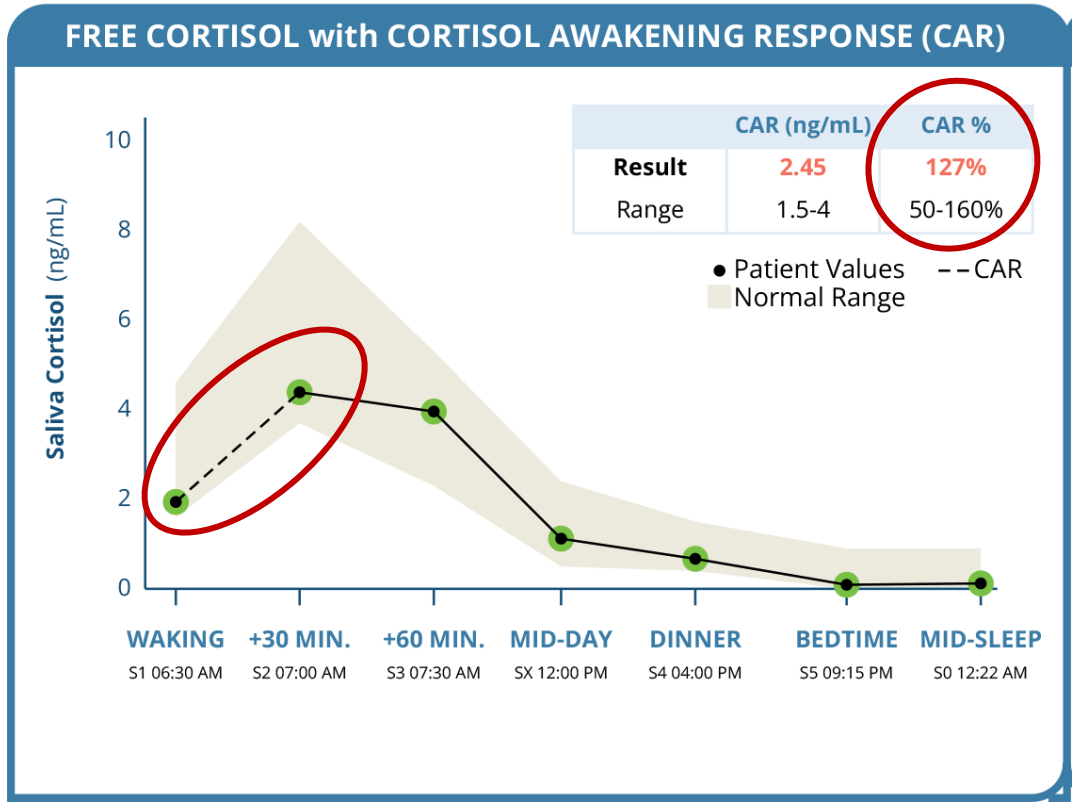
Highlights

- Dried urine is an alternative to liquid urine for measuring cortisol and its metabolites.
- 4-spot urine sampling is representative of a 24-hour urine collection.
- 4-spot urinary cortisol measures reflect the diurnal pattern of change in cortisol.
- Comprehensive profiling of cortisol is viable using both saliva plus

dutch

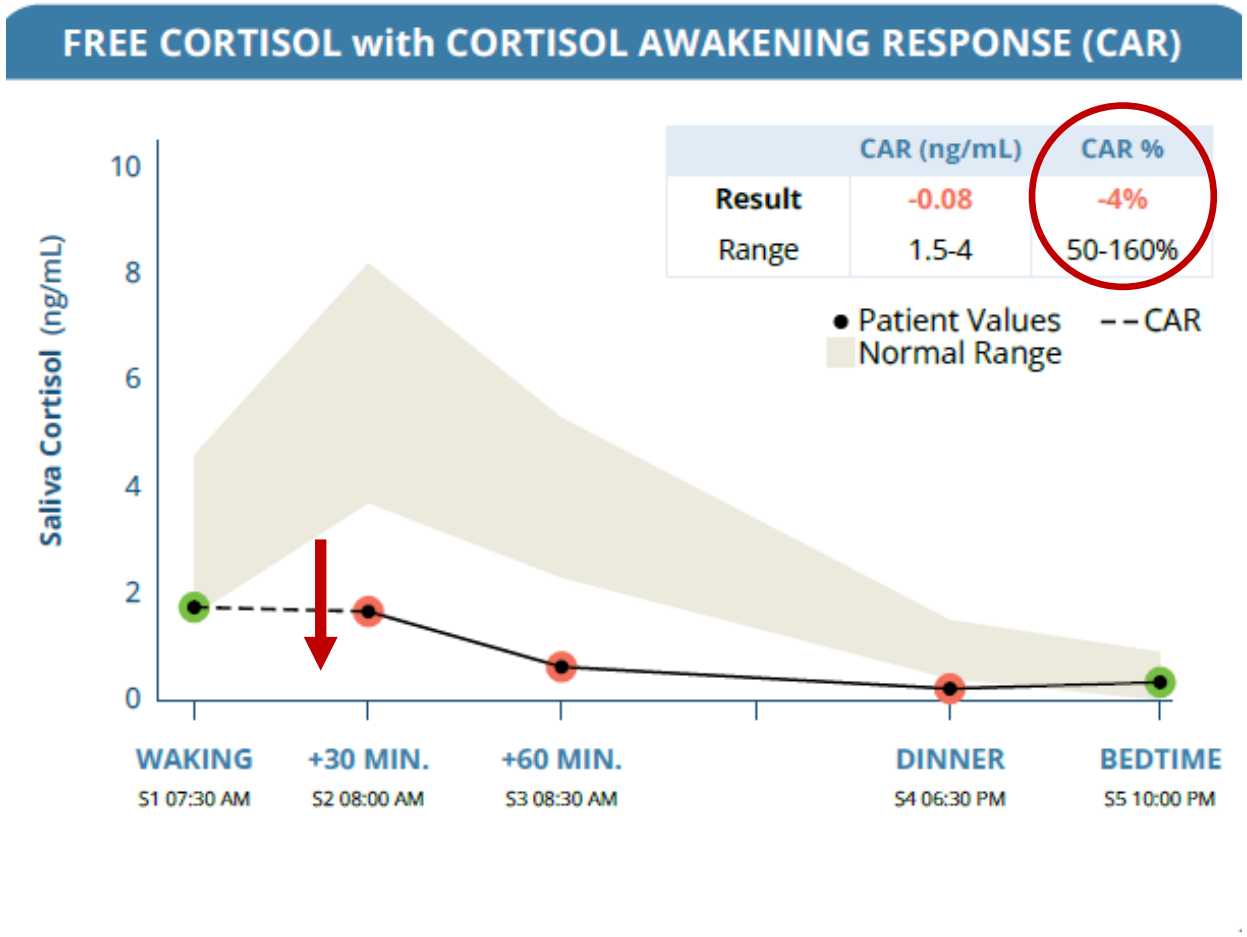
Saliva Cortisol – great for assessing Free Cortisol in real time

With saliva you can assess the **Cortisol Awakening Response (CAR)**



- The **CAR** is measured as the percent difference between the waking and 30-minute free cortisol.
- In healthy adults (assuming the test is timed correctly) the magnitude of the CAR was found to range between 50-160% or a rise of 1.5-4.0ng/mL.
- It estimates a **person's HPA axis resiliency and their ability to cope** with stress
  - **Low CAR** = **under**-response to stress
  - **High CAR** = **over**-response to stress
- May be helpful for patients with sleep issues, mood issues, stressful lifestyles, fatigue, symptoms present in the mornings, etc.

Saliva Cortisol Awakening Response (CAR)



Low CAR

For example, this person has an ***under-response*** to stress.

The CAR is like **the "mini-stress test" for the day**.

Did they have an appropriate "kick start" for the day?

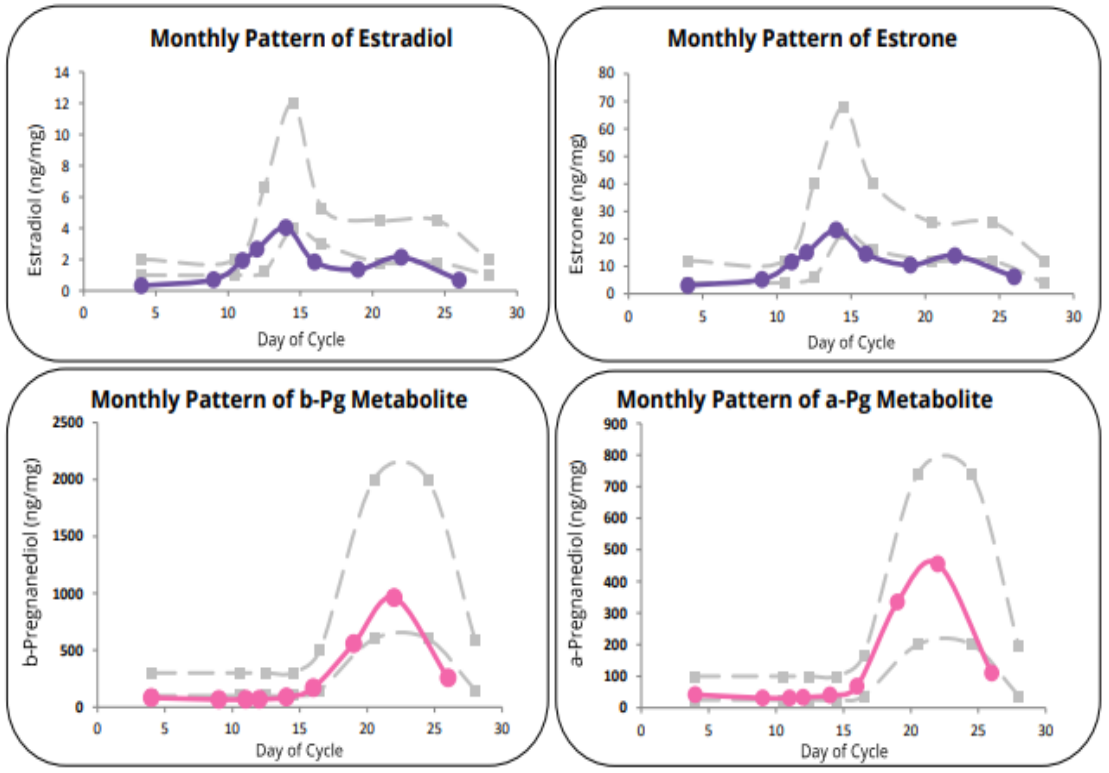
Cycling Patients using Cycle Mapping – 4-spot dried Urine

Cycling Patients using Cycle Mapping – 4-spot dried Urine

Providers can get a good view into:

- Ovulation – did it occur?
- Peak progesterone levels
- Timing of events during the cycle (ovulation, luteal phase length, etc.)
- Estrogen exposure during an entire cycle.

Estrogen (E) patterns can be seen below in purple. Progesterone (Pg) patterns can be seen below in pink. Normal ranges are within the gray dashed lines. See page 2 for more information.

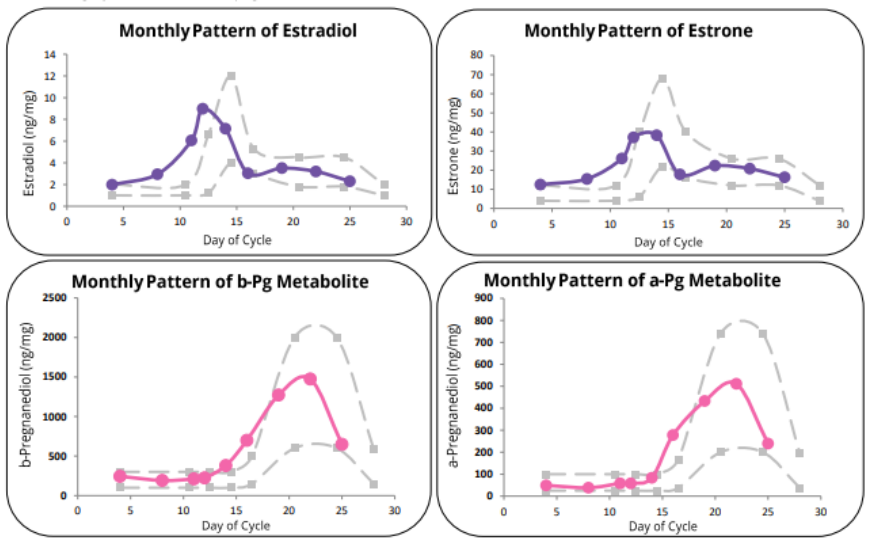


All values given in ng/mg creatinine

Getting multiple data points for bigger picture

25-DAY CYCLE

OVULATION AROUND DAY 12, PG PEAK DAY 22



All values given in ng/mg creatinine

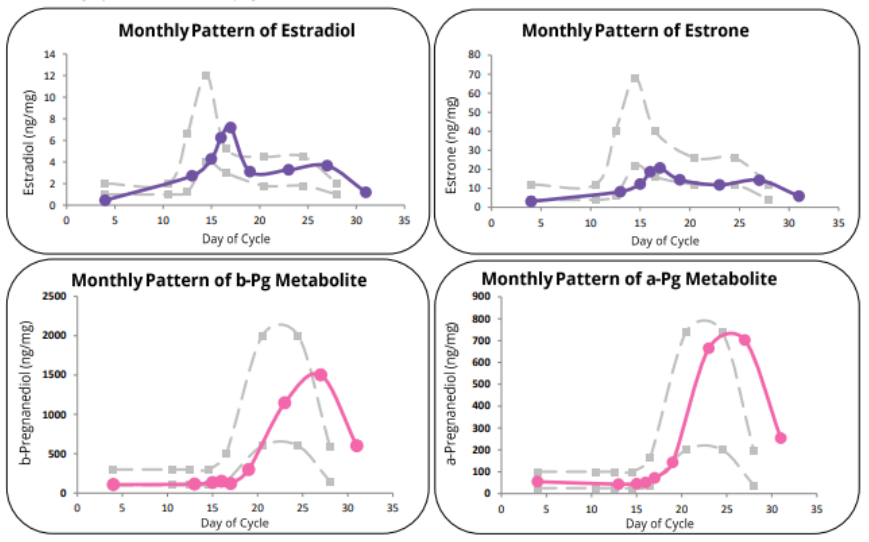
Measurement	1	2	3	4	5	6	7	8	9
Day(s) of Cycle	4	8	11	12	14	16	19	22	25

The days listed above were used for measurements. Two samples are used and listed for long cycles or patients without a normal cycle.

Estradiol (E2)	1.98	2.95	6.06	8.98	7.15	3.04	3.51	3.20	2.30
Estrone (E1)	12.4	15.4	26.1	37.2	38.2	17.8	22.3	20.8	16.3
a-Pregnanediol	49	38	58	58	83	278	433	511	239
b-Pregnanediol	244	191	210	224	379	698	1271	**1475**	647
b-Pregnanediol/E2 Ratio	123	65	35	25	53	230	362	461	281
Creatinine		0.62	0.28	0.21	0.50	0.15	0.52	0.29	0.42

31-DAY CYCLE

OVULATION AROUND DAY 17, PG PEAK DAY 27



All values given in ng/mg creatinine

Measurement	1	2	3	4	5	6	7	8	9
Day(s) of Cycle	4	13	15	16	17	19	23	27	31

The days listed above were used for measurements. Two samples are used and listed for long cycles or patients without a normal cycle.

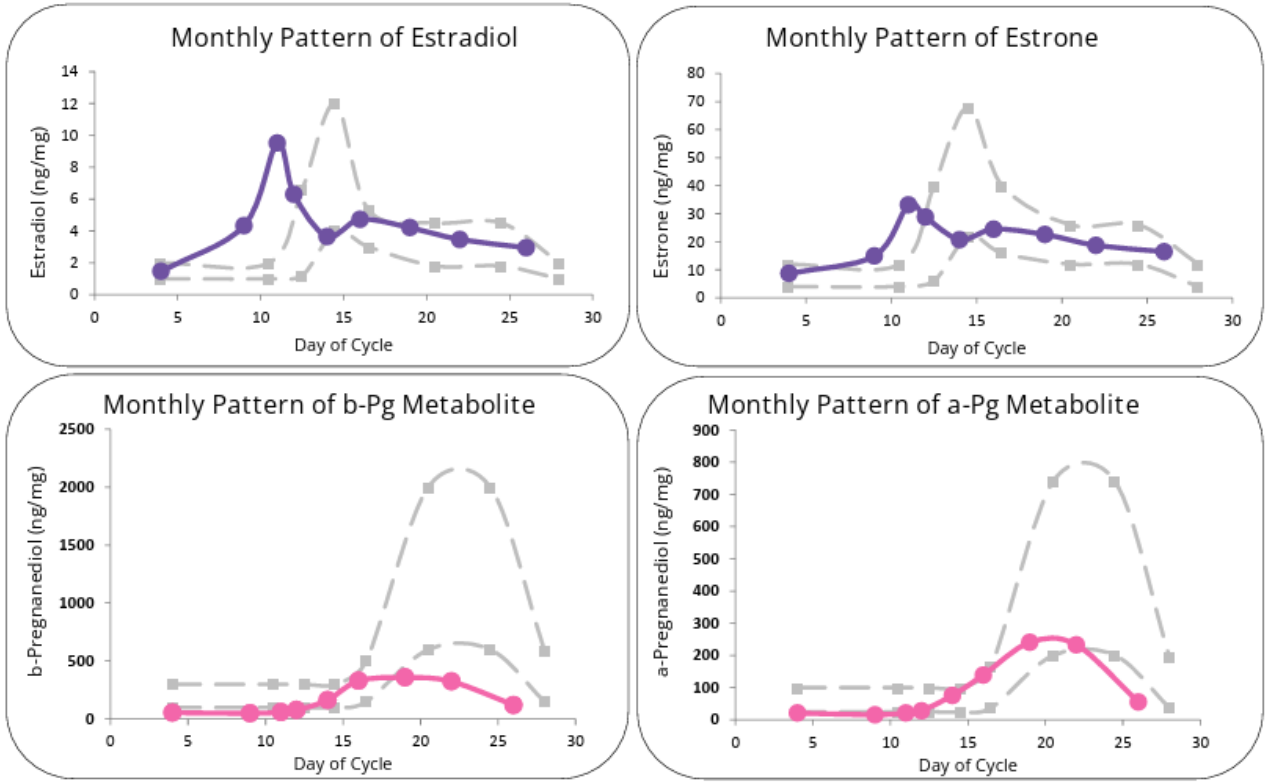
Estradiol (E2)	0.45	2.72	4.27	6.24	7.19	3.11	3.27	3.65	1.19
Estrone (E1)	3.1	8.2	12.2	18.7	20.7	14.5	11.8	14.3	5.8
a-Pregnanediol	54	43	45	51	71	142	664	703	254
b-Pregnanediol	108	113	134	149	120	298	1146	**1501**	600
b-Pregnanediol/E2 Ratio	237	42	31	24	17	96	350	411	504
Creatinine		0.86	0.71	1.24	0.88	0.74	0.53	0.93	0.80

dutch®

Example: 49-yo with Heavy Menses & Sleep Disturbance

- Estrogen levels are normal, however there was a weak progesterone rise that may not be adequate to balance out the estrogen.

- Will this help guide or change treatment plans with this expanded view of the cycle?



All values given in ng/mg creatinine

Measurement	1	2	3	4	5	6	7	8	9
Day(s) of Cycle	4	9	11	12	14	16	19	22	26

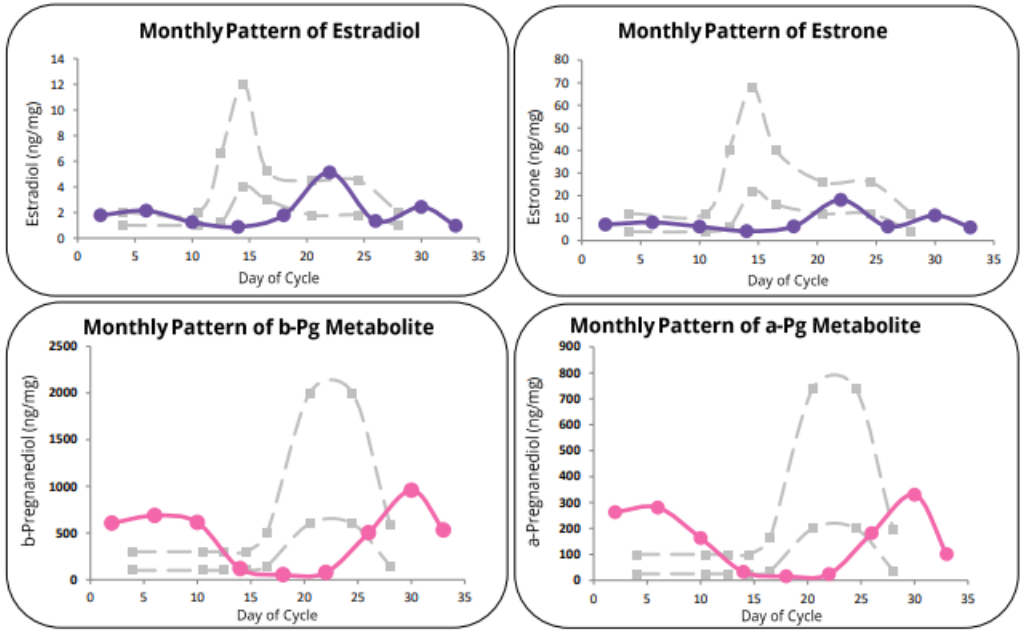
The days listed above were used for measurements. Two samples are used and listed for long cycles or patients without a normal cycle.

Estradiol (E2)	1.47	4.33	9.52	6.29	3.65	4.73	4.21	3.46	2.95
Estrone (E1)	8.7	14.9	33.1	28.8	20.7	24.5	22.6	18.7	16.5
a-Pregnanediol	21	16	21	28	75	139	244	233	55
b-Pregnanediol	55	49	60	78	163	330	398	325	121
b-Pregnanediol/E2 Ratio	38	11	6	12	45	70	95	94	41
Creatinine		1.00	1.45	1.15	1.06	1.18	0.89	0.68	0.85

Example: Uterine ablation

Not getting a menses due to ablation, however ovaries are still intact and cycling

Estrogen (E) patterns can be seen below in purple. Progesterone (Pg) patterns can be seen below in pink. Normal ranges are within the gray dashed lines. See page 2 for more information.



All values given in ng/mg creatinine

Measurement	1	2	3	4	5	6	7	8	9
Day(s) of Cycle	1,3	5,7	9,11	13,15	17,19	21,23	25,27	29,31	33

The days listed above were used for measurements. Two samples are used and listed for long cycles or patients without a normal cycle.

Estradiol (E2)	1.78	2.14	1.24	0.87	1.79	5.12	1.33	2.44	0.97
Estrone (E1)	7.2	8.1	6.3	4.2	6.3	18.1	6.3	11.2	5.9
a-Pregnanediol	263	280	163	33	16	24	182	330	101
b-Pregnanediol	606	686	613	119	53	77	503	**958**	532
b-Pregnanediol/E2 Ratio	340	321	494	137	30	15	377	393	550
Creatinine	1.17	1.06	1.11	1.87	1.37	0.99	2.03	1.08	

Overview of Hormone Testing Methods

Immunoassay vs. LC-MS/MS

Sensitivity and Specificity

Sensitivity

- The proportion of people **with** the disease who test positive
- High sensitivity → few false negatives → good at **"ruling out"** disease when the test is negative
- SnNOut — **Sen**sitive test, **N**egative result, Rules **Out**

Specificity

- The proportion of people **without** the disease who test negative
- High specificity → few false positives → good at **"ruling in"** disease when the test is positive
- SpPIn — **Sp**ecific test, **P**ositive result, Rules **In**

LC-MS/MS = **Optimal** specificity and sensitivity
Immunoassay = **Limited** specificity and sensitivity

Immunoassay vs. LC-MS/MS

- Immunoassay
  - Limited specificity and sensitivity
  - Can have **cross-reactions** with off-target substances to produce false-positive or false-negative results
  - More **prone to interference**
    - e.g., biotin supplementation must be stopped 72 hours to a week prior
  - Reference ranges can **overlap**
  - Cost effective
- LC-MS/MS (liquid chromatography with tandem mass spectrometry)
  - DUTCH uses LC-MS/MS testing
  - Optimal specificity and sensitivity
  - Lower detection limits (important when **measuring low levels** of hormones)
  - Less prone to false-positive or false-negative results
  - May be cost prohibitive

Serum vs. Saliva – the analytical method matters

- **Compared to serum** tests, **salivary** hormone assays **were less reliable** for measuring estradiol and progesterone levels across the menstrual cycle
- Salivary estradiol immunoassays frequently overestimated estradiol concentrations and showed poor agreement with expected menstrual cycle patterns
- Performance of salivary progesterone had mixed results – depended on the method:
  - Enzyme-linked assays (ELISA) showed relatively poor performance
  - Tandem mass spectrometry (LC-MS/MS) performed better

Research article Get rights and content ↗

Not within spitting distance: Salivary immunoassays of estradiol have subpar validity for predicting cycle phase ☆, ☆☆

Ruben C. Arslan [a b], Khandis Blake [c], Laura J. Botzet [d], Paul-Christian Bürkner [e], Lisa DeBruine [f], Tom Fiers [g], Nicholas Grebe [h], Amanda Hahn [i], Ben C. Jones [j], Urszula M. Marcinkowska [k], Sunni L. Mumford [l], Lars Penke [d], James R. Roney [m], Enrique F. Schisterman [l], Julia Stern [n] Show more

Cite Add to Mendeley Share 10.1016/j.psyneuen.2022.105994 ↗

Access through your organization Purchase PDF Patient Access

Article preview Substances Recommended articles Cited by (55) Metrics

Highlights

- Research on puberty, the menstrual cycle, and menopause often measures steroids.
- Estradiol and progesterone are often measured with salivary steroid immunoassays.
- We found salivary assays have weaker-than-expected relationships with cycle phase.
- They seem to be outperformed by estimates based only on the day in cycle.
- Consequently, swaths of the literature need to be revisited.

Abstract

Salivary steroid immunoassays are widely used in psychoneuroendocrinological studies of menstrual cycle phase, puberty, and menopause. Though manufacturers advertise their assays as suitable, they have not been rigorously validated for these purposes. We collated

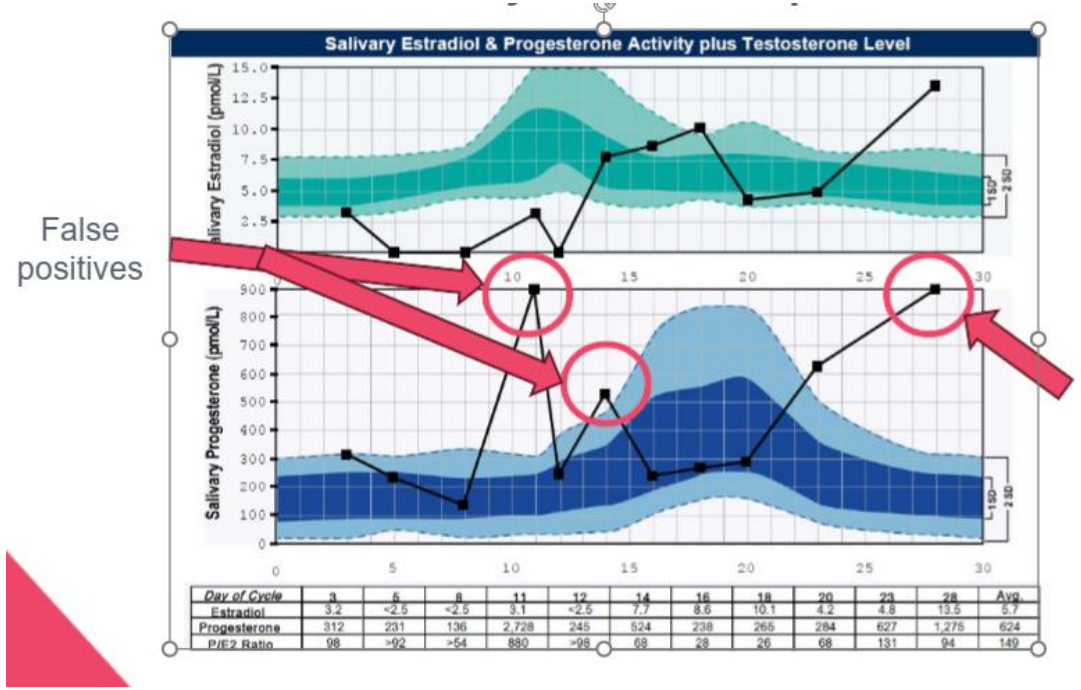
Article preview

Abstract
Introduction
Section snippets
References (61)

What does this mean clinically?

- If saliva estradiol is reporting higher than what is going on in circulation – we risk diagnosing someone with estrogen dominance or thinking their levels are normal
- Or if salivary progesterone levels are showing inconsistent levels throughout the month – how will we know if her progesterone levels are adequate for the luteal phase?

False positives



Immunoassay vs. LC-MS/MS

LC-MS/MS HAS LOWER DETECTION LIMITS

- Salivary immunoassays are less accurate at **measuring lower concentrations** of testosterone as compared to serum LC-MS/MS.
- In females, **salivary immunoassays overestimated** testosterone concentrations compared to LC-MS/MS

All EIAs in the current study tended to produce more inflated testosterone concentrations in samples assessed as having very low testosterone by LC–MS/MS (approximately <10 pg/mL). This was observed exclusively within female samples, who have lower concentrations of testosterone compared to men. This tendency to inflate very low concentrations of testosterone creates a substantial impediment to accurately assessing women's testosterone with EIAs. Researchers who are interested in studying testosterone in women may do so without this bias by using LC–MS/MS instead of EIAs. This systematic error in EIAs for assessing women's testos-

Welker, et al. Psychoneruoendocrinology. 2016. 71:180-188.

What does this mean clinically?

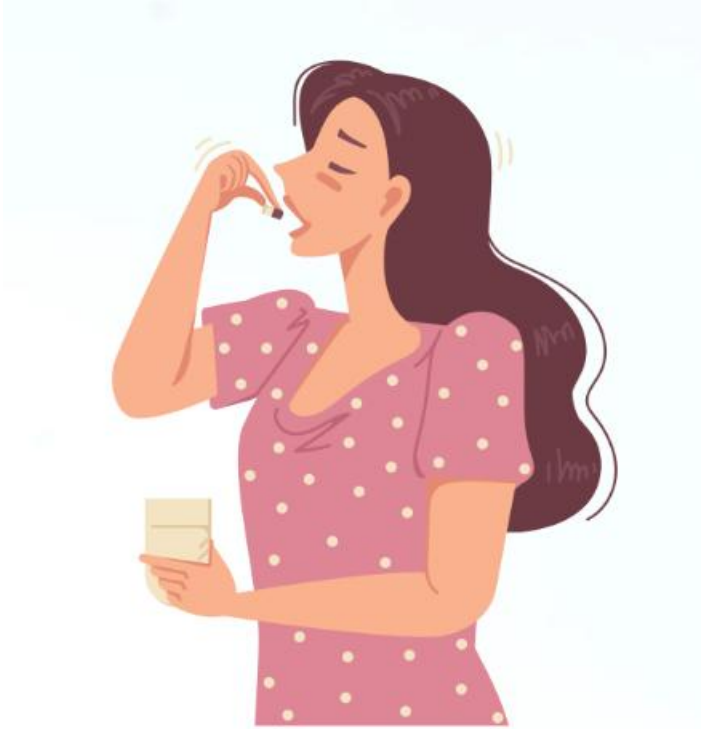
- This discrepancy poses a potential challenge for accurately measuring testosterone using salivary immunoassay, especially among women and children, who typically have lower testosterone levels

Immunoassay vs. LC-MS/MS

Cross-Reactivity: Oral Progesterone

After swallowing oral progesterone:

- Most of it gets metabolized right away in the gut and liver during first pass metabolism
- The result is a flood of progesterone metabolites in the body
- If a **serum immunoassay** is used to measure progesterone, results will be falsely elevated due to cross-reactivity with these *progesterone metabolites*



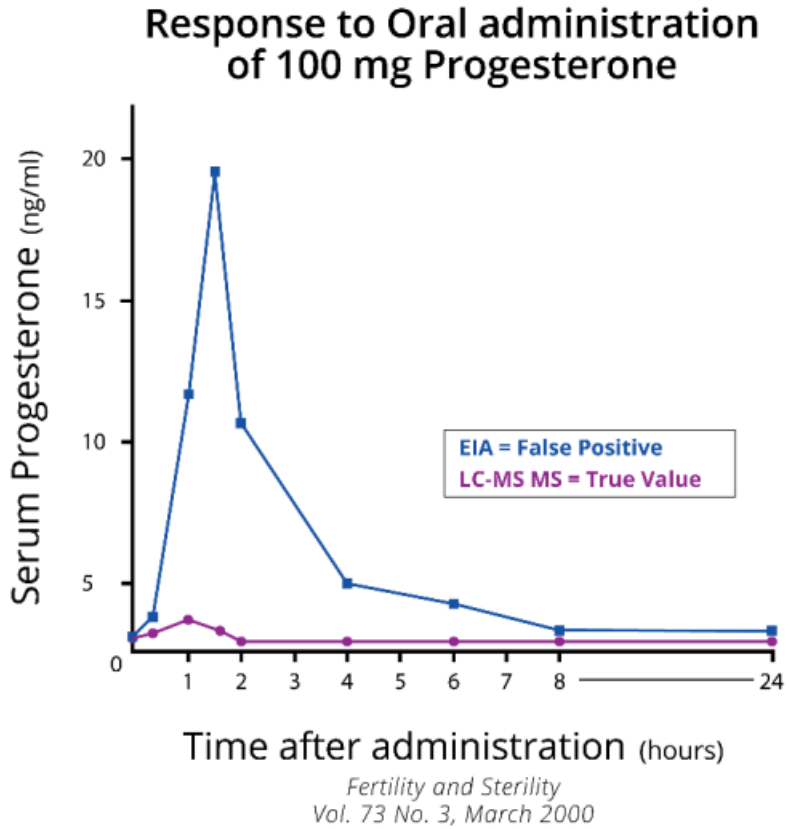
Levine H, Watson N. Fertil Steril. 2000 Mar;73(3):516-21.

Immunoassay vs. LC-MS/MS

Cross-Reactivity

After taking 100mg oral progesterone (OMP):

- With typical **immunoassay** testing (RIA) progesterone is **artificially increased due to cross-reactivity** with *progesterone metabolites* made in the gut.
- Results in limited specificity!
- However, **LC-MS/MS** testing tells us that peak serum progesterone levels are not that big, and levels return to baseline quickly.



Levine H, Watson N. Fertil Steril. 2000 Mar;73(3):516-21.

Immunoassay vs. LC-MS/MS

Immunoassay Reference Range Overlap

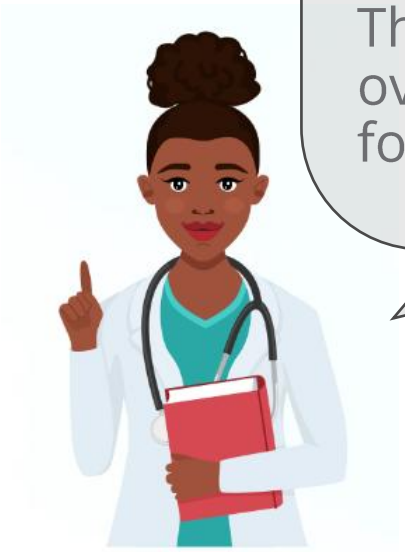
- **Serum** E2 using Immunoassay (ECLIA) [1]
  - Follicular: 12.5-166.0 pg/mL
  - Ovulation: 85.8-498.0 pg/mL
  - Luteal: 43.8-211.0 pg/mL
  - Postmenopausal: <6.0-54.7 pg/mL
- **Saliva** E2 using Immunoassay (EIA and LIA) [2]
  - Follicular: 2.8-8.8 pmol/L
  - Peak (days 11 and 12): 4.5-19.1 pmol/L
  - Luteal: 2.8-8.2 pmol/L
  - Menopausal: 3.7-9.4 pmol/L

1. Based off serum ranges for estradiol using ECLIA. Ranges are lab and method dependent.
2. Based off saliva ranges for estradiol using EIA and LIA. Ranges are lab and method dependent.

Immunoassay vs. LC-MS/MS

Immunoassay Reference Range Overlap

- **Serum** E2 using Immunoassay (ECLIA) [1]
  - Follicular: 12.5-166.0 pg/mL
  - Ovulation: 85.8-498.0 pg/mL
  - Luteal: 43.8-211.0 pg/mL
  - Postmenopausal: <6.0-54.7 pg/mL



My 50-year-old patient's **immunoassay serum** E2 is **44 pg/mL**.

Is she in the postmenopausal range or the follicular range? Are her ovaries still producing E2?

The postmenopausal range overlaps with both the follicular and luteal ranges!

1. Based off serum ranges for estradiol using ECLIA. Ranges are lab and method dependent.

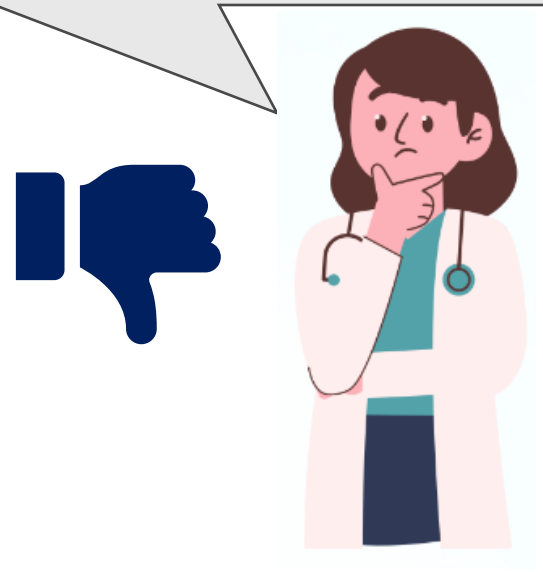
Immunoassay vs. LC-MS/MS

Immunoassay Reference Range Overlap

- **Saliva** E2 using Immunoassay (EIA and LIA) [2]
  - Follicular: 2.8-8.8 pmol/L
  - Peak (days 11 and 12): 4.5-19.1 pmol/L
  - Luteal: 2.8-8.2 pmol/L
  - Menopausal: 3.7-9.4 pmol/L

My 25-year-old patient collected her saliva on cycle day 20. Her **immunoassay saliva** E2 is **4.0 pmol/L**.

Is this normal? Or low? The follicular range almost completely overlaps with the luteal range!



2. Based off saliva ranges for estradiol using EIA and LIA. Ranges are lab and method dependent.

Immunoassay vs. LC-MS/MS

LC-MS/MS Reference Ranges with MINIMAL Overlap

- **Serum E2** using LC-MS/MS[1]
  - Follicular: 30.0-100.0 pg/mL
  - Luteal: 70.0-300.0 pg/mL
  - Postmenopausal: <15.0 pg/mL

- **Urine (4-Spot) E2** using LC-MS/MS [2]
  - Follicular: 1.0-2.0 ng/mg
  - Luteal: 1.8-4.5 ng/mg
  - Postmenopausal: 0.2-0.7 ng/mg

1. Based off serum ranges for estradiol using LC-MS/MS. Ranges are lab and method dependent.
2. Based off 4-spot dried urine ranges for estradiol using LC-MS/MS. Ranges are lab and method dependent.

Immunoassay vs. LC-MS/MS

LC-MS/MS Reference Ranges

- **Serum** E2 using LC-MS/MS[1]
  - Follicular: 30.0-100.0 pg/mL
  - Luteal: 70.0-300.0 pg/mL
  - Postmenopausal: <15.0 pg/mL

My 50-year-old patient's **LC-MS/MS serum** E2 is **12 pg/mL**.

She's in the postmenopausal range! Her ovaries are likely not producing E2.

1. Based off serum ranges for estradiol using LC-MS/MS. Ranges are lab and method dependent.

Immunoassay vs. LC-MS/MS

LC-MS/MS Reference Ranges

- **Urine** (4-Spot) E2 using LC-MS/MS [2]
  - Follicular: 1.0-2.0 ng/mg
  - Luteal: 1.8-4.5 ng/mg
  - Postmenopausal: 0.2-0.7 ng/mg

My 25-year-old patient collected her urine on cycle day 20. Her **LC-MS/MS urine** E2 is **3.0 ng/mg**.

I can be assured that this is normal and within the luteal range. It's not in the follicular or postmenopausal ranges!

1. Based off serum ranges for estradiol using LC-MS/MS. Ranges are lab and method dependent.
2. Based off 4-spot dried urine ranges for estradiol using LC-MS/MS. Ranges are lab and method dependent.

Immunoassay vs. LC-MS/MS Summary

- LC-MS/MS is more accurate than immunoassay, especially when **measuring low hormones levels**
- LC-MS/MS is the **preferred** method; however, it may be cost-prohibitive
- Immunoassay may be adequate in certain situations (see next slide)

- **Note:** Comparing immunoassay results with LC-MS/MS results can be misleading because the methods may differ in analytical specificity, calibration, and susceptibility to interference, and therefore **may not produce directly comparable results**.

When should I use LC-MS/MS or when is it okay to use Immunoassay

- Children
  - LC-MS/MS: for all hormones when suspecting premature puberty

- Cycling women
  - LC-MS/MS: luteal E2, Total T, Free T by *Vermeulen Equation
  - Immunoassay may be adequate: luteal Pg, DHEA-S

- Postmenopausal women
  - LC-MS/MS: for all hormones

- Men
  - LC-MS/MS: Total T (hypogonadal men) and E2 (all men)
  - Immunoassay may be adequate: Total T (in men who are not hypogonadal), DHEA-S

*Note: Calculated free testosterone (Vermeulen equation), derived from total testosterone (LC-MS/MS), SHBG, and albumin, is generally preferred over the standard direct free testosterone immunoassays because it provides a more accurate estimate of free testosterone in most patients.

Serum vs. Saliva vs. Dried Urine

Serum vs. Saliva vs. Urine – What Are We Testing?

Serum

- Hormones (**mostly non-bioavailable**) that are in transit TO tissues. Collection represents blood levels at that single point in time.
- Production
  ↓
  Blood Stream

Saliva

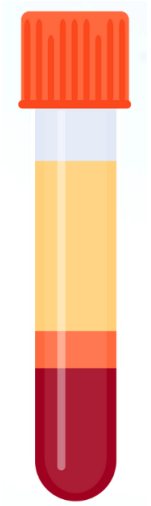
- **Free hormones** that have been taken up by *salivary* tissues. Collection represents salivary levels at that single point in time.
- Blood stream
  ↓
  Salivary gland tissue uptake

Urine (4-Spot)

- **Free & bioavailable hormones** that have been taken up by tissues *throughout the body* AND metabolized. Collection represents average levels over hours of time.
- Blood stream
  ↓
  Whole body tissue uptake
  ↓
  Utilization metabolism
  ↓
  Blood stream → Kidneys

Urine x 4 samples

Serum



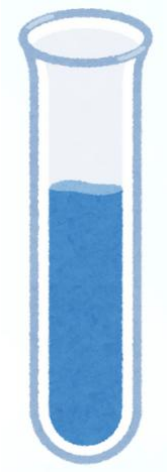
Pros

- Both LC-MS/MS and immunoassay are offered.
- Immunoassays are cost effective.
- Gold standard for measuring hormone levels.
- Reference ranges available for children and teens.

Cons

- Only captures hormone levels at a **moment** in time.
- Patients usually must travel to an office or lab for the blood draw.
- Needle sticks can cause anxiety, worry, and pain.
- Offers limited to no information about hormone metabolism – what happened at the tissues?
- Immunoassays cannot accurately measure low hormones and reference ranges overlap.

Saliva



Pros

- Immunoassays are cost effective.
- Can be collected at home.
- Great for free cortisol and cortisone assessments
- Able to evaluate the Cortisol Awakening Response (CAR)

Cons

- Usually only cost-effective to offer immunoassay.
- Only captures hormone levels at a moment in time.
- Salivary gland uptake does not represent all cellular uptake, and salivary tissue appears to concentrate some hormones (i.e., progesterone) in greater amounts than do other tissues (such as reproductive tissues).
- Collection may be difficult for those who cannot produce much saliva.
- Offers no information about hormone metabolism.
- Immunoassay cannot accurately measure low hormones and reference ranges overlap.
- Common commercially available saliva immunoassays* tend to be less accurate than serum immunoassays.
- Many validation studies utilizing saliva immunoassay testing for hormone therapy monitoring have failed. As results are often not clinically reliable, this is not considered a viable testing option for monitoring of hormone therapy.

*It is possible that salivary LC-MS/MS could provide more accurate results than common commercially available saliva immunoassays.

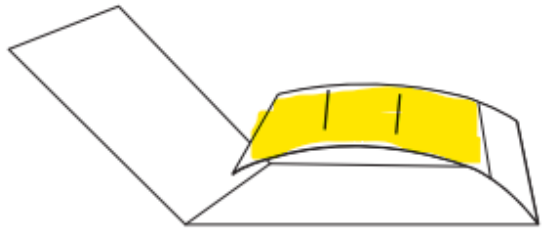
Urine

Pros

- Ultra-sensitive LC-MS/MS
- Can be collected at home
- Captures average hormone levels over **hours** of time (4-spot)
- Provides information about hormone metabolism

Cons

- Not accurate in children <10 years old or people with kidney disease.*
- Patients with UGT deletion will have falsely low T in the urine



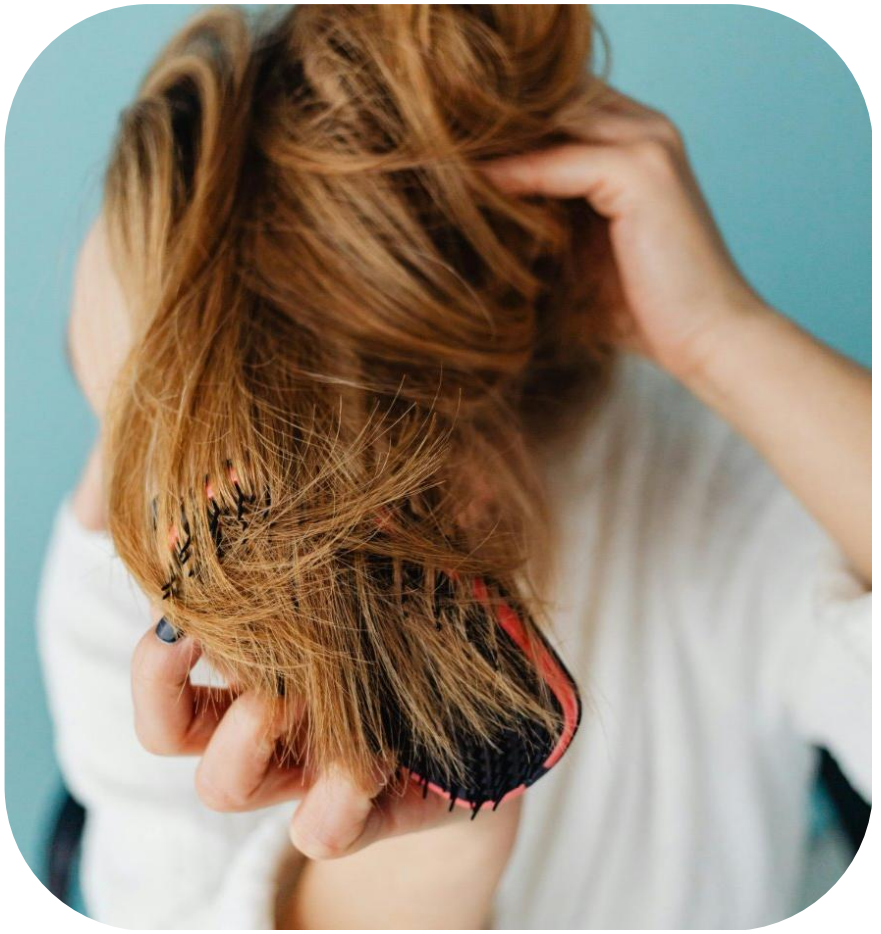
*This is because results are normalized to urine creatinine and creatinine excretion in children and people with kidney disease is different than in healthy adults.

Clinical Case for Baseline Hormone Testing

Hair Loss in a 37-Year-Old Cycling Female

- CC: **Hair loss** steady for the past 4 years
  - Reports weight loss resistance
  - Has always had **regular cycles**

Medications: none
Supplements: vitamin D3 (5,000 IU/day)



Hair Loss in a 37-Year-Old Cycling Female

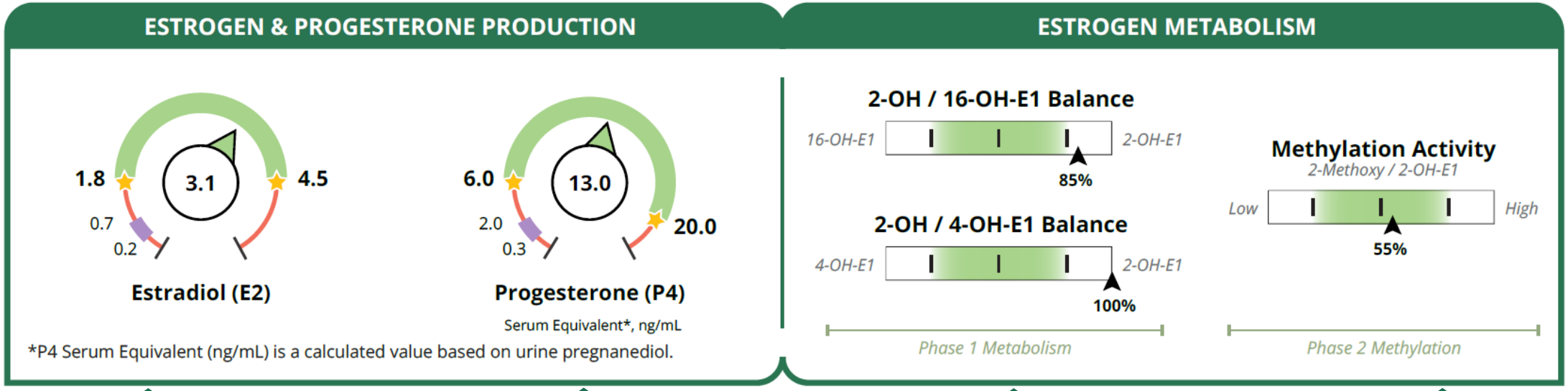
POSITIVE SERUM LAB FINDINGS:

- Elevated A1c 5.7
- Elevated hs-CRP
- Elevated Fasting Glucose

PERTINENT NEGATIVE SERUM LAB FINDINGS:

- CBC
- CMP (except elevated fasting glucose)
- Total T and free T, DHEA-S all within range
- Day 3 FSH and E2 normal/within luteal range
- Day 21 Progesterone normal/within luteal range
- Thyroid labs
- Lipid panel
- Iron panel
- Ferritin
- ANA screen

Hair Loss: Estrogen and Progesterone



Assess estrogen levels given the patient's reproductive status

E2 is within the luteal range.

Assess progesterone levels given the patient's reproductive status

Progesterone is within the luteal range. Ovulation occurred!

Assess 2-OH preference in phase 1 metabolism

2-OH is preferred over 4-OH and 16-OH.

Assess methylation of 2-OH estrogens

Methylation is within range.

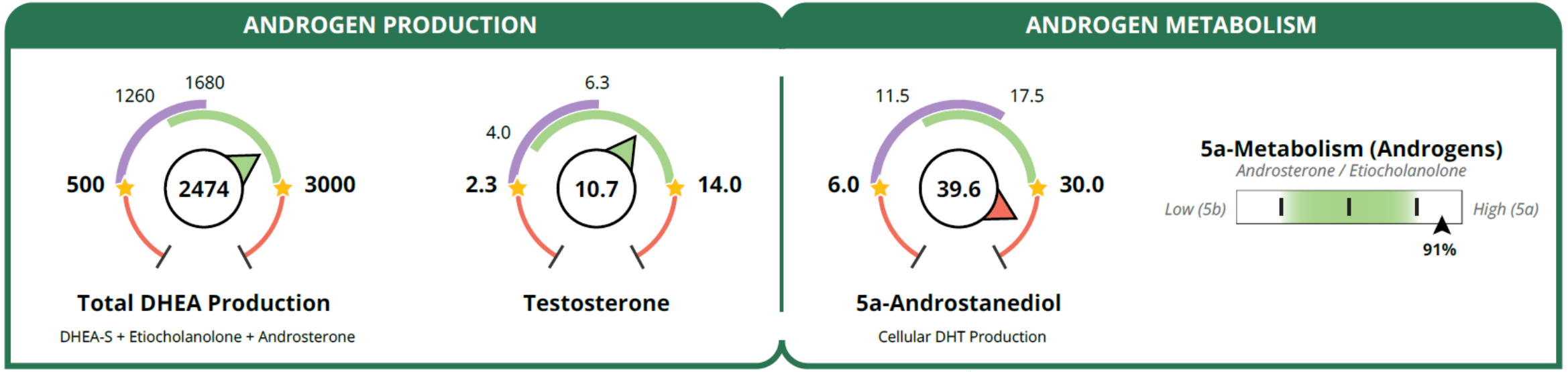
dutch

Hair Loss: DUTCH testing

Estrogen and Progesterone Findings:

- ✓ Normal estrogen levels
- ✓ Normal progesterone Levels
- ✓ Normal estrogen phase 1 metabolism (2-OH preference is the goal!)
- ✓ Normal estrogen phase 2 methylation activity

Hair Loss: Androgens



Assess adrenal androgen levels (Total DHEA)

Assess Testosterone levels

Assess cellular production of 5a-DHT via 5a-Androstanediol

Assess if there is a preference for the more potent alpha metabolism of the androgens

Total DHEA is within range.

Testosterone is within range.

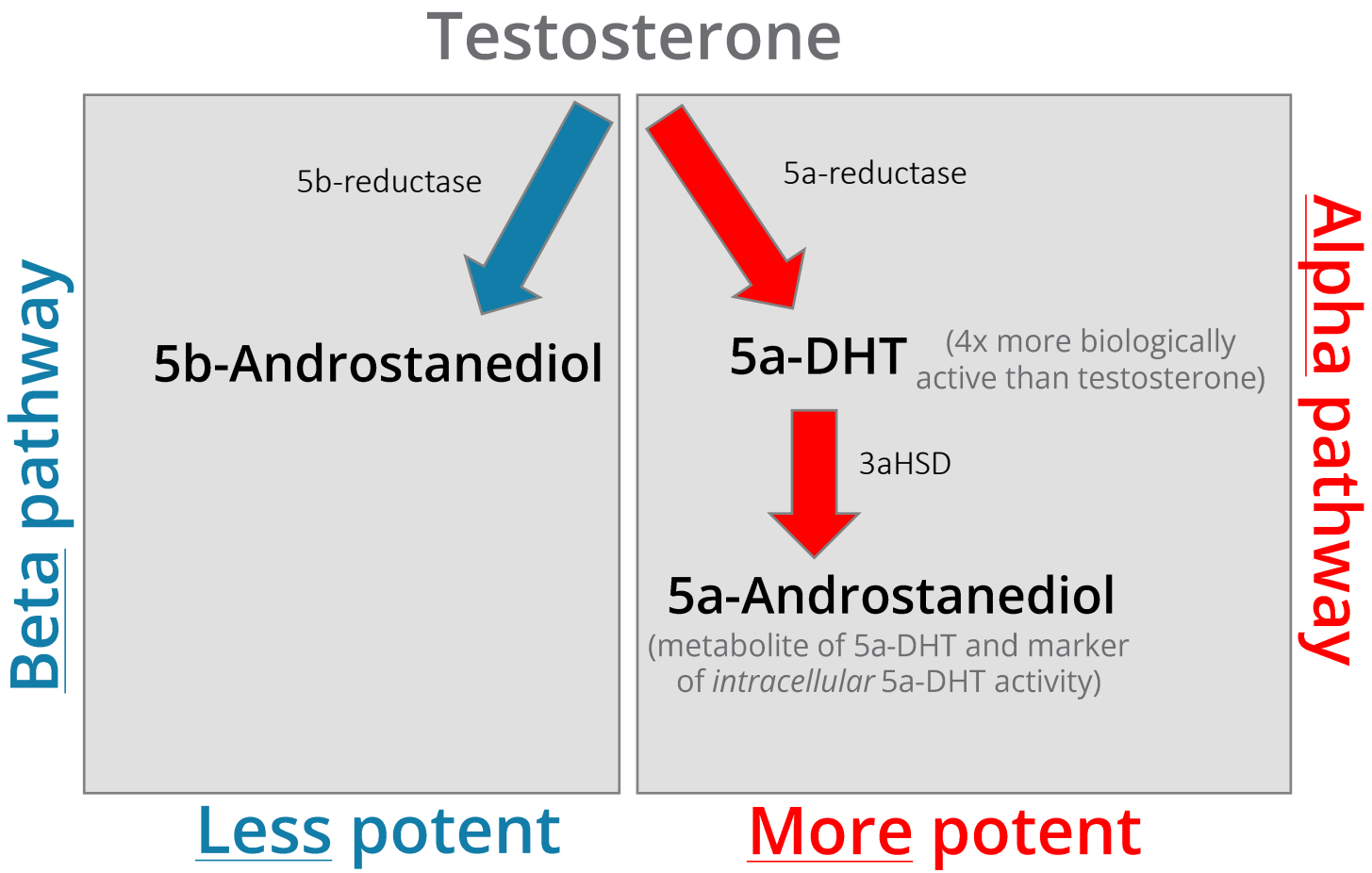
5a-Androstanediol is above range.

High 5a-reductase activity!

dutch

Androgens: DUTCH testing

- ✓ Normal DHEA levels
- ✓ Normal Testosterone Levels
- ↑ 5a-Androstanediol levels
  - ↑ (high intracellular androgen activity)
- ↑ Increased 5a-Reductase activity



What does this mean clinically?

- Serum labs ruled out thyroid, infection, anemia, etc. for hair loss
- The Androgens in serum were WNL
- Serum also showed some blood sugar dysregulation and inflammation (HbA1c and hsCRP)
- However, the pertinent findings in urine showed that there was an issue with hormone metabolism that may be contributing to symptoms
  - High 5a-Androstanediol – a marker that shows high cellular DHT production – leads to high androgen symptoms (acne, excessive facial hair, scalp hair loss, etc.)
  - High 5a-Androgen metabolism of DHEA metabolites
- The issue was in the tissue, not in production

How does this impact treatment?

- Instead of focusing on trying to reduce androgen production (remember the T and DHEA were normal in serum and urine)
- The focus in this case shifts to reasons why 5a-metabolism of androgens is up-regulated
- What can cause an increase in 5a-Reductase?
  - Inflammation
  - High stress
  - Obesity
  - Blood sugar dysregulation
  - PCOS

Did these findings change your treatment plan?

- What treatments may help improve 5a-reductase and improve high androgen symptoms?
  - 5a-Reductase inhibitors can push away from 5a to the 5b (less androgenic) pathway.
  - 5a- Reductase inhibitors can help with symptoms but not fix the root cause
    - Ex: Saw Palmetto, Stinging Nettle, Pygeum, Finasteride, etc.
- However, it does not fix the underlying root problem of why 5a is upregulated in the first place

Goals of Treatment

4-spot Urine Test Goals

- Lower 5a-metabolism preference (androgens)
- Lower 5a-Androstanediol

Blood Lab Goals

- Lower HbA1c
- Lower fasting glucose
- Improve underlying inflammation/elevated CRP

Lifestyle Goals

- Regular aerobic exercise
- Encourage weight loss if appropriate
- Improve Omega 3:Omega 6 ratio with diet choices
- Increase fiber

Sample Treatment Plan

Diet and Lifestyle

- **Consider 4-5 days a week of moderate intensity exercise – incorporating weight resistance training:** to improve weight loss, improve insulin resistance, decrease inflammation
- **Decrease 5a-Reductase activity:** Correct insulin resistance, encourage weight loss, reduce inflammation
- **Decrease Inflammation:** correct insulin resistance, increase exercise, improve diet and veggie intake, improve Omega3:Omega 6 ratio with diet choices, increase fiber intake

Supplements

- **Myoinositol** 2 grams daily (to improve blood sugar)
- **Fish oil** EPA/DHA to decrease inflammation
- **Saw Palmetto/Stinging Nettle x 3 months:** decrease 5a-reductase activity

Key Takeaways – Baseline Testing

Baseline Testing: Pros and Cons

	Lab Methods Commonly Available	Amount of Time Represented	Collection	Metabolism Patterns	Other
Serum	Immunoassay and ultra sensitive LC/MS-MS	Only captures a moment in time	Usually performed at an office or lab with a needle stick	Offers limited information about hormone metabolites (e.g., 5a-DHT, estrogen detox)	Serum is the gold standard for most hormones. Reference ranges (RR) available for children/teens. For immunoassay, cannot accurately measure low hormones and RR overlap.
Dried Urine (4-Spot)	Ultra sensitive LC/MS-MS	Captures average hormone levels over **hours** of time	Easy to collect at home	Able to see hormone metabolism patterns	Not accurate in children <10 or in patients with kidney disease. Patients with UGT deletion will have falsely low testosterone in the urine
Saliva	Usually only cost effective to offer immunoassay LC/MS available with some labs	Only captures a moment in time	Only easy to collect if able to produce sufficient saliva	NOT able to see hormone metabolism patterns	Cannot accurately measure low hormones and RR overlap. Common commercially available salivary immunoassays not as accurate as serum immunoassays. Great for Cortisol Awakening Response (CAR) evaluation.

Baseline Testing Take-Aways – Sex Hormones

Hormone and ROA	Serum	Urine	Saliva
Progesterone (Pg)	✓	✓	
Pg Metabolism	✗	✓	
Estrogen	✓	✓	
Estrogen Metabolism	✗	✓	
Total T	✓	✗	
Free/Bioavailable T	✓ (Vermeulen equation)	✓ (Serum gold standard)*	
T Metabolism	✗ (Limited)	✓	
DHEA	✓ (DHEA-S)	✓ ("Total DHEA")**	
DHEA Metabolism	✗	✓	

Not best option due to lower sensitivity and specificity of common, commercially available *salivary* immunoassay testing.

*Serum is gold standard due to the possibility of falsely low urinary T when UGT deletion is present
**"Total DHEA" = DHEA-S + Etiocholanolone + Androsterone (4-Spot Urine Test)

Baseline Testing Take-Aways - Cortisol

Hormone and ROA	Serum	Urine	Saliva
Total Cortisol	✓	✗	✗
Free Cortisol/Cortisone	✗	✓	✓
Free Cortisol/Cortisone pattern	✗	✓	✓
Cortisol Awakening Response (CAR)	✗	✗	✓
Metabolized Cortisol	✗	✓	✗

Menopause and Monitoring Hormone Therapy

Intro to Menopause & Monitoring Hormone Therapy

- **Menopause** is the cessation of the monthly hormone cycle and fertility in women.
- Average age of menopause is 51 (range 40-58).
- After cycles cease, the patient is **postmenopausal**.
- Due to ovarian activity fluctuation, menopause is not confirmed until cycles have **ceased for 12 full months**.

Casper R. In: Post T, editor. UpToDate. Waltham, MA 2023.
Shifren JL, et al. Menopause. 2014;21(10):1038-1062.

Transdermal (TD) Estradiol (E2)

TD E2 FDA-approved indications*:

- Moderate to severe VMS
- Moderate to severe vulvovaginal symptoms: vaginal atrophy (VVA) and dyspareunia
- Prevention of osteoporosis in postmenopausal women
- Hypoestrogenism from hypogonadism, surgical menopause (bilateral oophorectomy), or POI

Estrogen therapy other benefits:

- Cardiovascular protection
- Sleep
- Dementia (some populations)
- Muscle mass
- Weight management
- Mood disorders
- Skin elasticity
- Joint pain
- Quality of life

*Review specific products and doses for approved indications.

NAMS. Menopause. 2022;29(7):767-794.
de Villiers TJ, et al. Climacteric. 2016;19(4):313-315.
Shifren JL, et al. Menopause. 2014;21(10):1038-1062.

Progesterone

Progesterone is often added on, especially if the patient has a uterus.

Progesterone FDA-approved indications:

- Oral micronized progesterone (OMP) is FDA-approved for the prevention of endometrial hyperplasia in nonhysterectomized postmenopausal women.

Progesterone may also be beneficial for:

- Optimizing bone mineral density (BMD). Estradiol (E2) prevents bone resorption while progesterone makes new bone.
- Mood disorders due to progesterone's alpha metabolite modulation of GABA(A) receptors.
- Vasomotor symptoms (e.g., hot flashes).
- Potential neurobiological (brain protective) effects.

NAMS. Menopause. 2022;29(7):767-794.

Intro to Menopause & Monitoring Hormone Therapy

- **Transdermal estradiol (E2)** is preferred over oral E2 due to its lower risk of VTE, stroke, and gallbladder disease.
- **Micronized progesterone (MP)** preparations are preferred over synthetic progestins due to their lower risk of cardiovascular and thromboembolic events, and breast cancer.
- **Oral micronized progesterone (OMP)** (FDA-approved) and vaginal micronized progesterone (VMP) are the preferred forms for endometrial protection, not transdermal (TD) micronized progesterone.

Abou-Ismail MY, et al. Thromb Res. 2020;192:40-51.
Archer DF. Menopause. 2003;10(6):516-521.
Goldštajn MŠ, et al. Arch Gynecol Obstet. 2022;307(6):1727-1745.
NAMS. Menopause. 2022;29(7):767-794.
Renoux C, et al. BMJ. 2010;340(jun03 4):c2519-c2519.
Stevenson JC, et. al. Drugs Context. 2020 Dec 2;9:2020-10-1.

But First – Why Not TD Progesterone?

CAUTION: Endometrial hyperplasia and TD progesterone

- TD progesterone in combination with estrogen therapy **has NOT been shown to adequately protect the endometrium from hyperplasia.**
- Most studies FAIL to show protection with TD progesterone.
- Skin penetration of TD progesterone is variable and fails to ensure adequate circulating levels of this hormone.
- Of note, a single anecdotal study in 2003 suggested that TD progesterone may elicit protective effects on endometrial tissue, however, the authors concluded that due to the short study duration and limited number of participants **they did not recommend TD progesterone as a safe option in HRT,** and additional studies were necessary to confirm the findings (Leonetti HB, 2003).

Leonetti HB, et al. Fertil Steril. 2003; 79 (1): 221-222.
Stute P, et al. Climacteric. 2016; 19: 316-328.
Wren BG, et al. Climacteric. 2000; 3: 155-160.
Vashisht A, et al. Gynecol Endocrinol. 2005; 21: 101-105.

Transdermal E2 FDA approved indications*:

Formulation	DOSE	VMS	PREVENTION OF POSTMENOPAUSAL OSTEOPOROSIS	VVA / GSM	HYPOESTROGENISM DUE TO POI, INDUCED MENOPAUSE
E2 Patch	0.014 - 0.1 mg	✓	✓	✓	✓
E2 Gel	0.25 - 1 mg	✓		✓	
Compounded Creams (Not FDA approved)	0.25-1.5 mg				

Oral Micronized Progesterone (OMP) FDA approved indications*:

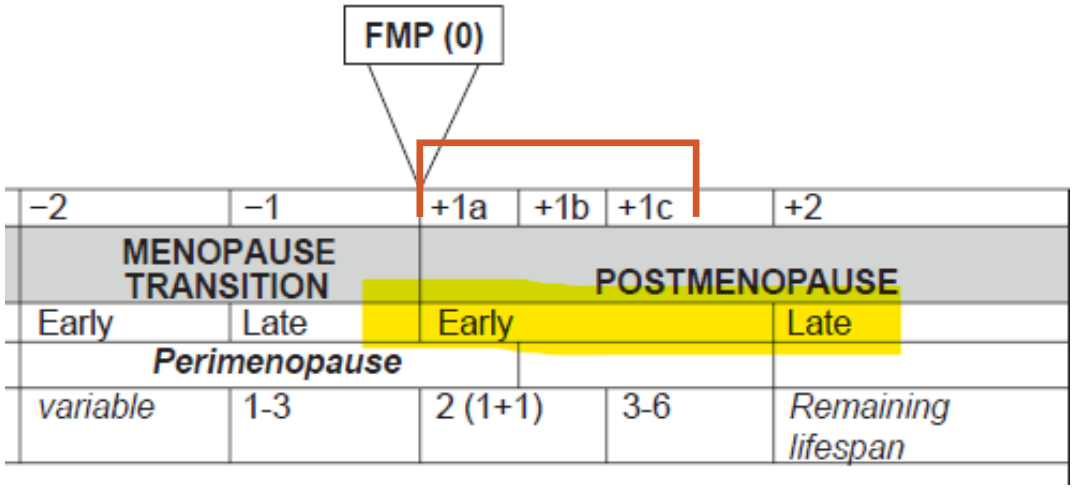
Formulation	DOSE	PREVENTION OF ENDOMETRIAL HYPERPLASIA
Oral capsule: Prometrium (FDA-approved), or compounded OMP	100 mg nightly; 200 mg nightly or 12-14 consecutive nights per month	✓

*Indications may vary by brand and dose. Always refer to package insert for the FDA-approved indications.

When to treat?

- MHRT can be used for hormone imbalances and symptoms at any time during this process.
- Typical use begins after the final menstrual period (FMP).
- Some patients begin MHRT earlier for symptomatic perimenopause.

The Red Brackets show the typical timeframe for starting MHRT:
Early menopause to control bothersome VMS.

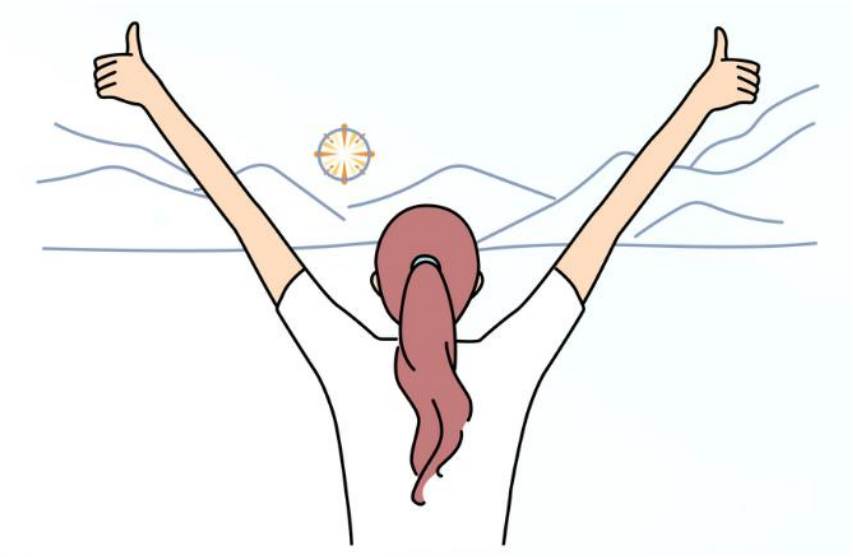


NAMS. Menopause. 2022;29(7):767-794.
Harlow SD, et al. Climacteric. 2012;15(2):105-114.

Why is Monitoring Hormone Therapy important?

But First… Why Monitor Estrogen Therapy?

- To optimize long-term benefits and minimize risks, of course!
- The amount of hormone that is absorbed is different from person to person, from ROA to ROA, and even from brand to brand!
- Because every patient's response to HRT is unique, and because we practice individualized medicine, hormone testing allows us to understand if a person is getting enough (or too much!) hormone.



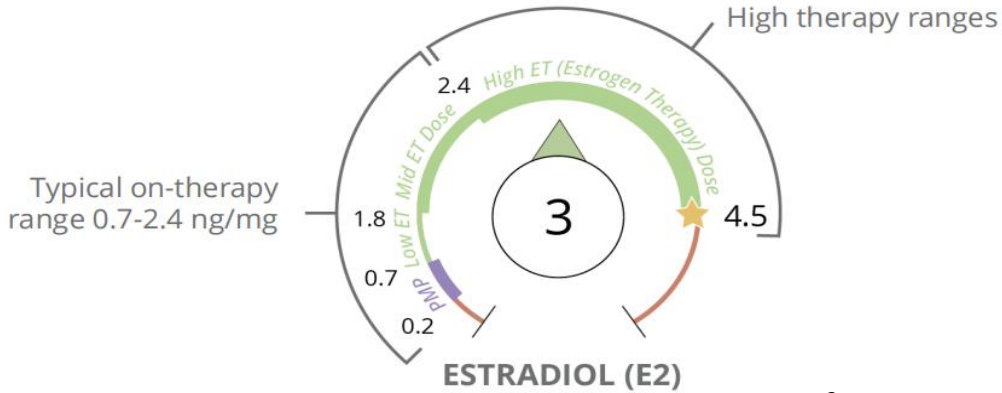
ROA = route of administration

Reference Ranges

- There is evidence that **TD patch doses that raise E2 levels above the postmenopausal range provide clinical benefit** (e.g., prevent or delay bone loss).

- **Postmenopausal E2 ranges**
  - Serum: **<15 pg/mL**[1]
  - Urine (DUTCH dried urine): **0.2-0.7** ng/mg[2]

- **Observed on-therapy E2 ranges**
  - Serum: **20-60** pg/mL (**75-220** pmol/L)
  - Urine (DUTCH dried urine): **0.7-2.4** ng/mg[2]



Urine (DUTCH) E2 Reference Ranges[2]

High dose therapy may be appropriate for some cases but is not considered typical. For example: surgical menopause, POI, etc. Always individualize care.

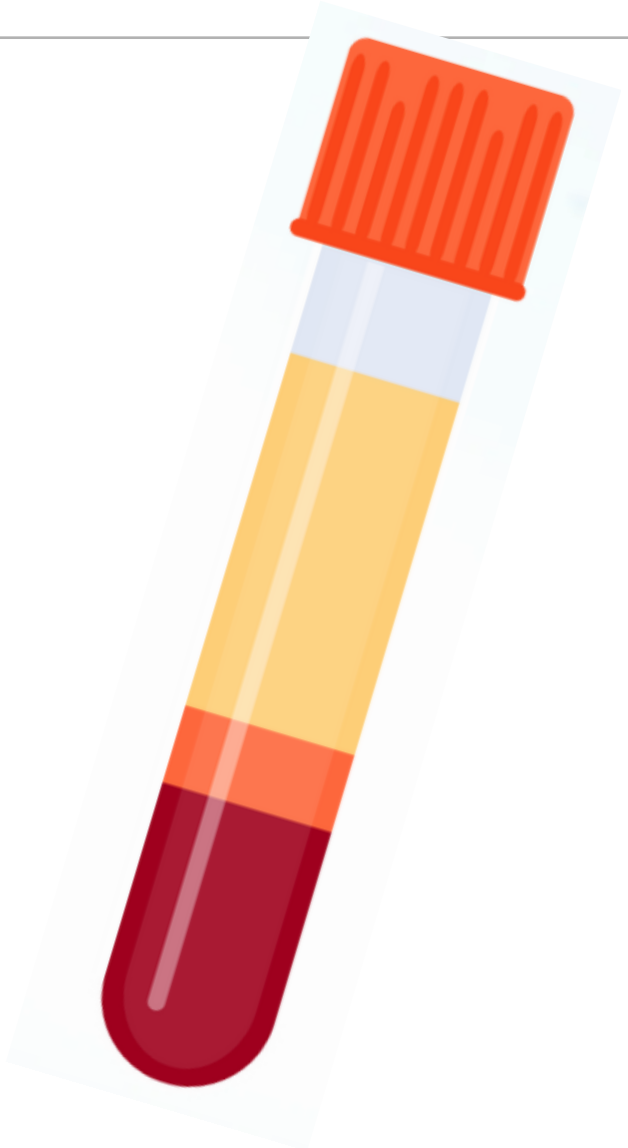
1. Based off serum ranges for estradiol using **LC-MS/MS**. Ranges are lab and method dependent.
2. Based off a DUTCH urine lab's range for estradiol using **LC/MS-MS**. Ranges are lab and method dependent.

Jarvinen A, et al. Maturitas. 2001;38(2):189-196.

Monitoring TD Estrogen Therapy Using Serum

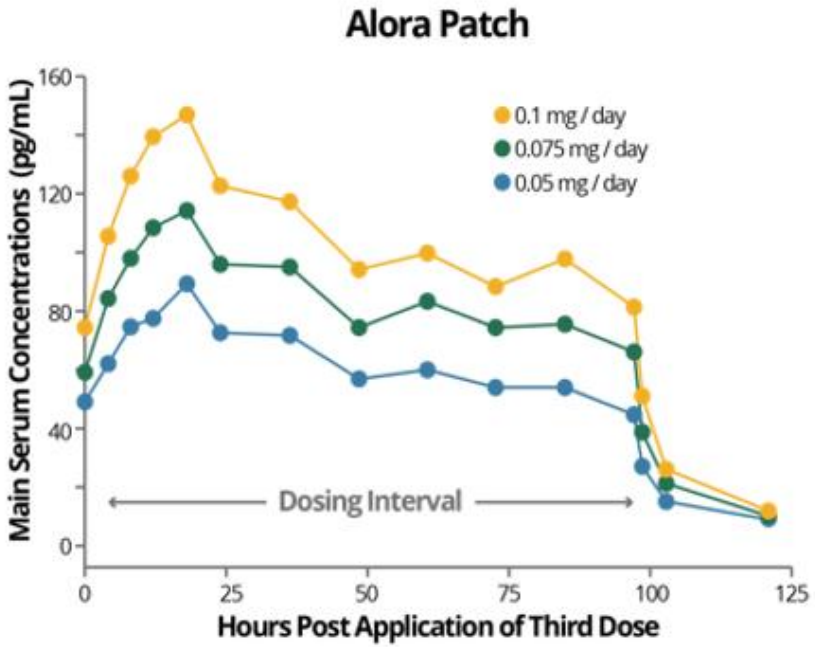
Monitoring Estrogen Therapy: Serum

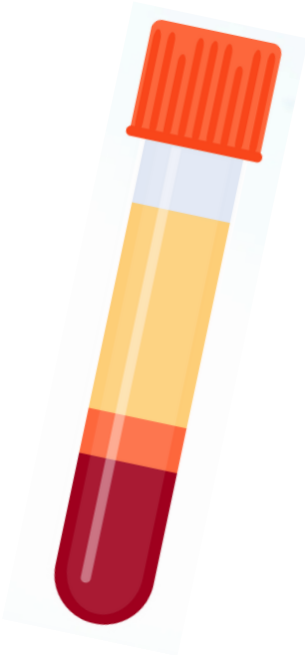
- **Serum** – as opposed to urine or saliva – is the most represented in the research.
- **Reflects a moment in time.**
- When pharmacokinetic patterns are **stable (E2 patches)**, serum can work well.



Monitoring Estrogen Therapy: Serum

- For example, a single serum draw performed **midway** in between E2 patch applications may provide clinically meaningful data.
- However, if the blood is drawn too soon after applying the patch, a provider may think they are overdosing the patient!





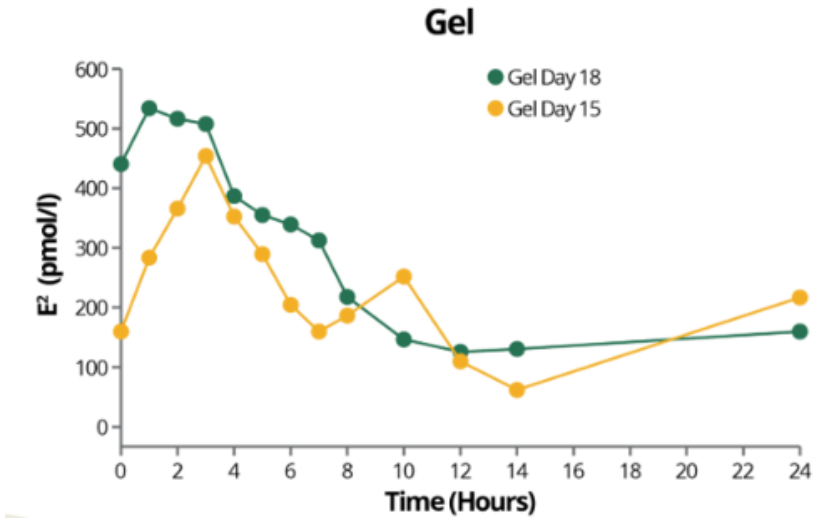
ALORA (estradiol transdermal system)[package insert]. Madison, NJ. Allergan USA Inc., 2020.

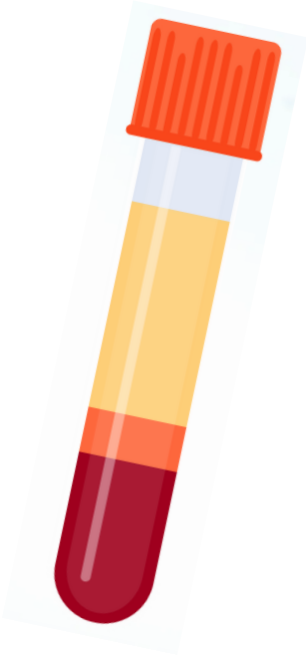
Monitoring Estrogen Therapy: Serum

- **When pharmacokinetic patterns are not stable…**
  - TD E2 creams/gels

…serum does not work as well.

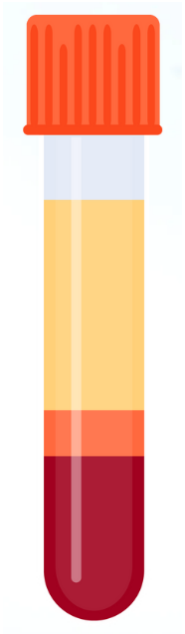
- This is because E2 levels fluctuate **A LOT** after supplementation and serum testing will yield different measurements depending on when the blood is drawn.





Jarvinen A, et al. Maturitas. 2001;38(2):189-196.

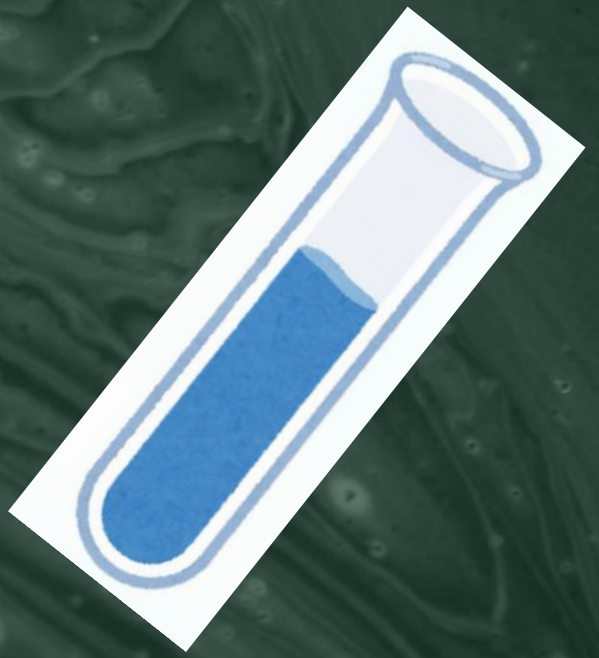
Monitoring Estrogen Therapy: Serum



- Best for monitoring:
  - Transdermal E2 patches *(blood draw should be done midway in between patch applications – for a patch applied twice weekly, this would be about 48 hours after patch application.*
- May not be the best for monitoring:
  - Transdermal E2 creams/gels

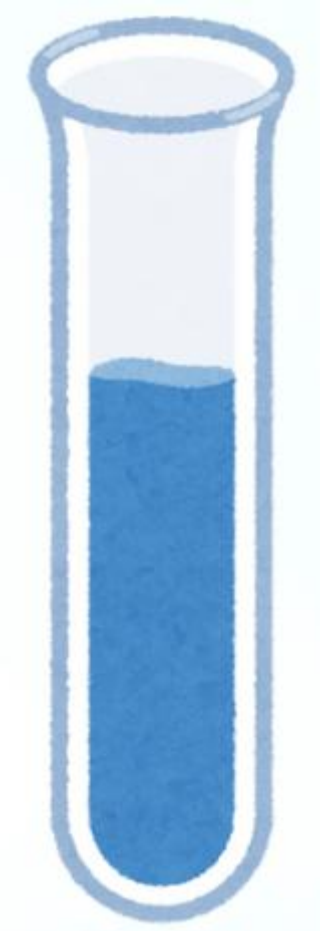
Remember – you must use ultra sensitive **LC-MS/MS** (not immunoassay) for measuring baseline and "on-therapy" estrogen levels in postmenopausal women.

Monitoring TD Estrogen Therapy Using Saliva

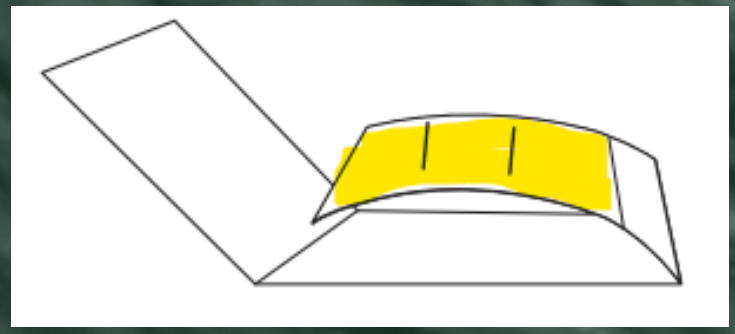


Monitoring Estrogen Therapy: Saliva

- Saliva testing is generally not recommended for monitoring estrogen therapy.
- Saliva data is limited, and **there are no saliva testing outcome studies** and there are no known commercially available E2 saliva assays with successful serum correlation data.
- When TD E2 is used, **salivary results don't correlate with clinical outcomes**, (e.g., bone mineral density, vaginal atrophy, etc.).
- The above statements are also true for salivary LC-MS assays, not just salivary immunoassays.

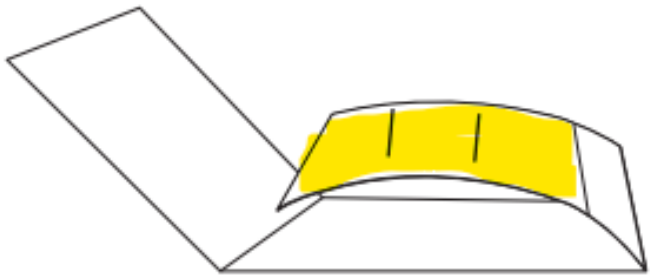


Monitoring Estrogen Therapy Using DUTCH Dried Urine



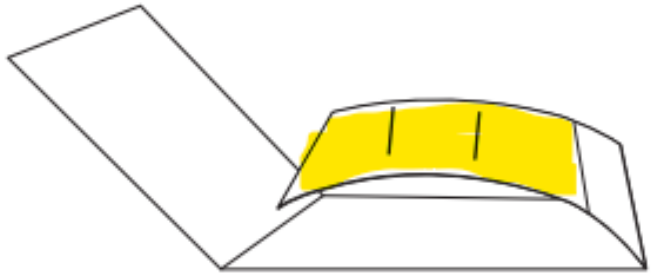
Monitoring Estrogen Therapy: DUTCH Urine

- DUTCH urine may be ideal for monitoring **transdermal (TD) estradiol.**
  - TD E2 = gels, creams, mists, patches applied topically to the skin.
- 4 dried urine samples provide exposure over 12-16 **hours** versus a single time point (like serum and saliva).
- Urine results parallel serum but provide a better estimate of overall estrogen exposure.



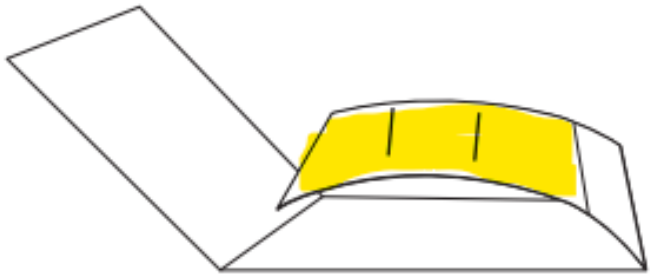
Monitoring Estrogen Therapy: DUTCH Urine

- Best for monitoring:
  - Transdermal E2 creams and gels
  - Transdermal E2 patches



Monitoring Estrogen Therapy: DUTCH Urine

- **Remember:** Urine testing can provide insight into **HOW hormones are being metabolized** and whether they are being directed toward potentially more problematic pathways associated with increased risk or symptoms.
- These findings can help guide treatment decisions or adjustments in therapy based on the individual's results and clinical picture



Monitoring Oral Micronized Progesterone (OMP)

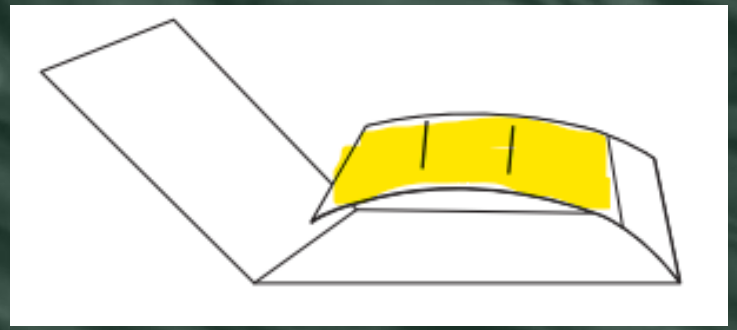
Monitoring Oral Micronized Progesterone (OMP)

Monitoring for prevention of endometrial hyperplasia

- **Remember:** There is currently no serum, urine, or saliva hormone test that can **guarantee endometrial protection** in patients receiving progesterone therapy.
- **Endometrial biopsy can evaluate the endometrium when clinically indicated,** but routine biopsy is not generally required solely to monitor progesterone therapy.
- Therefore, **evidence-based progestogen regimens, dose, duration, and ROAs** are used to provide endometrial protection. For example, evidence supports that **OMP 100-200 mg nightly, or 200 mg 12-14 sequential days per month protects the endometrium.**

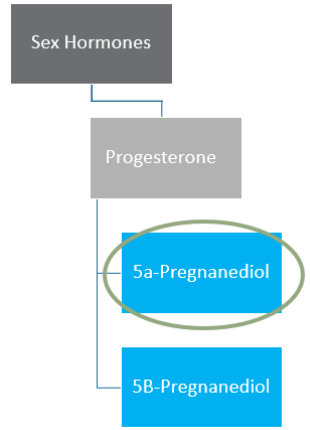
ACOG, ASRM. Obstet Gynecol. 2012 Aug;120(2 Pt 1):411-5.
Hamoda H. Post Reprod Health. 2022 Mar;28(1):40-46.
NAMS. Menopause. 2022;29(7):767-794.

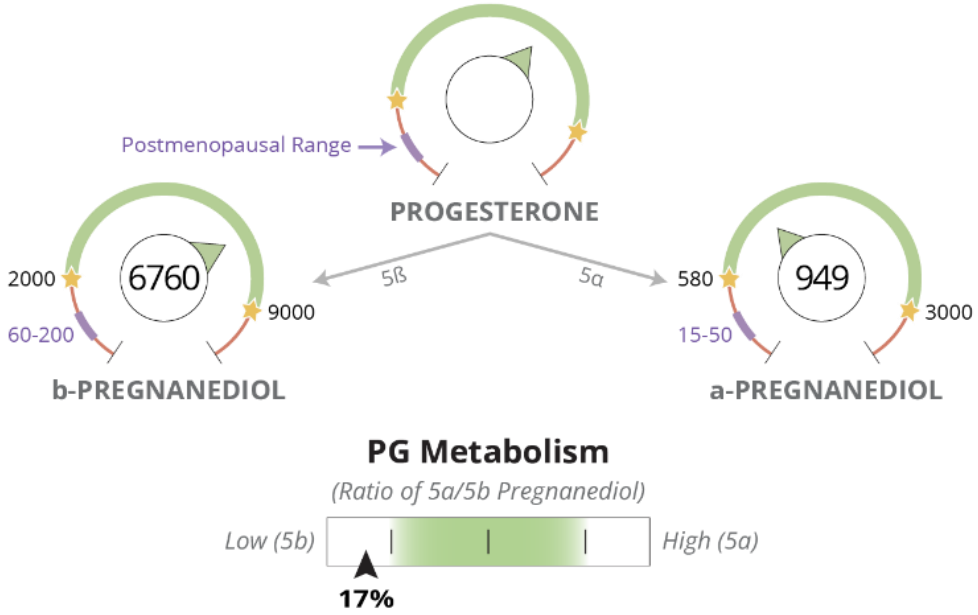
But with urine testing, you can see how the oral progesterone is being metabolized …



Why is hormone metabolism important: DUTCH testing

- Progesterone metabolism patterns may be helpful to assess, as the **alpha progesterone metabolites** modulate GABA(A) receptors and generally support mood and sleep.
- For example, when OMP is supplemented, women favoring the beta pathway (as shown below) ***may*** need a higher dose than women favoring the alpha pathway to see similar improvements in mood and sleep.





What is UGT and its impacts on urinary testosterone levels

What is UGT and its impacts on urinary testosterone levels

- In some people, the body processes and removes testosterone differently because of a genetic variation in an enzyme called **UGT2B17**. This enzyme helps the body prepare testosterone for removal into the urine (makes it water-soluble to be excreted in urine).
- Because of this variation, some people may show **lower testosterone levels on a urine test even when their actual testosterone levels in the body are normal**. This may be especially worth considering when urine testosterone is low, but other markers, such as **epitestosterone, 5α-androstanediol, and Total DHEA Production**, look normal.
- **This is not necessarily a problem or a sign that anything is wrong.** It simply means that, for some people, they excrete their testosterone differently, and it is not detected in the urine, which may not give the complete picture of their testosterone status.
- If testosterone appears very low on the DUTCH Test and this pattern is suspected, confirm the result with a **blood (serum) testosterone test**, including both **total and free testosterone**, before considering testosterone treatment.
- **In short:** a low urine testosterone result doesn't always mean your body has low circulating testosterone. Confirm in serum.

Case Study

53-Year-Old Female on HRT with Persistent Hot Flashes, Anxiety, and Insomnia

Case Study: Persistent Hot Flashes

- 53-year-old female
- Chief complaint of **hot flashes, anxiety, and insomnia** despite starting:

  OMP 100 mg and TD E2 cream 0.3mg 3 months ago
- **Saliva E2 (Immunoassay): 4.5 pmol/L**

- Saliva E2 using Immunoassay (EIA and LIA) [1]
  - Follicular: 2.8-8.8 pmol/L (0.21 – 2.54 pg/mL)
  - Peak (days 11 and 12): 4.5-19.1 pmol/L (1.30-5.51 pg/mL)
  - Luteal: 2.8-8.2 pmol/L (0.21 – 2.37 pg/mL)
  - Menopausal: 3.7-9.4 pmol/L (1.07 – 2.71 pg/mL)

1. Based off commercially available saliva ranges for estradiol using EIA and LIA. Ranges are lab and method dependent.

Case Study: Persistent Hot Flashes

- Saliva E2 (Immunoassay): **4.5 pmol/L**
  - **Is her E2 in the postmenopausal range?** Yes!
  - **Is her E2 in the follicular range?** Yes!
  - **Is her E2 in the luteal range?** Yes!
  - **Is her E2 in the ovulatory range?** Yes!

- Saliva E2 using Immunoassay (EIA and LIA) [1]
  - Follicular: 2.8-8.8 pmol/L (0.21 – 2.54 pg/mL)
  - Peak (days 11 and 12): 4.5-19.1 pmol/L (1.30-5.51 pg/mL)
  - Luteal: 2.8-8.2 pmol/L (0.21 – 2.37 pg/mL)
  - Menopausal: 3.7-9.4 pmol/L (1.07 – 2.71 pg/mL)

1. Based off commercially available saliva ranges for estradiol using EIA and LIA. Ranges are lab and method dependent.

Case Study: Persistent Hot Flashes

- Saliva E2 (Immunoassay): **4.5 p**
  - **Is her E2 in the pe**
  - **Is her E2 i**

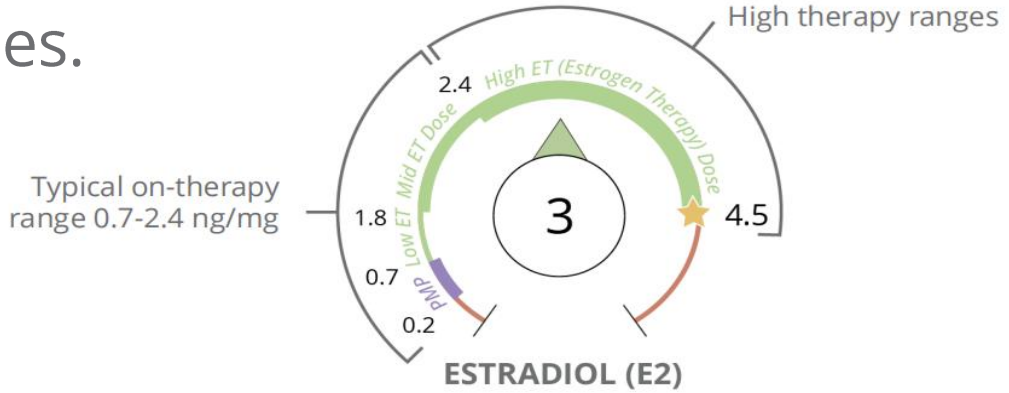
How is this saliva E2 result helpful?

2.71 pg/mL)

1. ...ilable saliva ranges for estradiol using EIA and LIA. Ranges are lab and method dependent.

Case Study: Persistent Hot Flashes

- 4-Spot Urine E2 (LC-MS/MS): **0.4 ng/mg**
- We can easily see that her E2 result falls solely within the postmenopausal range.
- No wonder she is still experiencing hot flashes.



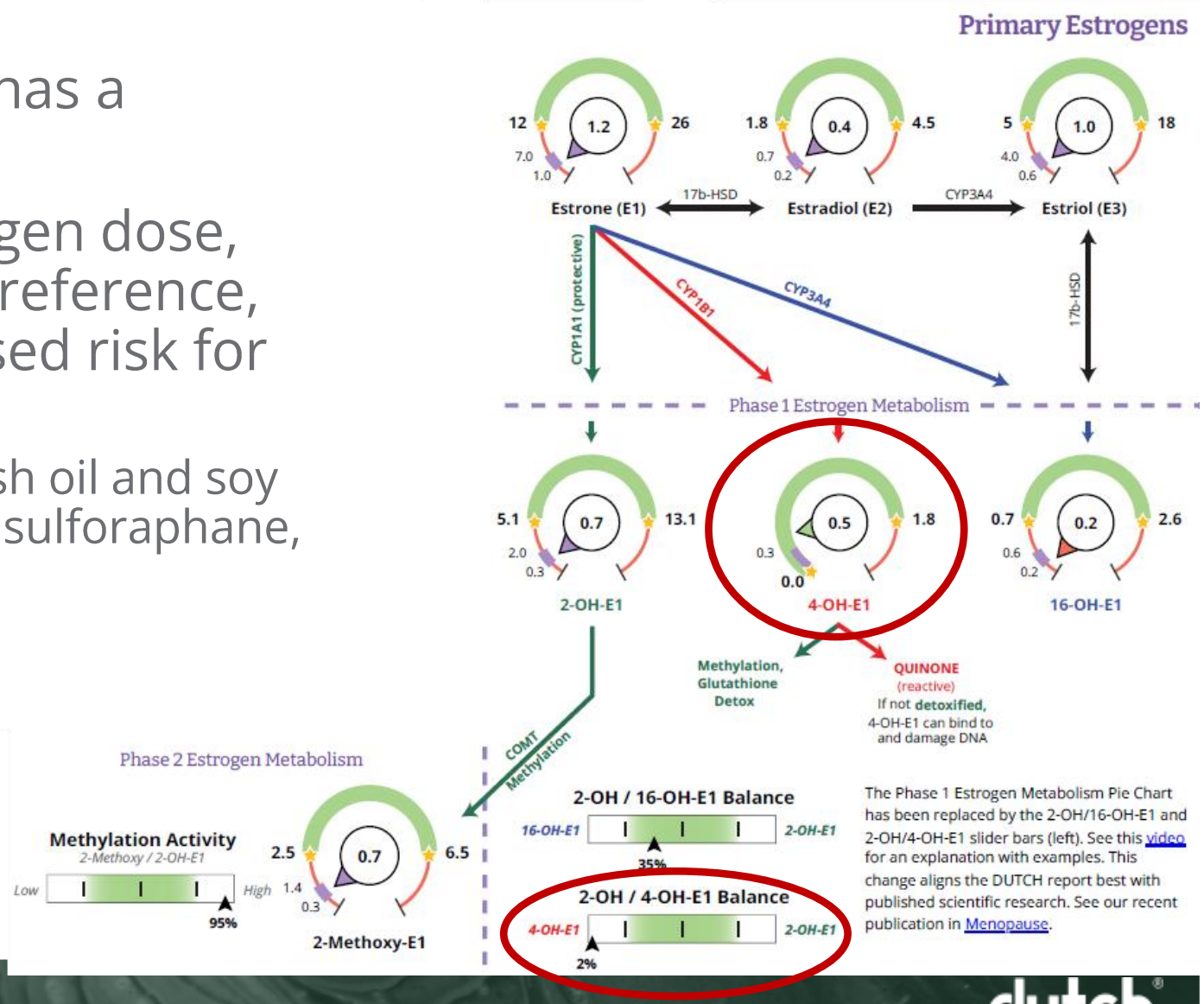
Urine (4-Spot) E2 using LC-MS/MS [1]

Follicular: 1.0-2.0 ng/mg
Ovulatory: 4.0-12.0 ng/mg
Luteal: 1.8-4.5 ng/mg
Postmenopausal: 0.2-0.7 ng/mg

1. Based off Precision Analytical DUTCH urine ranges for estradiol using LC-MS/MS. Ranges are lab and method dependent

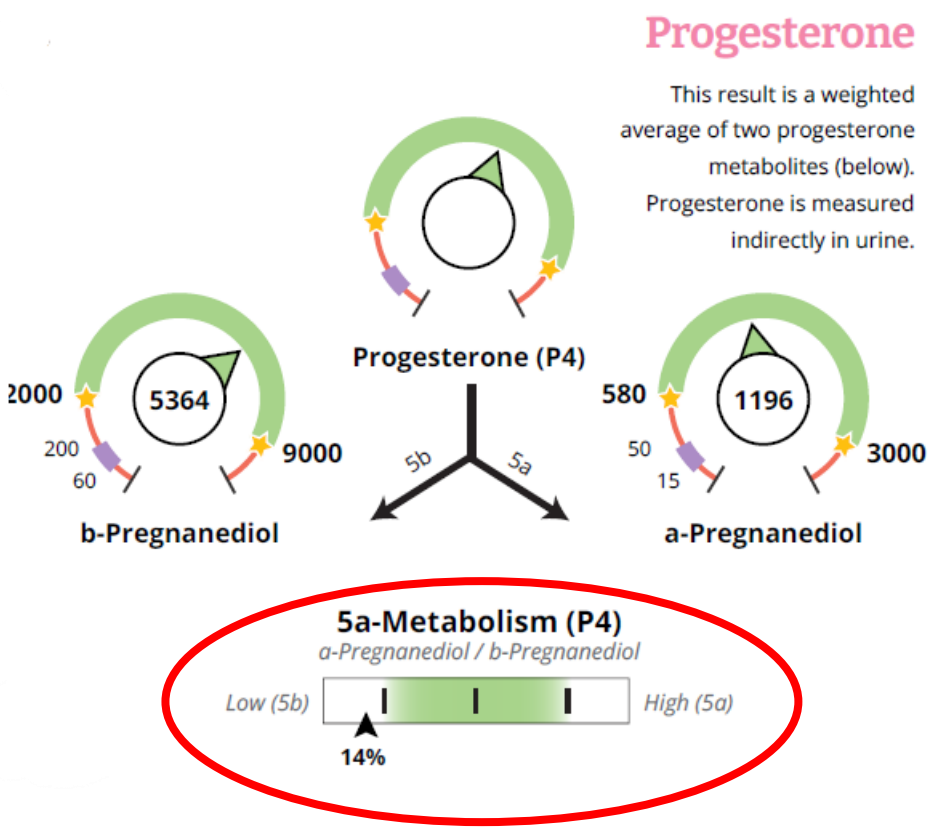
Case Study: Persistent Hot Flashes

- Additionally, we can see that she has a significant 4-OH preference.
- If we decide to increase her estrogen dose, we may want to lower the 4-OH preference, as 4-OH is associated with increased risk for breast cancer.
  - Flaxseeds, cruciferous vegetables, fish oil and soy (from reputable sources), rosemary, sulforaphane, lower alcohol/sugar intake.

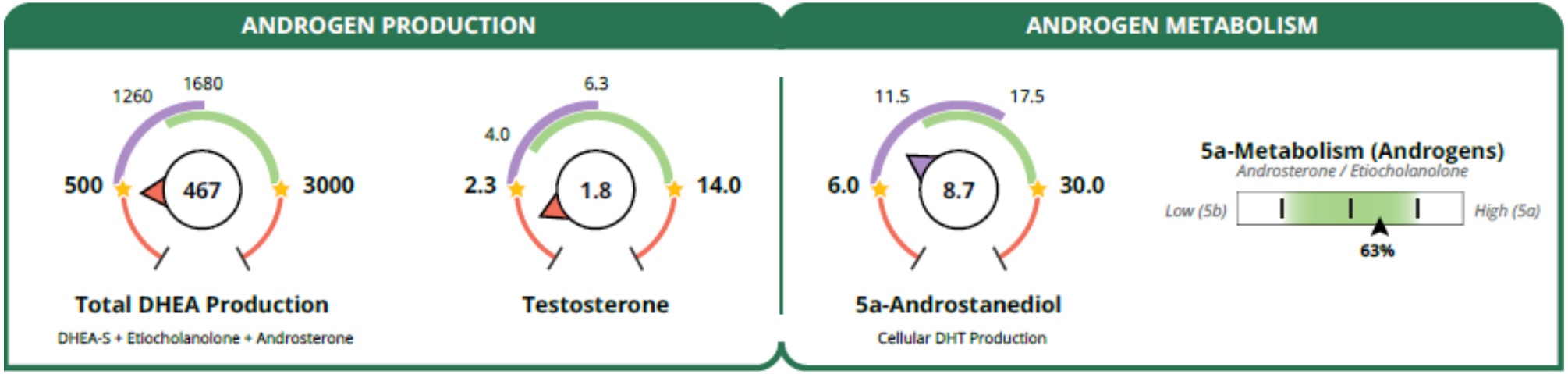


Case Study: Persistent Hot Flashes

- Also, her progesterone metabolism is favoring the beta pathway; therefore, she *may* benefit from a higher dose of OMP to see further improvements in mood and sleep.
- Remember, we don't monitor OMP with urine testing (or serum or saliva) to assess endometrial protection when a standard dose of E2 is used concurrently.



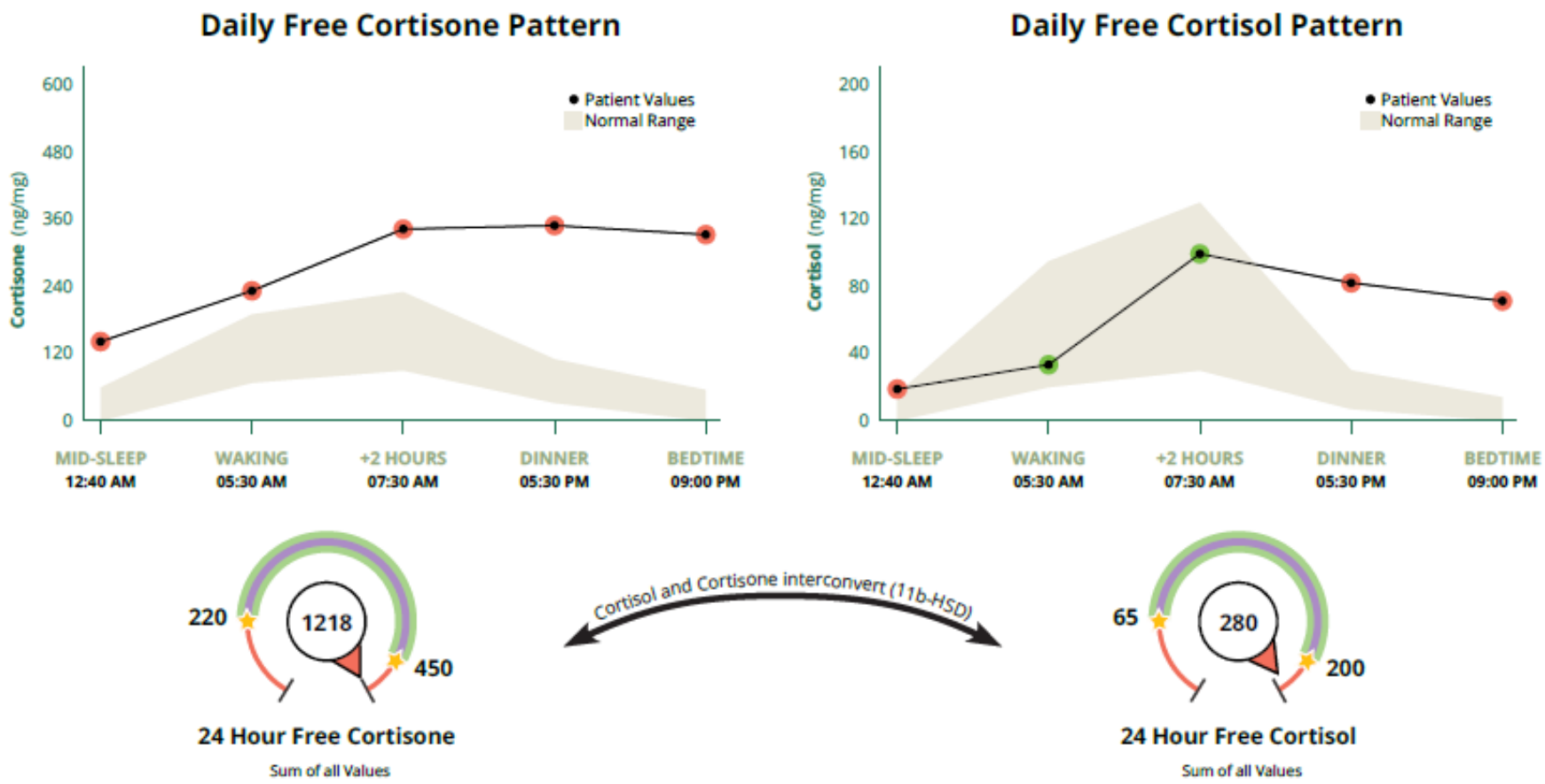
Case Study: Persistent Hot Flashes



- Her androgens are also low for her age.
- After menopause, most estrogen is made in the fat tissue from the aromatization of androgens.
- Thus, improving her androgen levels may also improve her estrogen and hot flashes.

Case Study: Persistent Hot Flashes

- Moreover, her high cortisol found on DUTCH Testing could also be contributing to her hot flashes, anxiety, and insomnia…



Case Study: Persistent Hot Flashes

- Elevated indican can signify that dysbiosis is present.
- And suboptimal gut health can contribute to mood issues!

Organic Acid Tests (OATs)

TEST		RESULT	UNITS	NORMAL RANGE
Nutritional Organic Acids (Urine)				
Vitamin B12 Marker - May be deficient if high				
Methylmalonate (MMA)	Within range	1.3	ug/mg	0 - 2.5
Vitamin B6 Markers - May be deficient if high				
Xanthurenate	Within range	0.68	ug/mg	0.12 - 1.2
Kynurenate	Within range	3.0	ug/mg	0.8 - 4.5
Biotin Marker - May be deficient if high				
b-Hydroxyisovalerate	Within range	8.8	ug/mg	0 - 12.5
Glutathione Marker - May be deficient if low or high				
Pyroglutamate	Within range	38.6	ug/mg	28 - 58
Gut Marker - Potential gut putrefaction or dysbiosis if high				
Indican	Above range	122.9	ug/mg	0 - 100
Neuro-Related Markers (Urine)				
Dopamine Metabolite				
Homovanillate (HVA)	Within range	8.3	ug/mg	3 - 11
Norepinephrine/Epinephrine Metabolite				
Vanilmandelate (VMA)	Within range	4.7	ug/mg	2.2 - 5.5
Neuroinflammation Marker				
Quinolinate	Within range	7.0	ug/mg	0 - 9.6
Additional Markers (Urine)				
Melatonin - Waking				
6-OH-Melatonin-Sulfate	Within range	31.6	ng/mg	10 - 85
Oxidative Stress / DNA Damage				
8-Hydroxy-2-deoxyguanosine (8-OHdG)	Within range	2.5	ng/mg	0 - 5.2

dutch®

Case Study: Persistent Hot Flashes

- Because we ran a 4-spot dried urine test, we were able to get additional information that will help us develop a treatment plan to improve her hot flashes, anxiety, and insomnia:

- E2 still low
- 4-OH preference (breast cancer risk!)
- Beta preference for progesterone metabolism
- Low androgens
- High cortisol
- High indican (dysbiosis!)

Key Takeaways for Monitoring Hormone Therapy

Takeaways – Monitoring Hormone therapy

- Serum can be used to monitor E2 patches but consider the DUTCH Test to evaluate estrogen metabolism patterns.
- Consider DUTCH urine (LC-MS/MS) over serum for monitoring TD E2 (especially TD creams and gels!), as it captures average hormone levels over 12-16 **hours** of time, includes hormone **metabolism patterns**, and can easily be collected at home.
- Additionally, the DUTCH Test may offer other insights into the patient's health status (hormone metabolism, androgen levels, cortisol, organic acids, etc.) that can improve treatment plans.

Takeaways – Monitoring Hormone Therapy

Overall Summary:

LC-MS/MS	Immunoassay	Serum LC-MS/MS	Urine (DUTCH Dried) LC-MS/MS	Saliva
• Estradiol • Testosterone[1]	• Progesterone (baseline) • DHEA-S	• Oral E2[2] • TD E2 patches[3]	• TD E2 gels, creams • TD E2 patches[3] • Vaginal E2 • OMP (to assess metabolism patterns)[4] Can be used to monitor estrogen *metabolism* for **ALL** E2 formulations.	Not best option due to lower specificity of salivary immunoassay testing and reference range overlap seen with saliva testing. Also, saliva results are very high when TD E2 is used, and results do not parallel serum. This may lead to providers underdosing E2 therapy.

1. Serum is gold standard for measuring T. Bioavailable T in the urine may be falsely low in patients with the UGT2B17 deletion. When using serum to measure total T, calculate free T by Vermeulen equation using total T, albumin, and SHBG.
2. Get blood drawn ~6-12 hours after taking oral E2.
3. Test midway between switching out patches. For twice weekly patch, test 48 hours after application.
4. **Currently there is no lab testing that can be used to guarantee endometrial protection for patients on any progestogen therapy.** Consider DUTCH urine (LC-MS/MS) with OMP to assess alpha vs beta progesterone metabolism patterns, as this may be helpful to assess the OMP's effect on mood and sleep.

Thank You!

Lindsey Szczepanski, MSN, APRN, WHNP-BC

August 25, 2026

American College of Apothecaries Group Training

dutch®

References

- Abboud, M. (2022). *Vitamin D supplementation and sleep: A systematic review and meta-analysis of intervention studies*. *Nutrients, 14*(5), Article 1076. https://doi.org/10.3390/nu14051076
- Anderson, T., & Wideman, L. (2023). The association between the cortisol and cortisone awakening responses. *Psychoneuroendocrinology*, *152*, 106075. https://doi.org/10.1016/j.psyneuen.2023.106075
- Arslan, R. C., Blake, K., Botzet, L. J., Bürkner, P. C., DeBruine, L., Fiers, T., Grebe, N., Hahn, A., Jones, B. C., Marcinkowska, U. M., Mumford, S. L., Penke, L., Roney, J. R., Schisterman, E. F., & Stern, J. (2023). Not within spitting distance: Salivary immunoassays of estradiol have subpar validity for predicting cycle phase. *Psychoneuroendocrinology, 149*, 105994. https://doi.org/10.1016/j.psyneuen.2022.105994
- Baudrand, R., Domínguez, J. M., Carvajal, C. A., Riquelme, A., Campino, C., Macchiavello, S., Bozinovic, M., Morales, M., Pizarro, M., Solis, N., Escalona, A., Boza, C., Arrese, M., & Fardella, C. E. (2011). Overexpression of hepatic 5α-reductase and 11β-hydroxysteroid dehydrogenase type 1 in visceral adipose tissue is associated with hyperinsulinemia in morbidly obese patients. *Metabolism: clinical and experimental*, *60*(12), 1775–1780. https://doi.org/10.1016/j.metabol.2011.05.001
- Bulman, A., D'Cunha, N. M., Marx, W., Turner, M., McKune, A., & Naumovski, N. (2025). The effects of L-theanine consumption on sleep outcomes: A systematic review and meta-analysis. *Sleep medicine reviews*, *81*, 102076. https://doi.org/10.1016/j.smrv.2025.102076
- Clavero-Jimeno, A., Martin-Olmedo, J. J., Migueles, J. H., Camacho-Cardenosa, A., Molina-Fernandez, M., Dote-Montero, M., Merino, J., Muñoz-Torres, M., Labayen, I., & Ruiz, J. R. (2026). *Sleep efficiency predicts next-day glycaemia and daytime glycaemia influences sleep in free-living adults at risk of type 2 diabetes*. *Diabetes, Obesity and Metabolism, 28*(6), 4890–4900. https://doi.org/10.1111/dom.70675

References

- Duan, M., Yang, M., Li, C., Wu, X., Yin, X., & Zhu, H. (2026). Effects of inositol in women with polycystic ovary syndrome: an umbrella review of meta-analyses from randomized controlled trials. *Frontiers in endocrinology*, *17*, 1741509. https://doi.org/10.3389/fendo.2026.1741509
- Gao, Q., Kou, T., Zhuang, B., Ren, Y., Dong, X., & Wang, Q. (2018). *The association between vitamin D deficiency and sleep disorders: A systematic review and meta-analysis*. *Nutrients, 10*(10), Article 1395. https://doi.org/10.3390/nu10101395
- Genova Diagnostics. (2025). *EndoSelect sample report* (Report No. 4119). https://www.gdx.net/core/sample-reports/Endoselect-4119-Sample-Report.pdf
- Greff, D., Juhász, A. E., Váncsa, S., Váradi, A., Sipos, Z., Szinte, J., Park, S., Hegyi, P., Nyirády, P., Ács, N., Várbíró, S., & Horváth, E. M. (2023). Inositol is an effective and safe treatment in polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. *Reproductive biology and endocrinology : RB&E*, *21*(1), 10. https://doi.org/10.1186/s12958-023-01055-z
- Hachey, D. L., Dawling, S., Roodi, N., & Parl, F. F. (2003). Sequential action of phase I and II enzymes cytochrome p450 1B1 and glutathione S-transferase P1 in mammary estrogen metabolism. *Cancer research*, *63*(23), 8492–8499.
- Hanna, I. H., Dawling, S., Roodi, N., Guengerich, F. P., & Parl, F. F. (2000). Cytochrome P450 1B1 (CYP1B1) pharmacogenetics: association of polymorphisms with functional differences in estrogen hydroxylation activity. *Cancer research*, *60*(13), 3440–3444.

References

- Hardaker, N. J., Hume, P. A., Sims, S. T., Stewart, T., & King, D. A. (2025). Association between salivary and blood hormone concentrations using an automated electrochemiluminescence immunoassay technique: Challenges and pitfalls. *Experimental Physiology, 110*(12), 1795–1801. https://doi.org/10.1113/EP092542
- Jaferi, A. (2025). *5α-androstanediol: A marker of androgen status in women* (White paper). Precision Analytical Inc. (DUTCH Test). https://dutchtest.com/api/files/file/5a-Androstanediol%20in%20Women.pdf
- Jeon, B., Luyster, F. S., & Chasens, E. R. (2026). *Role of sleep disturbances and diabetes-related distress on glycemic control: A path analysis*. *Research in Nursing & Health, 49*(2), 137–145. https://doi.org/10.1002/nur.70047
- Jewson, M., Purohit, P., & Lumsden, M. A. (2020). Progesterone and abnormal uterine bleeding/menstrual disorders. *Best practice & research. Clinical obstetrics & gynaecology*, *69*, 62–73. https://doi.org/10.1016/j.bpobgyn.2020.05.004
- Kavyani, Z., Musazadeh, V., Fathi, S., Hossein Faghfouri, A., Dehghan, P., & Sarmadi, B. (2022). Efficacy of the omega-3 fatty acids supplementation on inflammatory biomarkers: An umbrella meta-analysis. *International immunopharmacology*, *111*, 109104. https://doi.org/10.1016/j.intimp.2022.109104
- Khabir, Z., Abdelhafez, A., Camponovo, F., Joyce, P., & Garcia-Bennett, A. (2026). Role of the EPA: DHA dosing ratio in omega-3 supplements on blood fatty acid profiles and inflammation: a systematic review and meta-analysis. *Critical reviews in food science and nutrition*, 1–22. Advance online publication. https://doi.org/10.1080/10408398.2026.2615693

References

- Labcorp. (n.d.). *Estradiol (Test No. 004515).* https://www.labcorp.com/tests/004515/estradiol
- Labcorp. (n.d.). *Estradiol, sensitive, LC/MS (Test No. 140244).* https://www.labcorp.com/tests/140244/estradiol-sensitive-lc-ms
- Lentini, G., Querqui, A., Monti, N., & Bizzarri, M. (2025). PCOS and Inositols - Advances and Lessons We are Learning. A Narrative Review. *Drug design, development and therapy*, *19*, 4183–4199. https://doi.org/10.2147/DDDT.S524718
- Levine, H., & Watson, N. (2000). Comparison of the pharmacokinetics of crinone 8% administered vaginally versus Prometrium administered orally in postmenopausal women(3). *Fertility and sterility*, *73*(3), 516–521. https://doi.org/10.1016/s0015-0282(99)00553-1
- Marks, L. S., Hess, D. L., Dorey, F. J., Luz Macairan, M., Cruz Santos, P. B., & Tyler, V. E. (2001). Tissue effects of saw palmetto and finasteride: use of biopsy cores for in situ quantification of prostatic androgens. *Urology*, *57*(5), 999–1005. https://doi.org/10.1016/s0090-4295(00)01052-9
- Memi, E., Pavli, P., Papagianni, M., Vrachnis, N., & Mastorakos, G. (2024). Diagnostic and therapeutic use of oral micronized progesterone in endocrinology. *Reviews in endocrine & metabolic disorders*, *25*(4), 751–772. https://doi.org/10.1007/s11154-024-09882-0

References

- Nahata, A., & Dixit, V. K. (2014). Evaluation of 5α-reductase inhibitory activity of certain herbs useful as antiandrogens. *Andrologia*, *46*(6), 592–601. https://doi.org/10.1111/and.12115
- Newman, M., & Curran, D. A. (2021). Reliability of a dried urine test for comprehensive assessment of urine hormones and metabolites. *BMC Chemistry, 15*(1), 18. https://doi.org/10.1186/s13065-021-00744-3
- Newman, M., Curran, D. A., & Mayfield, B. P. (2020). Dried urine and salivary profiling for complete assessment of cortisol and cortisol metabolites. *Journal of Clinical & Translational Endocrinology, 22*, 100243. https://doi.org/10.1016/j.jcte.2020.100243
- Newman, M. S., Mayfield, B. P., Saltiel, D., & Stanczyk, F. Z. (2023). Assessing estrogen exposure from transdermal estradiol patch therapy using a dried urine collection and a GC-MS/MS assay. *Steroids, 189*, 109149. https://doi.org/10.1016/j.steroids.2022.109149
- Newman, M. S., Saltiel, D., Mayfield, B. P., & Stanczyk, F. Z. (2022). Comparison of cyclic urinary estrogen and progesterone metabolite patterns between women reporting menses and women reporting menses. *Fertility and Sterility.* https://doi.org/10.1016/j.fertnstert.2022.08.600
- Nolan, B. J., Liang, B., & Cheung, A. S. (2021). Efficacy of Micronized Progesterone for Sleep: A Systematic Review and Meta-analysis of Randomized Controlled Trial Data. *The Journal of clinical endocrinology and metabolism*, *106*(4), 942–951. https://doi.org/10.1210/clinem/dgaa873

References

- Pani, A., Gironi, I., Di Vieste, G., Mion, E., Bertuzzi, F., & Pintaudi, B. (2020). *From prediabetes to type 2 diabetes mellitus in women with polycystic ovary syndrome: Lifestyle and pharmacological management*. *International Journal of Endocrinology, 2020*, Article 6276187. https://doi.org/10.1155/2020/6276187
- Pezza, M., Carlomagno, V., Sammarco, E., Trischitta, A., Ceddia, C., Vitiello, A., Baj, G., Citi, V., & Colletti, A. (2025). Association of Myo-Inositol and Microlipodispersed Magnesium in Androgen-Dependent Dermatological Diseases: A Retrospective Study. *Pharmaceuticals (Basel, Switzerland)*, *18*(2), 251. https://doi.org/10.3390/ph18020251
- Practice Committee of the American Society for Reproductive Medicine. (2021). Diagnosis and treatment of luteal phase deficiency: A committee opinion. *Fertility and Sterility, 115*(6), 1416–1423. https://doi.org/10.1016/j.fertnstert.2021.03.013
- Root-Bernstein, R., Podufaly, A., & Dillon, P. F. (2014). Estradiol Binds to Insulin and Insulin Receptor Decreasing Insulin Binding in vitro. *Frontiers in endocrinology*, *5*, 118. https://doi.org/10.3389/fendo.2014.00118
- Rosner, W., Auchus, R. J., Azziz, R., Sluss, P. M., & Raff, H. (2007). Utility, limitations, and pitfalls in measuring testosterone: An Endocrine Society position statement. *Journal of Clinical Endocrinology & Metabolism, 92*(2), 405–413. https://doi.org/10.1210/jc.2006-1864
- Rothman, M. S., Carlson, N. E., Xu, M., Wang, C., Swerdloff, R., Lee, P., Goh, V. H., Ridgway, E. C., & Wierman, M. E. (2011). Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. *Steroids, 76*(1–2), 177–182. https://doi.org/10.1016/j.steroids.2010.10.010

References

- Schofield, R. C., Ramanathan, L. V., Pessin, M. S., & Carlow, D. C. (2018). Data to support a simultaneous testosterone and estradiol assay in serum by LC-MS/MS. *Data in Brief, 20*, 1160–1165. https://doi.org/10.1016/j.dib.2018.08.077
- Schüle, C., Nothdurfter, C., & Rupprecht, R. (2014). The role of allopregnanolone in depression and anxiety. *Progress in neurobiology*, *113*, 79–87. https://doi.org/10.1016/j.pneurobio.2013.09.003
- Sharma, V. K., & Singh, T. G. (2020). Chronic Stress and Diabetes Mellitus: Interwoven Pathologies. *Current diabetes reviews*, *16*(6), 546–556. https://doi.org/10.2174/1573399815666191111152248
- Slettvoll, T., Wahlberg, J., Lood, Y., Josefsson, M., Aardal, E., & Salihovic, S. (2025). Non-invasive assessment of free steroid hormones: Development of a high-throughput LC-MS/MS method for salivary steroid hormone quantification. *Analytical and Bioanalytical Chemistry, 417*(21), 4883–4894. https://doi.org/10.1007/s00216-025-06005-9
- Solnier, J., Du, M., Zhang, Y., Roh, Y. S., Kuo, Y. C., Ibi, A., Wood, S., Hardy, M., Gahler, R. J., & Chang, C. (2026). A Targeted Metabolomic Assessment of Oral Glutathione Bioavailability and Safety in Humans: A Randomized Crossover Clinical Trial. *Antioxidants (Basel, Switzerland)*, *15*(3), 354. https://doi.org/10.3390/antiox15030354
- Stalder, T., Lupien, S. J., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Kirschbaum, C., Miller, R., Wetherell, M. A., Finke, J. B., Klucken, T., & Clow, A. (2022). Evaluation and update of the expert consensus guidelines for the assessment of the cortisol awakening response (CAR). *Psychoneuroendocrinology*, 146, 105946. https://doi.org/10.1016/j.psyneuen.2022.105946

References

- St-Onge, M. P., Grandner, M. A., Brown, D., Conroy, M. B., Jean-Louis, G., Coons, M., Bhatt, D. L., & American Heart Association Obesity, Behavior Change, Diabetes, and Nutrition Committees of the Council on Lifestyle and Cardiometabolic Health; Council on Cardiovascular Disease in the Young; Council on Clinical Cardiology; and Stroke Council (2016). Sleep Duration and Quality: Impact on Lifestyle Behaviors and Cardiometabolic Health: A Scientific Statement From the American Heart Association. *Circulation*, *134*(18), e367–e386. https://doi.org/10.1161/CIR.0000000000000444
- Sunena, & Mishra, D. N. (2022). Stress Etiology of Type 2 Diabetes. *Current diabetes reviews*, *18*(9), e240222201413. https://doi.org/10.2174/1573399818666220224140934
- Talbott, S. M., Talbott, J. A., & Pugh, M. (2013). Effect of Magnolia officinalis and Phellodendron amurense (Relora®) on cortisol and psychological mood state in moderately stressed subjects. *Journal of the International Society of Sports Nutrition*, *10*(1), 37. https://doi.org/10.1186/1550-2783-10-37
- Thibodeau, P. A., Kocsis-Bédard, S., Courteau, J., Niyonsenga, T., & Paquette, B. (2001). Thiols can either enhance or suppress DNA damage induction by catecholestrogens. *Free radical biology & medicine*, *30*(1), 62–73. https://doi.org/10.1016/s0891-5849(00)00446-9
- Tomlinson, J. W., Finney, J., Gay, C., Hughes, B. A., Hughes, S. V., & Stewart, P. M. (2008). Impaired glucose tolerance and insulin resistance are associated with increased adipose 11beta-hydroxysteroid dehydrogenase type 1 expression and elevated hepatic 5alpha-reductase activity. *Diabetes*, *57*(10), 2652–2660. https://doi.org/10.2337/db08-0495
- Walker B. R. (2001). Activation of the hypothalamic-pituitary-adrenal axis in obesity: cause or consequence?. *Growth hormone & IGF research : official journal of the Growth Hormone Research Society and the International IGF Research Society*, *11 Suppl A*, S91–S95. https://doi.org/10.1016/s1096-6374(01)80015-0

References

- Welker, K. M., Lassetter, B., Brandes, C. M., Prasad, S., Koop, D. R., & Mehta, P. H. (2016). A comparison of salivary testosterone measurement using immunoassays and tandem mass spectrometry. *Psychoneuroendocrinology*, *71*, 180–188. https://doi.org/10.1016/j.psyneuen.2016.05.022
- Williams, J. L., Everett, J. M., D'Cunha, N. M., Sergi, D., Georgousopoulou, E. N., Keegan, R. J., McKune, A. J., Mellor, D. D., Anstice, N., & Naumovski, N. (2020). The Effects of Green Tea Amino Acid L-Theanine Consumption on the Ability to Manage Stress and Anxiety Levels: a Systematic Review. *Plant foods for human nutrition (Dordrecht, Netherlands)*, *75*(1), 12–23. https://doi.org/10.1007/s11130-019-00771-5
- Zhang, Y., Gaikwad, N. W., Olson, K., Zahid, M., Cavalieri, E. L., & Rogan, E. G. (2007). Cytochrome P450 isoforms catalyze formation of catechol estrogen quinones that react with DNA. *Metabolism: clinical and experimental*, *56*(7), 887–894. https://doi.org/10.1016/j.metabol.2007.03.001