

# Menopause and HRT: How Hormone Testing Can Help

August 16, 2023 12:00 pm – 1:00 pm

Dr. Debbie Rice ND MPH

Director of Clinical Education

# 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.

# Objectives

---

- 1: Understand** Menopause and Perimenopause and the major hormones involved
- 2: Define** Hormone Replacement Therapy (HRT) and options for replacement
- 3: Familiarize** ourselves with testing options and how testing can enhance evaluation and treatment

# Menopause

Occurs around ages 45-55 yo

Multisystem effect:

Cardiovascular

Neurological

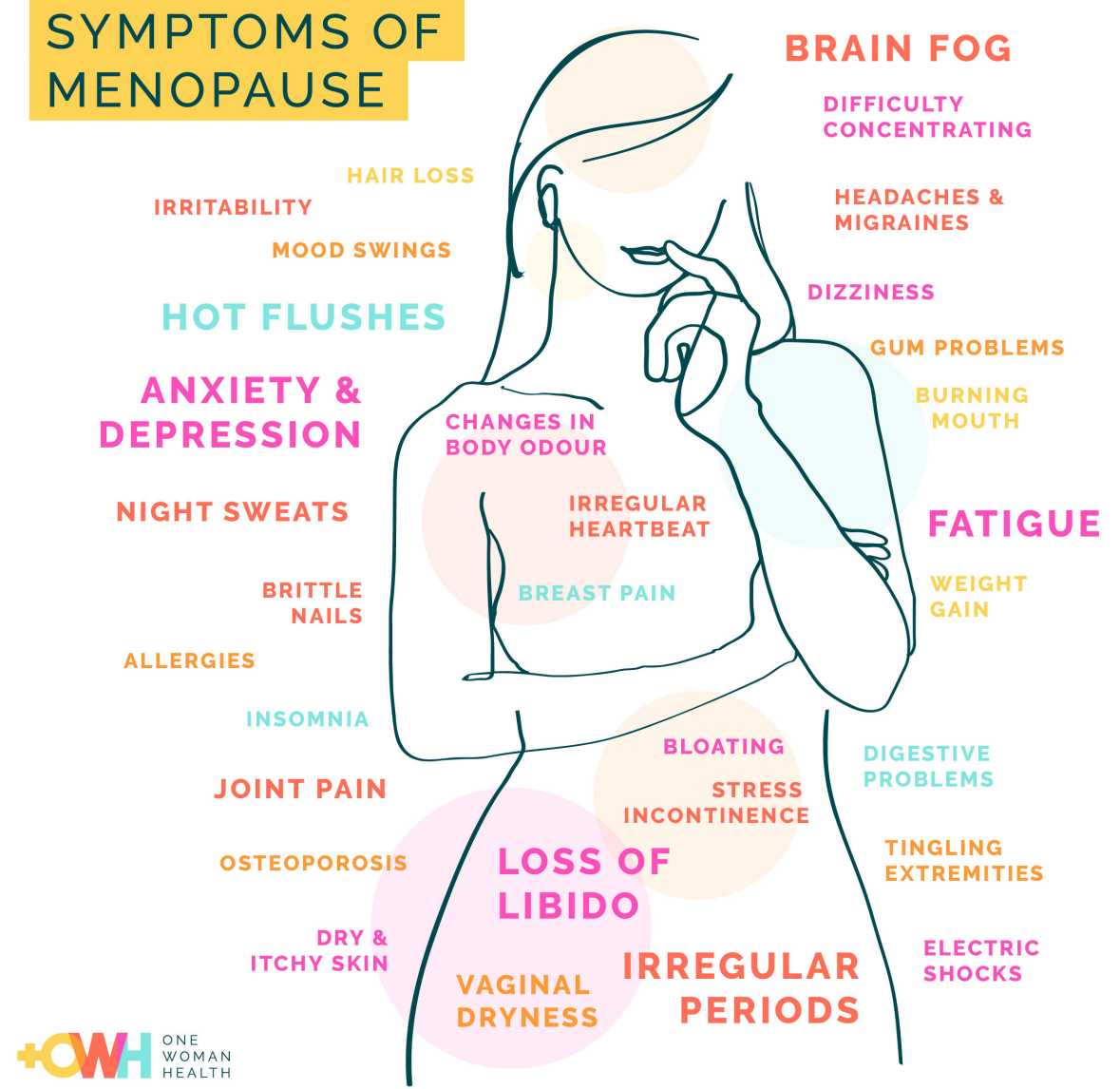
Digestion

Musculoskeletal/Bone health

Hair/Skin/Nails

Hormonal

## SYMPTOMS OF MENOPAUSE



# The Stages of Menstruation:

---

## Cycling Female:

- Generally cycle every 28-35 days with a 3-7 day bleed

## Peri-Menopause:

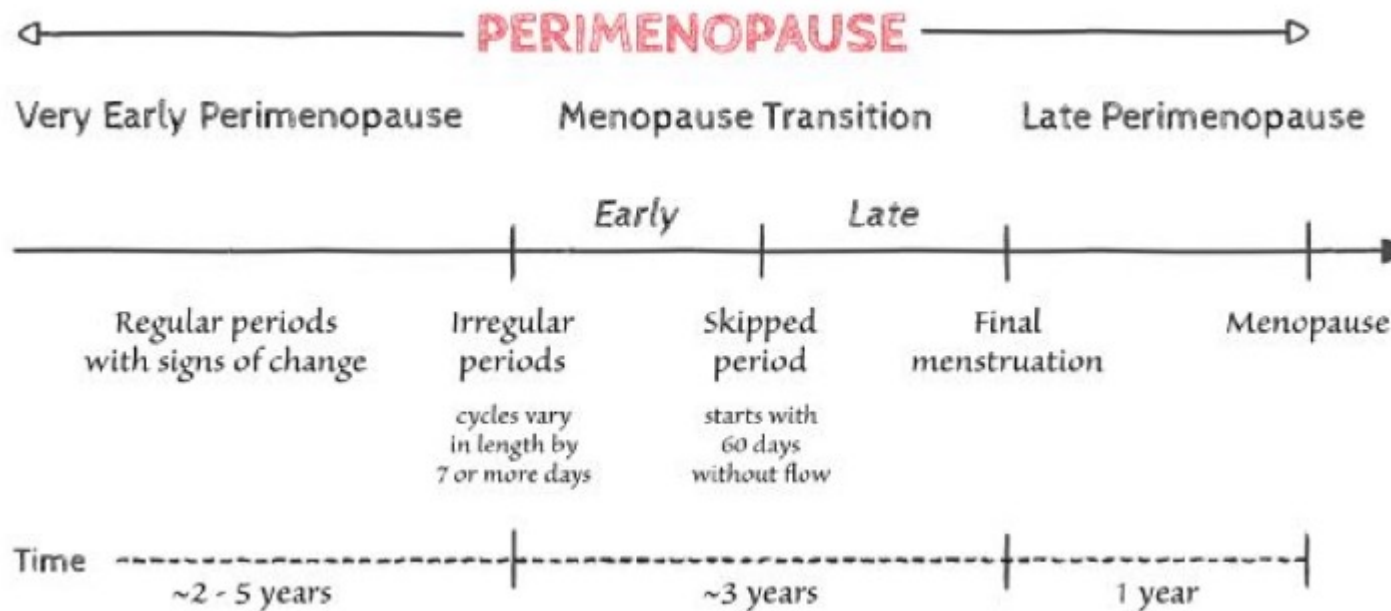
- Irregularities with cycling – sooner than 28 days, later than 35 days, bleeding more frequently, bleeding less than 3 days, or more than 7 days, and volume of blood may be a lot less, or a lot more

## Menopause:

- No bleeding for 12 consecutive months

# Phases of Perimenopause

1. very early perimenopause, when cycles are still regular
2. early menopause transition, from the onset of irregular periods
3. late menopause transition, from the first cycle of more than sixty days
4. late perimenopause, which is the twelve months from the final period
- +. menopause, which is the life phase that begins one year after your last period.



# Estradiol and Progesterone transition

Estradiol has significant variability at the beginning and end of menstruation – menarche and then again at menopause

Estrogen is needed to signal in a predictable and consistent manner

Once Estradiol is consistent, it can appropriately signal ovulation and then progesterone levels rise to support ovulation for that month

Perimenopause begins greater variability of estradiol signaling until it wanes to the low levels in menopause

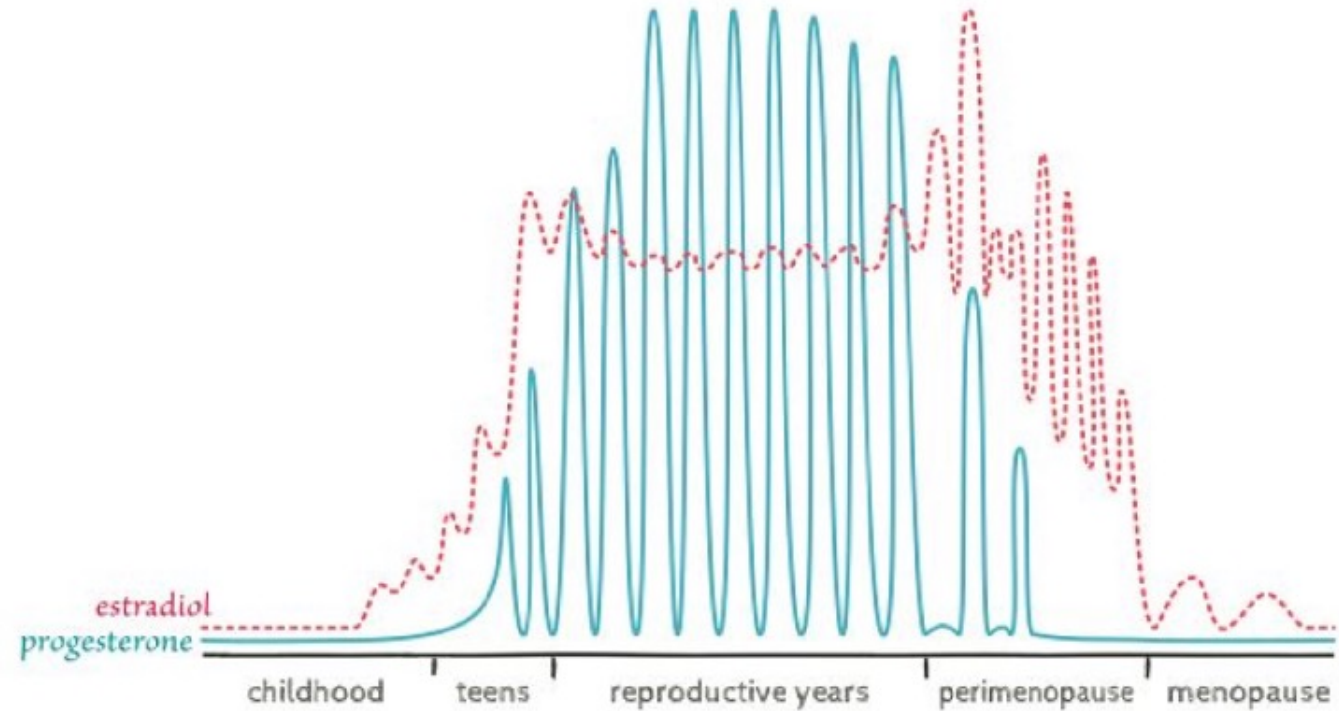


image 1 – hormones through the lifespan, adapted from JC Prior, “Perimenopause lost—reframing the end of menstruation.”

Briden, Lara. Hormone Repair Manual.

# Hormones involved in menopause:

---

- Estrogen
- Progesterone
- Testosterone
- Cortisol
- DHEA



Serum

Urine

- FSH, LH



Serum



# Hormones involved in menopause:

---

- Estrogen
- Progesterone
- Testosterone
- Cortisol
- DHEA
- FSH, LH

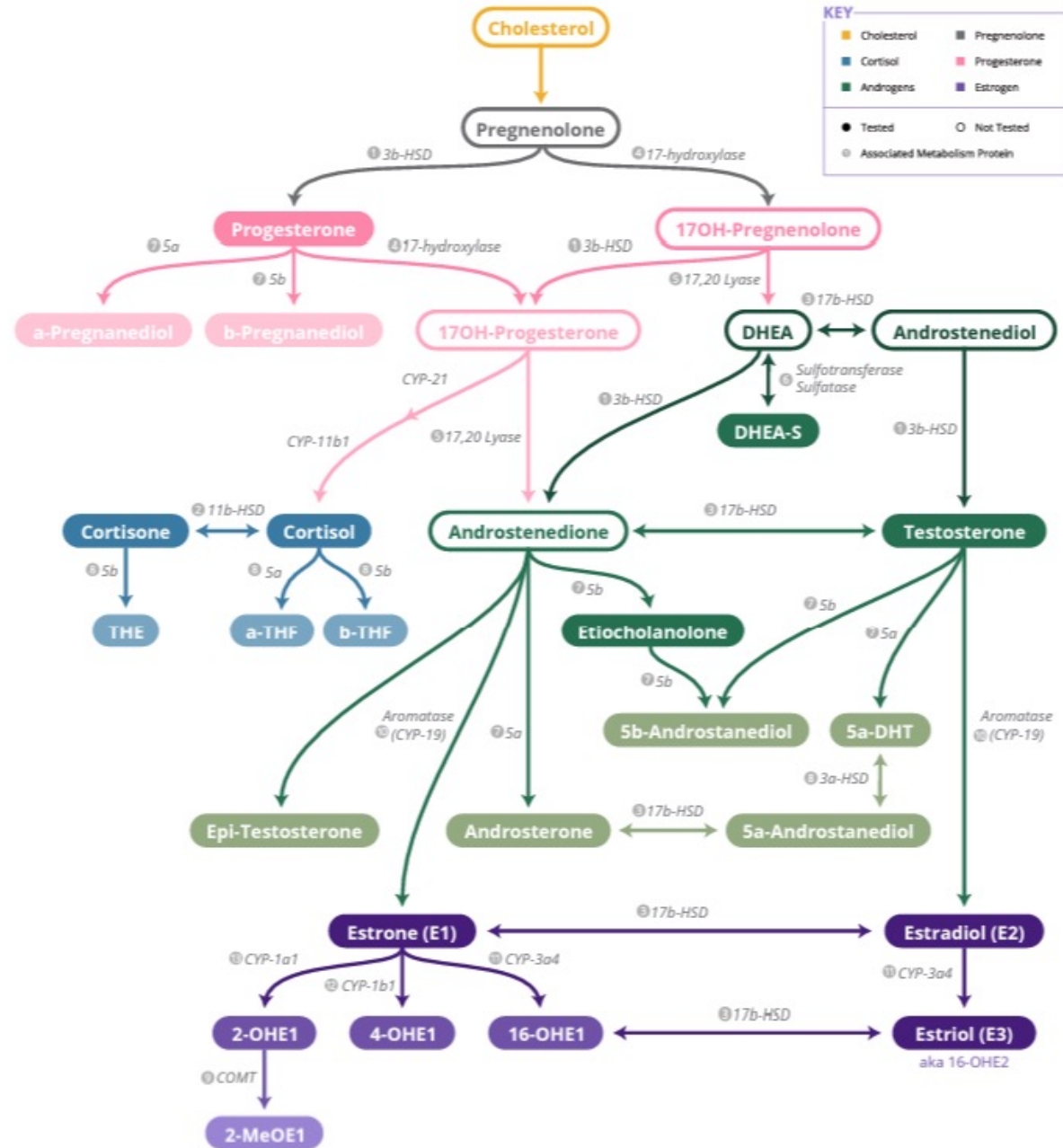
# Female Hormones

**Cholesterol** is the precursor to all steroid hormones → sex hormones and cortisol

**Estrogens** are the last stop on the steroid pathway

Estrogens are converted from **Testosterone** and **DHEA** (androgens) through aromatization

Aromatization is a process of conversion through an enzyme from the **androgens** to **estrogen**



## Estrogen

- Estrone (E1) – most abundant in menopause
  - Estradiol (E2) – most potent in cycling females
  - Estriol (E3) – most impactful in pregnancy
- 
- **What does Estrogen do?**
  - It is proliferative – it grows things!
  - Breast tissue, uterine tissue, ovarian tissue/follicles, bones
  - It is responsible for hair and breast growth in puberty
  - It is responsible for regulating our menstrual cycle

# Hormones: Estrogen

---

What are the **benefits** of Estrogen in females:

- Menstrual timing/regulation
- Sexual desire/libido
- Cholesterol metabolism
- Bone health
- Brain health (memory/mood)
- Skin and blood vessels (stretchy/elasticity)
- Cardiovascular Health
- Metabolic health – cellular metabolism
- E3 – some consider this to be anti-inflammatory (utilized in high doses for AI conditions)

# Hormones: Progesterone

---

**Progesterone** is dependent on Estrogen

- Estrogen signals progesterone to increase and prepare the endometrium for implantation after ovulation
- Progesterone is meant to “balance” estrogen in the luteal phase of the menstrual cycle
- Progesterone is made from the Corpus Luteum (left over from follicle created with ovulation)

# Hormones: Progesterone

---

Benefits of **progesterone** in post menopausal females:

- Improves mood
- Improves sleep
- Reduces hot flashes
- Improves Bone Mineral Density (BMD)

# Hormones: Testosterone

---

## Benefits of **testosterone** in females

- Muscle Mass
- Joint lubrication
- Sense of well-being
- Drive (libido and mental)
- Bone health
- Brain health – focus, spatial processing

# Hormones: DHEA

---

- A complicated hormone still needing more research
- It is a precursor hormone: precursor to Testosterone and Estrogen

## **DHEA**

- Balances the effects of cortisol
- Supports bone health
- Influences mood and sense of well-being
- Correlated to immune and disease states



# Perimenopause and Menopause:

---

## Symptoms and process

### Process:

- 1 - Initial loss of progesterone
- 2 - Variable levels of Estrogen
- 3 - Low levels of Estrogen
- 4 - Consequent drop in Testosterone

## Symptoms and Process

### Symptom presentation:

- **Loss of Progesterone:**
  - Initiation of vasomotor symptoms (VMS): hot flashes, night sweats
  - Poor sleep due to loss of GABA stimulation in the brain from progesterone
  - Poor mood due to loss of GABA in the brain
  - Brain fog
  - Poor mood due to fluctuations between progesterone and estrogen
    - Progesterone deficiency, and relative or blatant estrogen dominance

# Perimenopause and Menopause

---

## Symptoms and process

### Process:

- 1- Initial Loss of progesterone
- 2 – Variable levels of estrogen
- 3 – Low levels of estrogen
- 4 – Consequent drop of Testosterone

# Estrogen: Variable levels

---

## TOO LOW:

- Irregular cycles
- Hot Flashes/night sweats
- Mood changes
- Changes in focus
- Muscle/joint pain
- Headaches
- Loss of hair
- Migraines
- Loss of skin elasticity
- **Loss of blood vessel contractility**
- Changes in cholesterol levels
- **Loss of bone density**
- Change in body mass distribution (hour glass = blob)
- Changes in sleep
- Changes in energy
- Changes in bowels
- Dizziness
- Changes in urination/bladder symptoms – UTI type symptoms

# Estrogen: Variable levels

---

## **TOO HIGH:**

- Irregular cycles
- Hot flashes/night sweats
- Irritability
- Breast tenderness/enlargement
- Increased blood volume
- Fluctuations of estrogen occur due to inconsistent signaling of FSH from the brain
- Estrogen may appear dominant due to insufficient progesterone to balance estrogen

# Perimenopause and Menopause

---

## Symptoms and process

### Process:

- 1- Initial Loss of progesterone
- 2 – Variable levels of estrogen
- 3 – Low levels of estrogen
- 4 – Consequent drop of Testosterone

# Menopause and Perimenopause

---

## Low Estrogen symptoms:

- **Low Bone Mineral Density (BMD)** – osteopenia, osteoporosis, bone pain
- **Vasomotor symptoms (VMS)** – hot flashes, night sweats, burning from the inside
  - Most likely contributing to poor sleep, poor quality of life
- **Vulvo Vaginal Atrophy (VVA)**
  - Vaginal dryness, pain or discomfort with intimacy, wiping, urination, sitting, swimming/hot tubs/exercise become a problem
  - The labia shrink, vaginal cells become taught and tender
- **Mood Changes**
  - Low mood (depression symptoms), anxiety, brain fog, confusion

# Menopause and Perimenopause

---

## Symptoms and process

### Process:

- 1- Initial Loss of progesterone
- 2 – Variable levels of estrogen
- 3 – Low levels of estrogen
- 4 – Consequent drop of Testosterone



## Drop in Testosterone Levels

- Lower muscle mass
- Lowered sense of well-being
- Joint pain/muscle pain
- Increased fatigue
- Decreased motivation
- By menopause, **women lose up to  $\frac{3}{4}$  of their peak level of testosterone**; though research shows that by 65-70 yo, testosterone levels may return to similar levels as when they were younger

# Menopause Experience

I can't enjoy intimacy anymore – my body has betrayed me!

My mood is all over the place! Crying, laughing, irritable – ALL at the same time!

I feel like I don't know my body anymore

Why do I have acne? I thought I already did this in high school!

I am grieving losing my vitality, losing my youth

Why am I bleeding through my clothes?

My body is in pain – I can't do what I used to be able to do because of joint and muscle pain.

Why am I gaining weight?

I'm so TIRED! I can't sleep!

It is SO HOT IN HERE!

# Menopause Transition

- The idea of Second Puberty and variable levels of estrogen
- Loss of progesterone occurs around 5-10 years prior to loss of estrogen

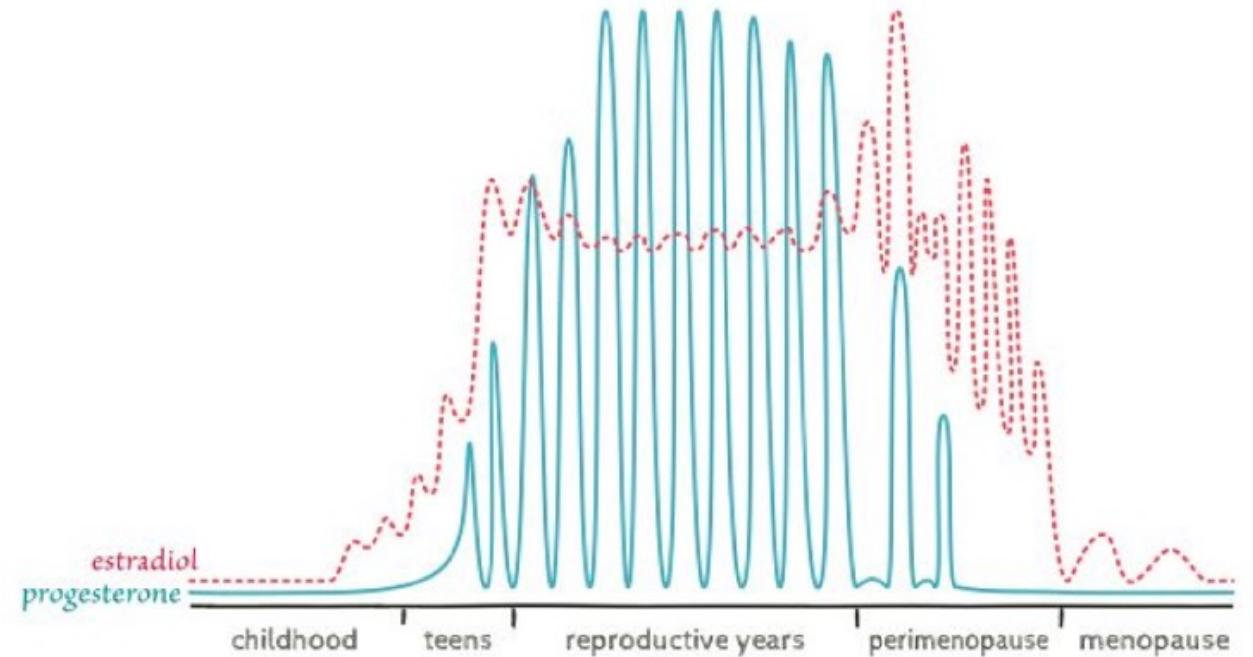


image 1 — hormones through the lifespan, adapted from JC Prior, "Perimenopause lost—reframing the end of menstruation."

# Menopause Interventions

---

Options for support during hormone changes:

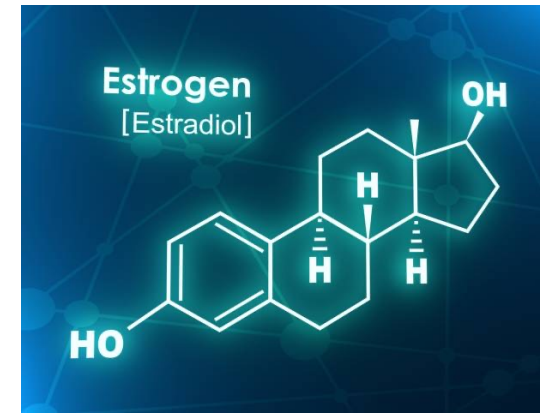
- **Hormone therapy:**
  - synthetic, bioidentical
- **Nutritional:**
  - protein intake, feeding adjustments (IF/macros)
- **Supplements including:**
  - Amino Acids, Creatine, Herbs, Vitamins
- **Lifestyle adjustments:**
  - Exercise, sleep patterns

# Options for hormone therapy replacement (HRT)

## HRT: Hormone Replacement Therapy

### Synthetic HRT:

Synthetic hormones are hormone compounds that act on our hormone receptors but may also act on other receptors. Examples of synthetic hormones include Premarin



### Bioidentical HRT (bHRT):

These hormones are made from plants and mimic how our own hormones influence our hormone receptors



# Hormone Replacement Therapy

---

- Estrogen Replacement Therapy (ERT)
- Progesterone Replacement Therapy
- Testosterone Replacement Therapy (TTh)

# Hormone Replacement Therapy: Estrogen

---

## Estrogen Replacement Therapy (ERT)

- We have 3 primary estrogens, so we can outline 3 possible options for estrogen therapy:
  - Estradiol (the most common), E2
  - Estriol (the next most common), E3
  - Estrone (the least common), E1
- 
- Bi:Est formulas usually contain E3:E2 (80:20), or (50:50) most commonly
  - Tri:Est formulas can also be used, but much less common

Usually a cream

# HRT: Estrogen

---

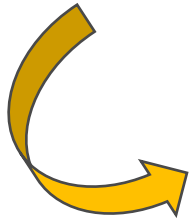
## ERT

- Topical
  - Creams, gels, patches
- Oral
  - Pills, troches/sublingual
- Vaginal
  - Cream, gel, troche, suppository
- Pellet
  - Injectable, or implant

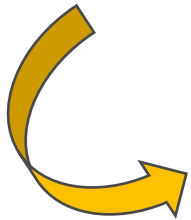


## Oral Estrogen risk discussion

Because oral estrogens are swallowed, they are then processed through the liver



The liver activates clotting factors in response to estrogen taken orally



Clotting factors increase the risk for blood clots

## NOT ALL PROGESTINS ARE CREATED EQUAL!

### Progesterone vs Progestin

- Progestin is a synthetic progesterone and has different effects on receptors than progesterone
- Progesterone is the natural form of progesterone
- KNOW which progestin you are taking

## Progesterone

- Oral – Prometrium
  - Oral Micronized Progesterone (OMP)
  - Sublingual falls under oral
- Topical
  - Cream, gel, oil
- Vaginal
  - Vaginal Micronized Progesterone (VMP)
  - Cream, gel, suppository
- Pellet
  - Injectable
- Injection
  - (more common with fertility treatments)

# Hormones: Testosterone

---

## Testosterone

- Topical
  - Cream, gel
  - *Most common*
- Vaginal
  - Cream, suppository, troche
- Pellet
- Injection
- Oral
  - *Least common, increased risk*

# FDA Approved E2 Gels

**Table 1: FDA Approved E2 Gels ("Low" = lowest recommended or studied dose)**

Brand of E2 Gel Product	Dose Level	Daily E2 Dose (mg)	Serum (pg/mL)	Vasomotor Symptoms (VMS)	Bone Mineral Density (BMD)	Vulvovaginal Atrophy (VVA)	References
EstroGel	Ultra-Low	0.27	11.7	Delayed	*	Failed	1
Divigel	Low	0.25	16	Delayed	*	Success	2,3
Elestrin	Low	0.52	9	Delayed	*	Success	4,5
Evamist	Low	1.53	19-23	Delayed	*	*	6,7
Estrasorb	Low	2.9	30	Success	*	*	8,9
EstroGel	Low	0.375	21	Success	*	Success	1
EstroGel	Moderate	0.75	33.5	Success	Delayed	Success	1,10,11,12,13
Divigel	Moderate	0.50	31	Success	*	Success	2,3
Elestrin	Moderate	1.04	32	Success	*	Success	4,5
Evamist	Moderate	3.06	24-32	Success	*	*	6,7
Estrasorb	Moderate	5.7	43	Success	*	*	8,9
EstroGel	High	1.5	65	Success	Success	Success	1,10,11,12,13
Divigel	High	1.0	62	Success	*	Success	2,3
Elestrin	High	1.56	60	Success	*	*	4,5
Evamist	High	4.59	31-40	Success	*	*	6,7
Estrasorb	High	8.6	63	Success	*	*	14
EstroGel	High+	3.0	102.9	Success	Success	*	12,15

All products listed above are considered E2 gels except Estrasorb, which is an emulsified product

# FDA Approved E2 Patches

**Table 2: FDA Approved E2 Patches**

Brand of E2 Patch	Total E2 in patch (mg)	Daily E2 Dose (mg)	Serum (pg/mL)	Vasomotor Symptoms (VMS)	Bone Mineral Density (BMD)	Vulvovaginal Atrophy (VVA)	References
Menostar	1	0.014	8.6, 13.7	Success	Success	Success	16,17,18
Alora	0.77	0.025	24.5	*	Success	*	19
Climara	2	0.025	22	Success	Success	*	20
Vivelle-Dot	0.39	0.025	*	*	Success	*	21
Vivelle-Dot	0.585	0.0375	34	Success	Success	*	21
Esclim	5	0.025	15.5	Success	*	*	22
Estraderm	*	0.025	*	*	Success	*	23
Alora	1.5	0.05	64	Success	Success	Success	19
Menorest	4.4	0.05	48.5	*	*	*	24
Climara	3.8	0.05	41	Success	Success	*	20,24
Vivelle-Dot	0.78	0.05	57	Success	Success	*	21
Esclim	10	0.05	26.3	Success	*	*	22
Estraderm	4	0.05	32	Success	*	*	23
Alora	3.1	0.1	98	Success	Success	Success	19
Climara	7.6	0.1	87	Success	Success	*	20
Vivelle-Dot	1.56	0.1	89	Success	Success	*	21
Esclim	20	0.1	51.4	Success	*	*	22
Estraderm	8	0.1	74	Success	*	*	23

# Compounded Hormones:

---

- Compounded hormones are not FDA approved at this time
- *Though* many Compounding Pharmacies service a need for compounded medications **including bioidentical hormone replacement** due to the need for individualized medicine and consideration of patient-mediated care
  - \*there are FDA approved bioidentical hormones as listed in the previous slide, just not compounded

So how do we test  
these levels?



# Urine

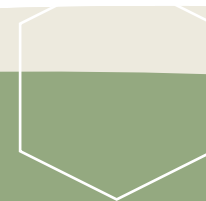
- Estrogen
- Estrogen (Phase 1 metabolism)
- Estrogen (Phase 2 methylation)
- Testosterone (available)
- Testosterone (metabolism)
- DHEA-S
- DHEA (metabolites)
- Progesterone metabolites
- Cortisol/cortisone (free pattern)
- Cortisol (CAR) – combined
- Cortisol/cortisone (metabolism)
- B6, B12,
- Glutathione deficiency
- Neurotransmitter balance
- Oxidative stress – 8OHdG
- Melatonin (production)
- Cycle Mapping – all month

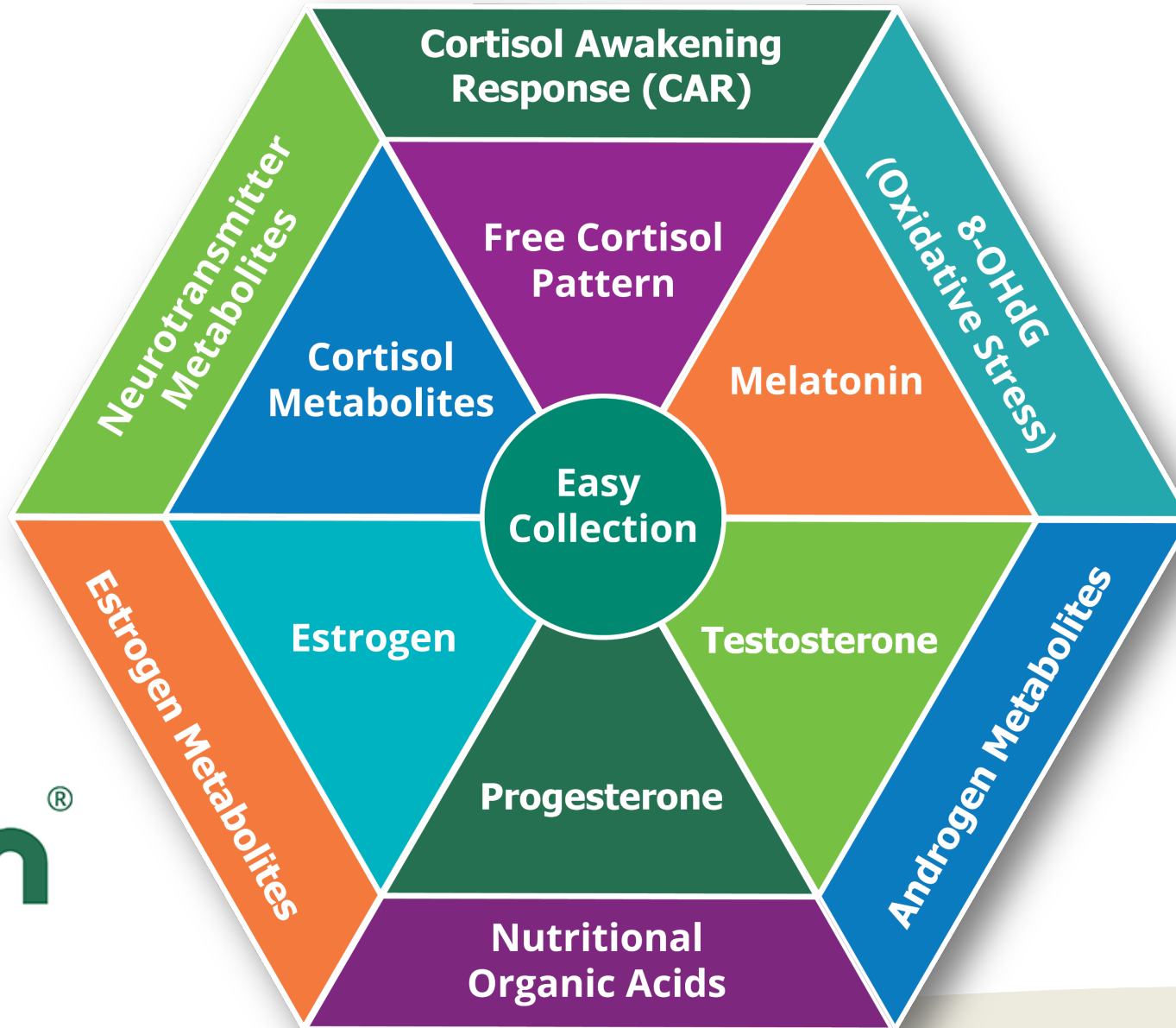
# Serum(blood)

- Estrogen
- No estrogen detoxification
- Testosterone (total/free)
- DHEA or DHEA-S
- Progesterone
- Cortisol (total, no diurnal pattern)

# Saliva

- Estrogen
- No estrogen detoxification
- Testosterone (available)
- DHEA or DHEA-S
- Progesterone
- Cortisol (free pattern)
- Cortisol (CAR)
- Melatonin
- Month long estrogen/progesterone



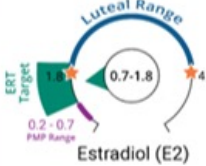


# Hormones Monitoring Options:

## MONITORING (B)HRT WITH LAB TESTING



Can serum or DUTCH, as a standalone test, effectively monitor HRT? ✓ Yes ✗ No ? Maybe

Oral Progesterone (OMP)	Estradiol (E2) Patches	E2 Gels & Creams (Skin)	Vaginal E2 & Testosterone (T)	Vaginal Progesterone (Pg)	Transdermal (TD) Testosterone	Testosterone Injections & Pellets
✓ DRIED URINE	✓ DRIED URINE	✓ DRIED URINE	✓ DRIED URINE	✗ DRIED URINE	? DRIED URINE	? DRIED URINE
<p>The DUTCH Test® provides useful feedback when using OMP in women with PMP sleep disturbances. 5a (more active) and 5b metabolites are measured to individualize OMP dosing. OMP's sleep effects are via its 5a metabolites, predominately allopregnanolone binding to the GABA receptor.</p> <p>No lab test reflects OMP's effect on the endometrium.</p>	<p>Values between the top of the postmenopausal range and the lower limit 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 because in addition to metabolites, dried urine averages out the daily up and down E2 patterns. This is particularly helpful with gels and creams that may have serum values that change rapidly over time.</p> 	<p>The DUTCH Test® is unique in that it removes potential contamination, and monitoring is helpful with E2 and T.</p> <p>Very low doses may impact local tissue without increasing lab values. For local (not systemic) E2 therapy, keep urine E2 in PMP range.</p>	<p>The DUTCH Test® is unique in that it removes potential contamination, and monitoring is helpful with E2 and T.</p> <p>Very low doses may impact local tissue without increasing lab values. For local (not systemic) E2 therapy, keep urine E2 in PMP range.</p>	<p>Pg is measured indirectly in urine by measuring pregnanediols. These metabolites may be underrepresented when Pg is taken vaginally. Serum Pg seems to increase to a higher degree than urine metabolites with vaginal Pg application.</p>	<p>Levels generally parallel changes in serum and clinical outcomes (increased lean body mass, erythrocytosis, etc. in men). Epi-testosterone (Epi-T) values can be used to assess gonadal suppression due to TRT (Epi-T levels in men decrease as TRT increases and are &lt;10ng/mg with complete suppression).</p>	<p>Injections and pellets increase levels, as expected, but the increase may exceed what is seen in serum testing. DUTCH allows for monitoring both the dosing of hormones as well as metabolic patterns.</p>
✗ SERUM	✓ SERUM	? SERUM	✓ SERUM	? SERUM	✓ SERUM	✓ SERUM
<p>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.</p>	<p>Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion.</p> <p>Most recommendations are to push serum E2 levels to 20-40pg/mL for clinical impact.</p>	<p>The only published data for E2 creams shows serum results move up and down within a few hours, so serum testing can easily underestimate clinical impact. DUTCH results average out the daily up and down pattern and may be a better option.</p>	<p>Serum results rise quite dramatically with what may seem like modest doses due to the high uptake of hormones across the mucosal membrane. However, values may rise and fall quickly, so be careful with the interpretation of both low and high results.</p>	<p>Serum values increase with dosing and likely represent systemic exposure to Pg. However, the uterine first-pass effect loads the uterus with high levels of Pg (which may be desirable) and serum does not reflect uterine hormone levels.</p>	<p>A great deal of published research shows that serum levels reflect clinical changes in both men and women taking TD T. Be aware of potential up and down patterns throughout the day, but serum is the best tool for monitoring doses of TD T in both men and women.</p>	<p>Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion.</p> <p>Test injections halfway between doses or right before a dose.</p>
✗ SALIVA	<p>The literature does not support salivary testing's use for monitoring TD hormone creams. The saliva data is limited and, in fact, there are no saliva testing outcome studies using TD creams, injections, estradiol patches, oral estradiol, or vaginal hormones. While salivary testing is the gold standard for free cortisol measurement, avoiding its use for monitoring HRT is advised. For situations where saliva testing may parallel the clinical impact, DUTCH or serum testing are better options (see above).</p>					
✗ Oral Estradiol, Estradiol Pellets, or Sublingual Hormones	<p>Though not recommended, if you choose to use either oral estradiol or estradiol pellets, serum testing can monitor both, whereas urine should only be used with pellet therapy. Sublingual hormones may be used in some situations but lab monitoring is not helpful in optimizing doses.</p>					
✗ Transdermal Progesterone	<p>In PMP women, the evidence does not support TD Pg's use to protect the endometrium. When prescribed, laboratory monitoring is not helpful for TD Pg dosing.</p>					

# Is there research for dried urine?

## Reliability of a dried urine test for comprehensive assessment of urine hormones and metabolites

Mark Newman\* and Desmond A. Curran

### Abstract

**Background:** Mass spectrometry allows for analysis of multiple hormone and organic acid metabolites from small urine volumes; however, to assess the full extent of daily hormone production, 24-h urine collections are usually required. The aims of this study were, first, to confirm that mass spectrometric analysis of an array of hormones and organic acids would yield similar results in both liquid and dried urine, and, second, to determine if collection of four dried spot urine samples could be substituted for a 24-h collection when measuring reproductive hormones.

**Methods:** Two study populations were included in this prospective observational study. Twenty individuals collected both a spot liquid urine and dried urine on filter paper to analyze eight organic acids. A second group of 26 individuals collected both a 24-h urine and four dried spot urines during waking hours throughout the same day for evaluation of 17 reproductive hormones and metabolites; data from 18 of these individuals were available to compare liquid versus dried urine results. Dried urine was extracted, hydrolyzed, and derivatized before analysis by mass spectrometry; all analytes from dried urine were normalized to urine creatinine.

**Results:** Reproductive hormone results from dried and liquid urine were in excellent agreement with intraclass correlation coefficients (ICCs) greater than 0.90; comparison of dried to liquid urine for organic acids showed good to excellent agreement (ICC range: 0.75 to 0.99). Comparison between the 4-spot urine collection and 24-h urine collection methods showed excellent agreement (ICC > 0.9) for 14 of the 17 urine metabolites and good agreement for the others (ICC 0.78 to 0.85) with no systematic differences between the two methods of collection.

**Conclusions:** The burden of urine collection can be reduced using collection of four spot dried urines on filter paper without compromising comparability with hormone results from a 24-h urine collection. A large number of urine analytes can be assessed from the dried urine with similar results to those from liquid urine. Given the ease of sample handling, this 4-spot dried urine assay would be useful for both clinical assessment of patients and for large epidemiologic studies.

**Keywords:** Dried urine testing, GC-MS/MS, LC-MS/MS, Reproductive hormones, Estrogen, Testosterone, Androgens, Organic acids

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

Mark Newman<sup>1\*</sup>, Suzanne M. Pratt<sup>2</sup>, Desmond A. Curran<sup>1</sup> and Frank Z. Stanczyk<sup>3</sup>

### 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 demonstrate that analysis of urine samples dried on filter paper by gas chromatography with tandem mass spectrometry (GC-MS/MS) provides results similar to serum analyzed by radioimmunoassay (RIA). Secondary aims were to show that collection of four samples during the day (4-spot method) can be substituted for a 24-h collection, and that analysis of urine from dried samples is equivalent to liquid urine samples.

**Methods:** This prospective observational study compared results of urine and serum analyses. Urine samples from women throughout the menstrual cycle and single samples from postmenopausal women were evaluated. Urine was collected onto filter paper and dried. Dried urine was extracted, hydrolyzed, and derivatized prior to analysis by GC-MS/MS. Hormone concentrations were normalized to creatinine. Single samples were used to compare results of 24-h urine collection to the 4-spot method from a separate population of women and men. A subset of these samples were used to compare results from dried urine to liquid urine.

**Results:** The primary study showed good reliability in the comparisons between the dried urine and serum assays. During the menstrual cycles of a subset of four women, urine metabolite concentrations followed the same pattern as serum concentrations. Comparison of 4-spot to 24-h urine collections and of dried to liquid urine measurements had intraclass correlation coefficients (ICC) greater than 0.95, indicating excellent agreement.

**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.

**Keywords:** Dried filter paper, DUTCH, Estradiol, GC-MS/MS, Hormone replacement therapy, Pregnanediol, Progesterone, Reproductive hormones, Subfertility

# Research for Dried Urine Testing

## Reliability of a dried urine test for comprehensive assessment of urine hormones and metabolites

Mark Newman

**Conclusions:** The burden of urine collection can be reduced using collection of four spot dried urines on filter paper without compromising comparability with hormone results from a 24-h urine collection. A large number of urine analytes can be assessed from the dried urine with similar results to those from liquid urine. Given the ease of sample handling, this 4-spot dried urine assay would be useful for both clinical assessment of patients and for large epidemiologic studies.

**Abstract Background:** urine require organized

dried spot urine samples could be substituted for a 24-h collection when measuring reproductive hormones.

**Methods:** Two study populations were included in this prospective observational study. Twenty individuals collected both a spot liquid urine and dried urine on filter paper to analyze eight organic acids. A second group of 26 individuals collected both a 24-h urine and four dried spot urines during waking hours throughout the same day for evaluation of 17 reproductive hormones and metabolites; data from 18 of these individuals were available to compare liquid versus dried urine results. Dried urine was extracted, hydrolyzed, and derivatized before analysis by mass spectrometry; all analytes from dried urine were normalized to urine creatinine.

**Results:** Reproductive hormone results from dried and liquid urine were in excellent agreement with intraclass correlation coefficients (ICCs) greater than 0.90; comparison of dried to liquid urine for organic acids showed good to excellent agreement (ICC range: 0.75 to 0.99). Comparison between the 4-spot urine collection and 24-h urine collection methods showed excellent agreement (ICC > 0.9) for 14 of the 17 urine metabolites and good agreement for the others (ICC 0.78 to 0.85) with no systematic differences between the two methods of collection.

**Conclusions:** The burden of urine collection can be reduced using collection of four spot dried urines on filter paper without compromising comparability with hormone results from a 24-h urine collection. A large number of urine analytes can be assessed from the dried urine with similar results to those from liquid urine. Given the ease of sample handling, this 4-spot dried urine assay would be useful for both clinical assessment of patients and for large epidemiologic studies.

**Keywords:** Dried urine testing, GC-MS/MS, LC-MS/MS, Reproductive hormones, Organic acids

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

Mark Newman<sup>1\*</sup>, Suzanne M. Pratt<sup>2</sup>, Desmond A. Curran<sup>1</sup>, and Frank Z. Stanczyk<sup>3</sup>

estrogen and progesterone in urine, instead of measuring estradiol and progesterone in urine, instead of measuring estradiol in patient care. The primary aim of this study was to evaluate the use of dried urine samples collected by gas chromatography with tandem mass spectrometry (GC-MS/MS) compared to those collected by radioimmunoassay (RIA). Secondary aims were to evaluate whether the 4-spot method (method) can be substituted for a 24-h collection, and that analysis of urine from dried samples is equivalent to liquid urine samples.

**Methods:** This prospective observational study compared results of urine and serum analyses. Urine samples from women throughout the menstrual cycle and single samples from postmenopausal women were evaluated. Urine was collected onto filter paper and dried. Dried urine was extracted, hydrolyzed, and derivatized prior to analysis by GC-MS/MS. Hormone concentrations were normalized to creatinine. Single samples were used to compare results of 24-h urine collection to the 4-spot method from a separate population of women and men. A subset of these samples were used to compare results from dried urine to liquid urine.

**Results:** The primary study showed good reliability in the comparisons between the dried urine and serum assays. During the menstrual cycles of a subset of four women, urine metabolite concentrations followed the same pattern as serum concentrations. Comparison of 4-spot to 24-h urine collections and of dried to liquid urine measurements had intraclass correlation coefficients (ICC) greater than 0.95, indicating excellent agreement.

**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.

# The Research for Dried Urine

## Monitoring:

E2 transdermal gel

E2 transdermal patch

Comparisons of E2 patches, gels, and creams

## Comparing Urinary Estrogen Profiles of Women on Transdermal Estradiol Patches, Gels, or Creams

Mark Newman, MS<sup>1</sup>; Doreen Saltiel, MD, JD<sup>1,2</sup>; Bryan P. Mayfield, PharmD<sup>1,3</sup>; Frank Stanczyk, PhD<sup>4</sup>

### OBJECTIVE

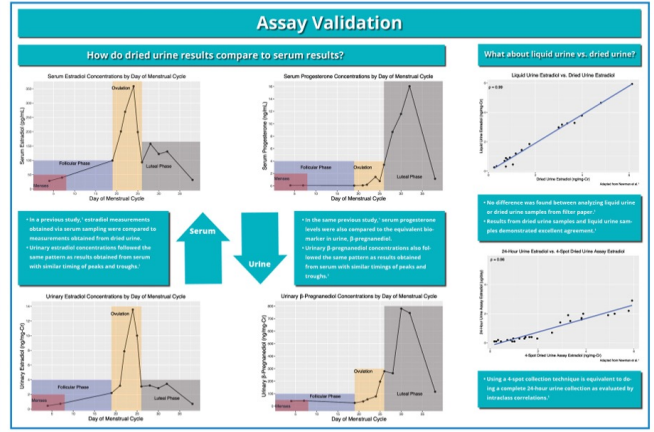
- Transdermal (TD) E2 patches and gels are the most commonly used types of TD E2 for hormone therapy, however, some providers choose to treat select patients with compounded E2 creams.
- A need exists to understand the comparative effects of these 3 different TD delivery systems given their unique and differing pharmacokinetics and the lack of data to support the efficacy of compounded E2 products.
- E2 levels are most often measured in serum, but these values may not be the most accurate, especially in those women and gels whose significant variation in E2 levels throughout the day, and across only represents 1 moment in time.
- In contrast, urine E2 levels may provide a representation of serum E2 concentrations over a 24-hour period.
- The aim of this study was to compare the effects of increasing doses of TD E2 patches, gels, and creams to urinary E2 concentrations by pharmacokinetic means.

### DESIGN

- This study utilized data from a retrospective observational study, Precision Analytical Retrospective Data Correlation (NCT04305093).
- The dataset from this larger study contained measures of multiple urinary markers obtained via a 4-spot dried urine sampling method.
- For this analysis, we used a subset of the data that included only the urinary E2 profiles of:
  - Premenopausal women not on menopausal hormone therapy (n = 11827)
  - Postmenopausal women using TD E2 gel (n = 24)
  - Urinary E2 was measured using gas chromatography/mass spectrometry (GC/MS/MS) with a lower limit of quantification of 0.092 ng/mL.
  - Doses were divided into 3 ranges:
    - Low-dose (0.25 mg, 0.30 mg, 0.375 mg, 0.5 mg, and 0.52 mg)
    - Mid-dose (0.75 mg and 1 mg)
    - High-dose (1.5 mg, 2.0 mg, 2.5 mg, and 3 mg)
  - The comparative, non-hormone Targeted (T) trend test was used to assess for ordered differences across groups to determine if dose-proportional increases in urinary E2 were seen with increasing dose ranges of TD E2 gel.

### DESIGN

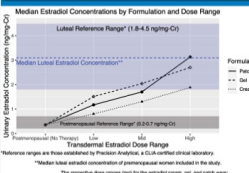
- In a previous study, estradiol measurements obtained via serum sampling were compared to measurements obtained via dried urine.
- Urinary estradiol concentrations followed the same pattern as results obtained from serum with similar timing of peaks and troughs.
- Comparisons between groups were made by statistical models using ANOVA and the non-parametric, Jonckheere-Terpstra (JT) trend test.



### RESULTS

- Analysis of the data demonstrated that for patches and gel groups, E2 concentrations were similar within the defined dose ranges and the E2 concentration for each of the 3 formulations showed an ordered trend with dose-proportional increases (P<0.0001 for patches and P<0.001 for gels).
- E2 concentrations for patches using creams also showed an ordered trend for dose-proportional increases (P<0.0001); however, the concentrations were lower than those observed with patches and gel in the same dose ranges.

### How do gels, patches, and creams compare?



### CONCLUSIONS

- Our results demonstrated the differences in urinary E2 profiles changes in response to increasing doses of E2 patches, gel, and creams.
- These results suggest that the validated dried urine assay used in this study may provide an attractive alternative to serum when monitoring TD E2 therapy.
- Future studies utilizing this assay may help determine the degree to which these measurements correlate with clinical impressions, which could lead to improved precision and personalization of MHT.

**Abbreviations:** E2, estradiol; E2C, estradiol cream; E2G, estradiol gel; E2P, estradiol patch; E2U, estradiol urine; E2U4, estradiol urine 4-spot; E2U8, estradiol urine 8-spot; E2U12, estradiol urine 12-spot; E2U16, estradiol urine 16-spot; E2U20, estradiol urine 20-spot; E2U24, estradiol urine 24-spot; E2U30, estradiol urine 30-spot; E2U36, estradiol urine 36-spot; E2U42, estradiol urine 42-spot; E2U48, estradiol urine 48-spot; E2U54, estradiol urine 54-spot; E2U60, estradiol urine 60-spot; E2U66, estradiol urine 66-spot; E2U72, estradiol urine 72-spot; E2U78, estradiol urine 78-spot; E2U84, estradiol urine 84-spot; E2U90, estradiol urine 90-spot; E2U96, estradiol urine 96-spot; E2U102, estradiol urine 102-spot; E2U108, estradiol urine 108-spot; E2U114, estradiol urine 114-spot; E2U120, estradiol urine 120-spot; E2U126, estradiol urine 126-spot; E2U132, estradiol urine 132-spot; E2U138, estradiol urine 138-spot; E2U144, estradiol urine 144-spot; E2U150, estradiol urine 150-spot; E2U156, estradiol urine 156-spot; E2U162, estradiol urine 162-spot; E2U168, estradiol urine 168-spot; E2U174, estradiol urine 174-spot; E2U180, estradiol urine 180-spot; E2U186, estradiol urine 186-spot; E2U192, estradiol urine 192-spot; E2U198, estradiol urine 198-spot; E2U204, estradiol urine 204-spot; E2U210, estradiol urine 210-spot; E2U216, estradiol urine 216-spot; E2U222, estradiol urine 222-spot; E2U228, estradiol urine 228-spot; E2U234, estradiol urine 234-spot; E2U240, estradiol urine 240-spot; E2U246, estradiol urine 246-spot; E2U252, estradiol urine 252-spot; E2U258, estradiol urine 258-spot; E2U264, estradiol urine 264-spot; E2U270, estradiol urine 270-spot; E2U276, estradiol urine 276-spot; E2U282, estradiol urine 282-spot; E2U288, estradiol urine 288-spot; E2U294, estradiol urine 294-spot; E2U300, estradiol urine 300-spot; E2U306, estradiol urine 306-spot; E2U312, estradiol urine 312-spot; E2U318, estradiol urine 318-spot; E2U324, estradiol urine 324-spot; E2U330, estradiol urine 330-spot; E2U336, estradiol urine 336-spot; E2U342, estradiol urine 342-spot; E2U348, estradiol urine 348-spot; E2U354, estradiol urine 354-spot; E2U360, estradiol urine 360-spot; E2U366, estradiol urine 366-spot; E2U372, estradiol urine 372-spot; E2U378, estradiol urine 378-spot; E2U384, estradiol urine 384-spot; E2U390, estradiol urine 390-spot; E2U396, estradiol urine 396-spot; E2U402, estradiol urine 402-spot; E2U408, estradiol urine 408-spot; E2U414, estradiol urine 414-spot; E2U420, estradiol urine 420-spot; E2U426, estradiol urine 426-spot; E2U432, estradiol urine 432-spot; E2U438, estradiol urine 438-spot; E2U444, estradiol urine 444-spot; E2U450, estradiol urine 450-spot; E2U456, estradiol urine 456-spot; E2U462, estradiol urine 462-spot; E2U468, estradiol urine 468-spot; E2U474, estradiol urine 474-spot; E2U480, estradiol urine 480-spot; E2U486, estradiol urine 486-spot; E2U492, estradiol urine 492-spot; E2U498, estradiol urine 498-spot; E2U504, estradiol urine 504-spot; E2U510, estradiol urine 510-spot; E2U516, estradiol urine 516-spot; E2U522, estradiol urine 522-spot; E2U528, estradiol urine 528-spot; E2U534, estradiol urine 534-spot; E2U540, estradiol urine 540-spot; E2U546, estradiol urine 546-spot; E2U552, estradiol urine 552-spot; E2U558, estradiol urine 558-spot; E2U564, estradiol urine 564-spot; E2U570, estradiol urine 570-spot; E2U576, estradiol urine 576-spot; E2U582, estradiol urine 582-spot; E2U588, estradiol urine 588-spot; E2U594, estradiol urine 594-spot; E2U600, estradiol urine 600-spot; E2U606, estradiol urine 606-spot; E2U612, estradiol urine 612-spot; E2U618, estradiol urine 618-spot; E2U624, estradiol urine 624-spot; E2U630, estradiol urine 630-spot; E2U636, estradiol urine 636-spot; E2U642, estradiol urine 642-spot; E2U648, estradiol urine 648-spot; E2U654, estradiol urine 654-spot; E2U660, estradiol urine 660-spot; E2U666, estradiol urine 666-spot; E2U672, estradiol urine 672-spot; E2U678, estradiol urine 678-spot; E2U684, estradiol urine 684-spot; E2U690, estradiol urine 690-spot; E2U696, estradiol urine 696-spot; E2U702, estradiol urine 702-spot; E2U708, estradiol urine 708-spot; E2U714, estradiol urine 714-spot; E2U720, estradiol urine 720-spot; E2U726, estradiol urine 726-spot; E2U732, estradiol urine 732-spot; E2U738, estradiol urine 738-spot; E2U744, estradiol urine 744-spot; E2U750, estradiol urine 750-spot; E2U756, estradiol urine 756-spot; E2U762, estradiol urine 762-spot; E2U768, estradiol urine 768-spot; E2U774, estradiol urine 774-spot; E2U780, estradiol urine 780-spot; E2U786, estradiol urine 786-spot; E2U792, estradiol urine 792-spot; E2U798, estradiol urine 798-spot; E2U804, estradiol urine 804-spot; E2U810, estradiol urine 810-spot; E2U816, estradiol urine 816-spot; E2U822, estradiol urine 822-spot; E2U828, estradiol urine 828-spot; E2U834, estradiol urine 834-spot; E2U840, estradiol urine 840-spot; E2U846, estradiol urine 846-spot; E2U852, estradiol urine 852-spot; E2U858, estradiol urine 858-spot; E2U864, estradiol urine 864-spot; E2U870, estradiol urine 870-spot; E2U876, estradiol urine 876-spot; E2U882, estradiol urine 882-spot; E2U888, estradiol urine 888-spot; E2U894, estradiol urine 894-spot; E2U900, estradiol urine 900-spot; E2U906, estradiol urine 906-spot; E2U912, estradiol urine 912-spot; E2U918, estradiol urine 918-spot; E2U924, estradiol urine 924-spot; E2U930, estradiol urine 930-spot; E2U936, estradiol urine 936-spot; E2U942, estradiol urine 942-spot; E2U948, estradiol urine 948-spot; E2U954, estradiol urine 954-spot; E2U960, estradiol urine 960-spot; E2U966, estradiol urine 966-spot; E2U972, estradiol urine 972-spot; E2U978, estradiol urine 978-spot; E2U984, estradiol urine 984-spot; E2U990, estradiol urine 990-spot; E2U996, estradiol urine 996-spot; E2U1002, estradiol urine 1002-spot; E2U1008, estradiol urine 1008-spot; E2U1014, estradiol urine 1014-spot; E2U1020, estradiol urine 1020-spot; E2U1026, estradiol urine 1026-spot; E2U1032, estradiol urine 1032-spot; E2U1038, estradiol urine 1038-spot; E2U1044, estradiol urine 1044-spot; E2U1050, estradiol urine 1050-spot; E2U1056, estradiol urine 1056-spot; E2U1062, estradiol urine 1062-spot; E2U1068, estradiol urine 1068-spot; E2U1074, estradiol urine 1074-spot; E2U1080, estradiol urine 1080-spot; E2U1086, estradiol urine 1086-spot; E2U1092, estradiol urine 1092-spot; E2U1098, estradiol urine 1098-spot; E2U1104, estradiol urine 1104-spot; E2U1110, estradiol urine 1110-spot; E2U1116, estradiol urine 1116-spot; E2U1122, estradiol urine 1122-spot; E2U1128, estradiol urine 1128-spot; E2U1134, estradiol urine 1134-spot; E2U1140, estradiol urine 1140-spot; E2U1146, estradiol urine 1146-spot; E2U1152, estradiol urine 1152-spot; E2U1158, estradiol urine 1158-spot; E2U1164, estradiol urine 1164-spot; E2U1170, estradiol urine 1170-spot; E2U1176, estradiol urine 1176-spot; E2U1182, estradiol urine 1182-spot; E2U1188, estradiol urine 1188-spot; E2U1194, estradiol urine 1194-spot; E2U1200, estradiol urine 1200-spot; E2U1206, estradiol urine 1206-spot; E2U1212, estradiol urine 1212-spot; E2U1218, estradiol urine 1218-spot; E2U1224, estradiol urine 1224-spot; E2U1230, estradiol urine 1230-spot; E2U1236, estradiol urine 1236-spot; E2U1242, estradiol urine 1242-spot; E2U1248, estradiol urine 1248-spot; E2U1254, estradiol urine 1254-spot; E2U1260, estradiol urine 1260-spot; E2U1266, estradiol urine 1266-spot; E2U1272, estradiol urine 1272-spot; E2U1278, estradiol urine 1278-spot; E2U1284, estradiol urine 1284-spot; E2U1290, estradiol urine 1290-spot; E2U1296, estradiol urine 1296-spot; E2U1302, estradiol urine 1302-spot; E2U1308, estradiol urine 1308-spot; E2U1314, estradiol urine 1314-spot; E2U1320, estradiol urine 1320-spot; E2U1326, estradiol urine 1326-spot; E2U1332, estradiol urine 1332-spot; E2U1338, estradiol urine 1338-spot; E2U1344, estradiol urine 1344-spot; E2U1350, estradiol urine 1350-spot; E2U1356, estradiol urine 1356-spot; E2U1362, estradiol urine 1362-spot; E2U1368, estradiol urine 1368-spot; E2U1374, estradiol urine 1374-spot; E2U1380, estradiol urine 1380-spot; E2U1386, estradiol urine 1386-spot; E2U1392, estradiol urine 1392-spot; E2U1398, estradiol urine 1398-spot; E2U1404, estradiol urine 1404-spot; E2U1410, estradiol urine 1410-spot; E2U1416, estradiol urine 1416-spot; E2U1422, estradiol urine 1422-spot; E2U1428, estradiol urine 1428-spot; E2U1434, estradiol urine 1434-spot; E2U1440, estradiol urine 1440-spot; E2U1446, estradiol urine 1446-spot; E2U1452, estradiol urine 1452-spot; E2U1458, estradiol urine 1458-spot; E2U1464, estradiol urine 1464-spot; E2U1470, estradiol urine 1470-spot; E2U1476, estradiol urine 1476-spot; E2U1482, estradiol urine 1482-spot; E2U1488, estradiol urine 1488-spot; E2U1494, estradiol urine 1494-spot; E2U1500, estradiol urine 1500-spot; E2U1506, estradiol urine 1506-spot; E2U1512, estradiol urine 1512-spot; E2U1518, estradiol urine 1518-spot; E2U1524, estradiol urine 1524-spot; E2U1530, estradiol urine 1530-spot; E2U1536, estradiol urine 1536-spot; E2U1542, estradiol urine 1542-spot; E2U1548, estradiol urine 1548-spot; E2U1554, estradiol urine 1554-spot; E2U1560, estradiol urine 1560-spot; E2U1566, estradiol urine 1566-spot; E2U1572, estradiol urine 1572-spot; E2U1578, estradiol urine 1578-spot; E2U1584, estradiol urine 1584-spot; E2U1590, estradiol urine 1590-spot; E2U1596, estradiol urine 1596-spot; E2U1602, estradiol urine 1602-spot; E2U1608, estradiol urine 1608-spot; E2U1614, estradiol urine 1614-spot; E2U1620, estradiol urine 1620-spot; E2U1626, estradiol urine 1626-spot; E2U1632, estradiol urine 1632-spot; E2U1638, estradiol urine 1638-spot; E2U1644, estradiol urine 1644-spot; E2U1650, estradiol urine 1650-spot; E2U1656, estradiol urine 1656-spot; E2U1662, estradiol urine 1662-spot; E2U1668, estradiol urine 1668-spot; E2U1674, estradiol urine 1674-spot; E2U1680, estradiol urine 1680-spot; E2U1686, estradiol urine 1686-spot; E2U1692, estradiol urine 1692-spot; E2U1698, estradiol urine 1698-spot; E2U1704, estradiol urine 1704-spot; E2U1710, estradiol urine 1710-spot; E2U1716, estradiol urine 1716-spot; E2U1722, estradiol urine 1722-spot; E2U1728, estradiol urine 1728-spot; E2U1734, estradiol urine 1734-spot; E2U1740, estradiol urine 1740-spot; E2U1746, estradiol urine 1746-spot; E2U1752, estradiol urine 1752-spot; E2U1758, estradiol urine 1758-spot; E2U1764, estradiol urine 1764-spot; E2U1770, estradiol urine 1770-spot; E2U1776, estradiol urine 1776-spot; E2U1782, estradiol urine 1782-spot; E2U1788, estradiol urine 1788-spot; E2U1794, estradiol urine 1794-spot; E2U1800, estradiol urine 1800-spot; E2U1806, estradiol urine 1806-spot; E2U1812, estradiol urine 1812-spot; E2U1818, estradiol urine 1818-spot; E2U1824, estradiol urine 1824-spot; E2U1830, estradiol urine 1830-spot; E2U1836, estradiol urine 1836-spot; E2U1842, estradiol urine 1842-spot; E2U1848, estradiol urine 1848-spot; E2U1854, estradiol urine 1854-spot; E2U1860, estradiol urine 1860-spot; E2U1866, estradiol urine 1866-spot; E2U1872, estradiol urine 1872-spot; E2U1878, estradiol urine 1878-spot; E2U1884, estradiol urine 1884-spot; E2U1890, estradiol urine 1890-spot; E2U1896, estradiol urine 1896-spot; E2U1902, estradiol urine 1902-spot; E2U1908, estradiol urine 1908-spot; E2U1914, estradiol urine 1914-spot; E2U1920, estradiol urine 1920-spot; E2U1926, estradiol urine 1926-spot; E2U1932, estradiol urine 1932-spot; E2U1938, estradiol urine 1938-spot; E2U1944, estradiol urine 1944-spot; E2U1950, estradiol urine 1950-spot; E2U1956, estradiol urine 1956-spot; E2U1962, estradiol urine 1962-spot; E2U1968, estradiol urine 1968-spot; E2U1974, estradiol urine 1974-spot; E2U1980, estradiol urine 1980-spot; E2U1986, estradiol urine 1986-spot; E2U1992, estradiol urine 1992-spot; E2U1998, estradiol urine 1998-spot; E2U2004, estradiol urine 2004-spot; E2U2010, estradiol urine 2010-spot; E2U2016, estradiol urine 2016-spot; E2U2022, estradiol urine 2022-spot; E2U2028, estradiol urine 2028-spot; E2U2034, estradiol urine 2034-spot; E2U2040, estradiol urine 2040-spot; E2U2046, estradiol urine 2046-spot; E2U2052, estradiol urine 2052-spot; E2U2058, estradiol urine 2058-spot; E2U2064, estradiol urine 2064-spot; E2U2070, estradiol urine 2070-spot; E2U2076, estradiol urine 2076-spot; E2U2082, estradiol urine 2082-spot; E2U2088, estradiol urine 2088-spot; E2U2094, estradiol urine 2094-spot; E2U2100, estradiol urine 2100-spot; E2U2106, estradiol urine 2106-spot; E2U2112, estradiol urine 2112-spot; E2U2118, estradiol urine 2118-spot; E2U2124, estradiol urine 2124-spot; E2U2130, estradiol urine 2130-spot; E2U2136, estradiol urine 2136-spot; E2U2142, estradiol urine 2142-spot; E2U2148, estradiol urine 2148-spot; E2U2154, estradiol urine 2154-spot; E2U2160, estradiol urine 2160-spot; E2U2166, estradiol urine 2166-spot; E2U2172, estradiol urine 2172-spot; E2U2178, estradiol urine 2178-spot; E2U2184, estradiol urine 2184-spot; E2U2190, estradiol urine 2190-spot; E2U2196, estradiol urine 2196-spot; E2U2202, estradiol urine 2202-spot; E2U2208, estradiol urine 2208-spot; E2U2214, estradiol urine 2214-spot; E2U2220, estradiol urine 2220-spot; E2U2226, estradiol urine 2226-spot; E2U2232, estradiol urine 2232-spot; E2U2238, estradiol urine 2238-spot; E2U2244, estradiol urine 2244-spot; E2U2250, estradiol urine 2250-spot; E2U2256, estradiol urine 2256-spot; E2U2262, estradiol urine 2262-spot; E2U2268, estradiol urine 2268-spot; E2U2274, estradiol urine 2274-spot; E2U2280, estradiol urine 2280-spot; E2U2286, estradiol urine 2286-spot; E2U2292, estradiol urine 2292-spot; E2U2298, estradiol urine 2298-spot; E2U2304, estradiol urine 2304-spot; E2U2310, estradiol urine 2310-spot; E2U2316, estradiol urine 2316-spot; E2U2322, estradiol urine 2322-spot; E2U2328, estradiol urine 2328-spot; E2U2334, estradiol urine 2334-spot; E2U2340, estradiol urine 2340-spot; E2U2346, estradiol urine 2346-spot; E2U2352, estradiol urine 2352-spot; E2U2358, estradiol urine 2358-spot; E2U2364, estradiol urine 2364-spot; E2U2370, estradiol urine 2370-spot; E2U2376, estradiol urine 2376-spot; E2U2382, estradiol urine 2382-spot; E2U2388, estradiol urine 2388-spot; E2U2394, estradiol urine 2394-spot; E2U2400, estradiol urine 2400-spot; E2U2406, estradiol urine 2406-spot; E2U2412, estradiol urine 2412-spot; E2U2418, estradiol urine 2418-spot; E2U2424, estradiol urine 2424-spot; E2U2430, estradiol urine 2430-spot; E2U2436, estradiol urine 2436-spot; E2U2442, estradiol urine 2442-spot; E2U2448, estradiol urine 2448-spot; E2U2454, estradiol urine 2454-spot; E2U2460, estradiol urine 2460-spot; E2U2466, estradiol urine 2466-spot; E2U2472, estradiol urine 2472-spot; E2U2478, estradiol urine 2478-spot; E2U2484, estradiol urine 2484-spot; E2U2490, estradiol urine 2490-spot; E2U2496, estradiol urine 2496-spot; E2U2502, estradiol urine 2502-spot; E2U2508, estradiol urine 2508-spot; E2U2514, estradiol urine 2514-spot; E2U2520, estradiol urine 2520-spot; E2U2526, estradiol urine 2526-spot; E2U2532, estradiol urine 2532-spot; E2U2538, estradiol urine 2538-spot; E2U2544, estradiol urine 2544-spot; E2U2550, estradiol urine 2550-spot; E2U2556, estradiol urine 2556-spot; E2U2562, estradiol urine 2562-spot; E2U2568, estradiol urine 2568-spot; E2U2574, estradiol urine 2574-spot; E2U2580, estradiol urine 2580-spot; E2U2586, estradiol urine 2586-spot; E2U2592, estradiol urine 2592-spot; E2U2598, estradiol urine 2598-spot; E2U2604, estradiol urine 2604-spot; E2U2610, estradiol urine 2610-spot; E2U2616, estradiol urine 2616-spot; E2U2622, estradiol urine 2622-spot; E2U2628, estradiol urine 2628-spot; E2U2634, estradiol urine 2634-spot; E2U2640, estradiol urine 2640-spot; E2U2646, estradiol urine 2646-spot; E2U2652, estradiol urine 2652-spot; E2U2658, estradiol urine 2658-spot; E2U2664, estradiol urine 2664-spot; E2U2670, estradiol urine 2670-spot; E2U2676, estradiol urine 2676-spot; E2U2682, estradiol urine 2682-spot; E2U2688, estradiol urine 2688-spot; E2U2694, estradiol urine 2694-spot; E2U2700, estradiol urine 2700-spot; E2U2706, estradiol urine 2706-spot; E2U2712, estradiol urine 2712-spot; E2U2718, estradiol urine 2718-spot; E2U2724, estradiol urine 2724-spot; E2U2730, estradiol urine 2730-spot; E2U2736, estradiol urine 2736-spot; E2U2742, estradiol urine 2742-spot; E2U2748, estradiol urine 2748-spot; E2U2754, estradiol urine 2754-spot; E2U2760, estradiol urine 2760-spot; E2U2766, estradiol urine 2766-spot; E2U2772, estradiol urine 2772-spot; E2U2778, estradiol urine 2778-spot; E2U2784, estradiol urine 2784-spot; E2U2790, estradiol urine 2790-spot; E2U2796, estradiol urine 2796-spot; E2U2802, estradiol urine 2802-spot; E2U2808, estradiol urine 2808-spot; E2U2814, estradiol urine 2814-spot; E2U2820, estradiol urine 2820-spot; E2U2826, estradiol urine 2826-spot; E2U2832, estradiol urine 2832-spot; E2U2838, estradiol urine 2838-spot; E2U2844, estradiol urine 2844-spot; E2U2850, estradiol urine 2850-spot; E2U2856, estradiol urine 2856-spot; E2U2862, estradiol urine 2862-spot; E2U2868, estradiol urine 2868-spot; E2U2874, estradiol urine 2874-spot; E2U2880, estradiol urine 2880-spot; E2U2886, estradiol urine 2886-spot; E2U2892, estradiol urine 2892-spot; E2U2898, estradiol urine 2898-spot; E2U2904, estradiol urine 2904-spot; E2U2910, estradiol urine 2910-spot; E2U2916, estradiol urine 2916-spot; E2U2922, estradiol urine 2922-spot; E2U2928, estradiol urine 2928-spot; E2U2934, estradiol urine 2934-spot; E2U2940, estradiol urine 2940-spot; E2U2946, estradiol urine 2946-spot; E2U2952, estradiol urine 2952-spot; E2U2958, estradiol urine 2958-spot; E2U2964, estradiol urine 2964-spot; E2U2970, estradiol urine 2970-spot; E2U2976, estradiol urine 2976-spot; E2U2982, estradiol urine 2982-spot; E2U2988, estradiol urine 2988-spot; E2U2994, estradiol urine 2994-spot; E2U3000, estradiol urine 3000-spot; E2U3006, estradiol urine 3006-spot; E2U3012, estradiol urine 3012-spot; E2U3018, estradiol urine 3018-spot; E2U3024, estradiol urine 3024-spot; E2U3030, estradiol urine 3030-spot; E2U3036, estradiol urine 3036-spot; E2U3042, estradiol urine 3042-spot; E2U3048, estradiol urine 3048-spot; E2U3054, estradiol urine 3054-spot; E2U3060, estradiol urine 3060-spot; E2U3066, estradiol urine 3066-spot; E2U3072, estradiol urine 3072-spot; E2U3078, estradiol urine 3078-spot; E2U3084, estradiol urine 3084-spot; E2U3090, estradiol urine 3090-spot; E2U3096, estradiol urine 3096-spot; E2U3102, estradiol urine 3102-spot; E2U3108, estradiol urine 3108-spot; E2U3114, estradiol urine 3114-spot; E2U3120, estradiol urine 3120-spot; E2U3126, estradiol urine 3126-spot; E2U3132, estradiol urine 3132-spot; E2U3138, estradiol urine 3138-spot; E2U3144, estradiol urine 3144-spot; E2U3150, estradiol urine 3150-spot; E2U3156, estradiol urine 3156-spot; E2U3162, estradiol urine 3162-spot; E2U3168, estradiol urine 3168-spot; E2U3174, estradiol urine 3174-spot; E2U3180, estradiol urine 3180-spot; E2U3186, estradiol urine 3186-spot; E2U3192, estradiol urine 3192-spot; E2U3198, estradiol urine 3198-spot; E2U3204, estradiol urine 3204-spot; E2U3210, estradiol urine 3210-spot; E2U3216, estradiol urine 3216-spot; E2U3222, estradiol urine 3222-spot; E2U3228, estradiol urine 3228-spot; E2U3234, estradiol urine 3234-spot; E2U3240, estradiol urine 3240-spot; E2U3246, estradiol urine 3246-spot; E2U3252, estradiol urine 3252-spot; E2U3258, estradiol urine 3258-spot; E2U3264, estradiol urine 3264-spot; E2U3270, estradiol urine 3270-spot; E2U3276, estradiol urine 3276-spot; E2U3282, estradiol urine 3282-spot; E2U3288, estradiol urine 3288-spot; E2U3294, estradiol urine 3294-spot; E2U3300, estradiol urine 3300-spot; E2U3306, estradiol urine 3306-spot; E2U3312, estradiol urine 3312-spot; E2U3318, estradiol urine 3318-spot; E2U3324, estradiol urine 3324-spot; E2U3330, estradiol urine 3330-spot; E2U3336, estradiol urine 3336-spot; E2U3342, estradiol urine 3342-spot; E2U3348, estradiol urine 3348-spot; E2U3354, estradiol urine 3354-spot; E2U3360, estradiol urine 3360-spot; E2U3366, estradiol urine 3366-spot; E2U3372, estradiol urine 3372-spot; E2U3378, estradiol

# Goal of Hormone Therapy

---

- Reduce symptoms and risks including:
- Vulvo-Vaginal Atrophy (VVA)
  - Vasomotor Symptoms (VMS)
  - Improve Bone Mineral Density (BMD)
  - Reduce Cardiovascular Disease Risk (CVD)
  - Improved quality of life



# Goal of Hormone Testing:

---

## Understand baseline:

- Evaluate levels of estrogen, progesterone, testosterone, DHEA, cortisol, and melatonin
- Understand metabolomics: how hormones are being metabolized
- Review HPA Axis influence on sex hormones

## Understand follow up:

- Evaluate changes in hormone levels with supplementation
- Be informed on metabolite preferences to optimize supplementation

# Goals of Hormone Testing and Using DUTCH

---

Utilize the research to ensure dosing of HRT is sufficient and effective to reduce symptoms and risk

- Research finds that serum or urine levels just outside the postmenopausal range and into the lower part of the premenopausal (luteal) range may be optimal targets for both E2 patches and gels.

**GOAL RANGE for E2:** Serum, 20-60pg/mL; DUTCH, 0.7-1.8ng/mg  
(for bone support)

# Goals for Hormone Testing

---

- Serum levels correlate to clinical improvements for both E2 patches and gels as it relates to VMS, BMD, VVA, and changes in FSH, lipids, SHBG, and the endometrium.
- **Urine levels generally parallel serum levels and clinical outcomes with TD E2 gels (DUTCH data) and patches (DUTCH and published data)**, although no studies have tied clinical outcomes directly to urine levels. The DUTCH test also includes estrogen metabolites.
- Saliva testing should not be used with creams or gels as its exaggerated values do not appear to correlate to any studied clinical outcomes.
- DUTCH may be optimal with E2 creams and gels given the rapid up-and-down serum pattern.

## Take Aways:

- Dried urine testing may be used as a surrogate for serum testing according to peer reviewed studies for E2 TD (patch/gels)
- Serum testing has been the gold standard against all other methods
- In non supplemented data, serum and dried urine correlate well
- In E2 patch and gel studies, dried urine parallels serum values

# QUICK NOTE ABOUT HRT and BEST PRACTICES

---

- Dr. **Doreen Saltiel** MD completed a webinar that walks through **all of** this information in detail – invaluable information

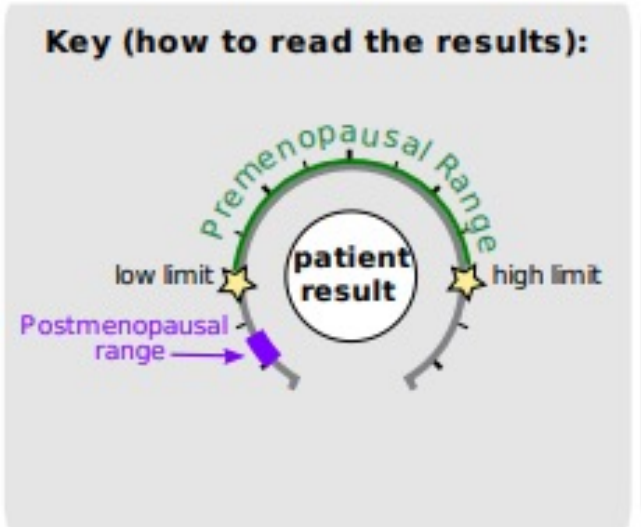
We agree moving forward:

- With any sort of E2 supplementation, OMP/VMP should be considered according to the research to ensure endometrial protection. This looks like OMP 200 mg, or VMP 50-100 mg
- Oral estrogens and testosterone come with significant risk; this is not a preferred method of supplementation

# How to Read and Understand the Numbers:

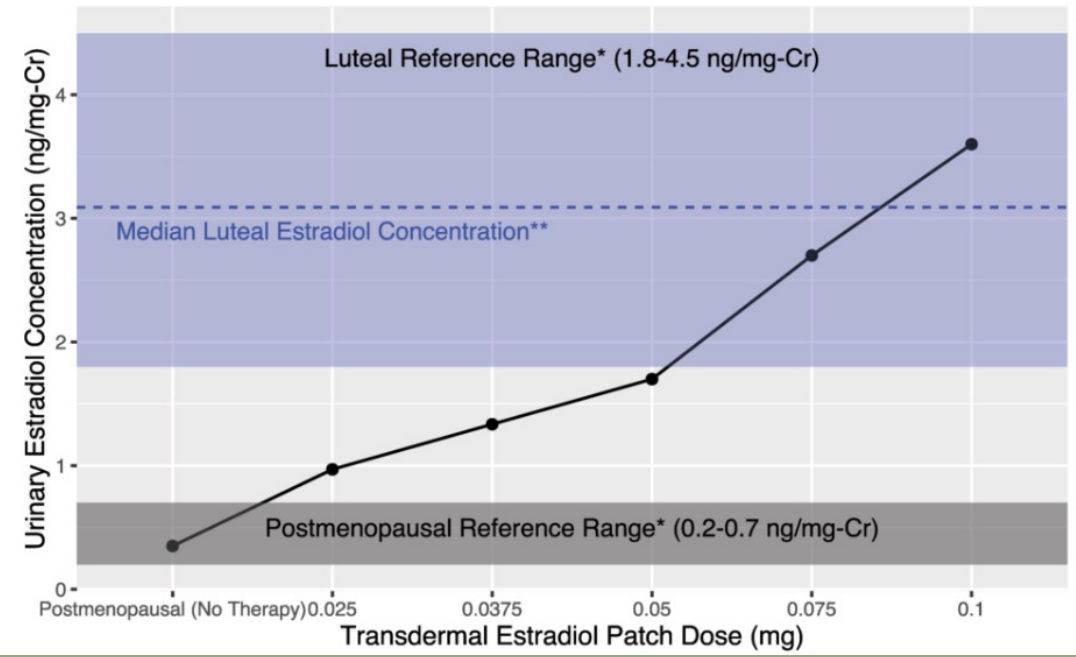
## Mind the Gap:

This represents the space between the postmenopausal range and the premenopausal ranges – these should not overlap. There should be a distinct difference between these ranges



We can see on this graph the distinct difference between postmenopausal range, application of an E2 patch, and the change from postmenopausal range into the luteal range

## Mind the Gap

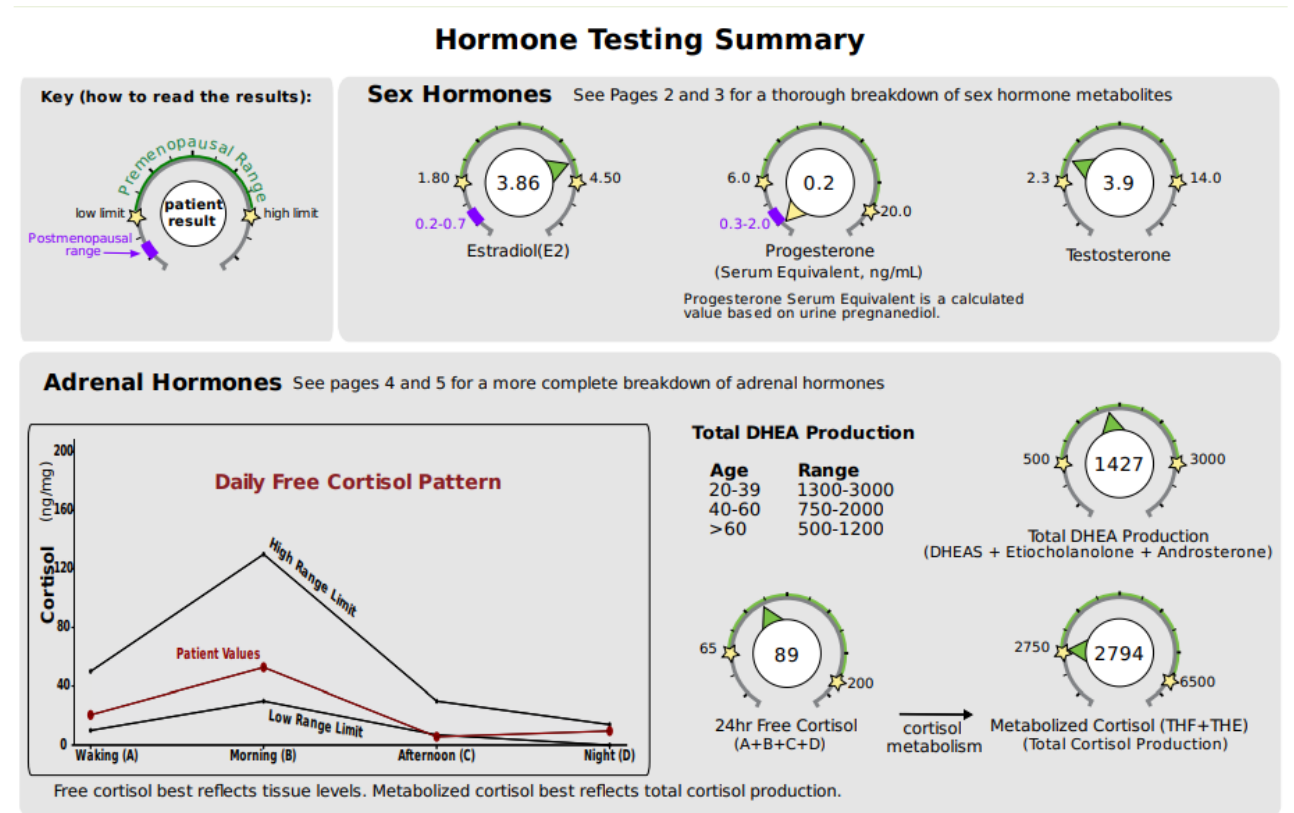


# Value of testing

Baseline levels of a 43 yo F with irregular cycles (perimenopausal)

We are evaluating:

- How variable are estrogen levels?
- What is the estrogen level relative to progesterone?
- What are the androgen levels?
- How is HPA Axis function?



The following videos (which can also be found on the website under the listed names along with others) may aid your understanding:

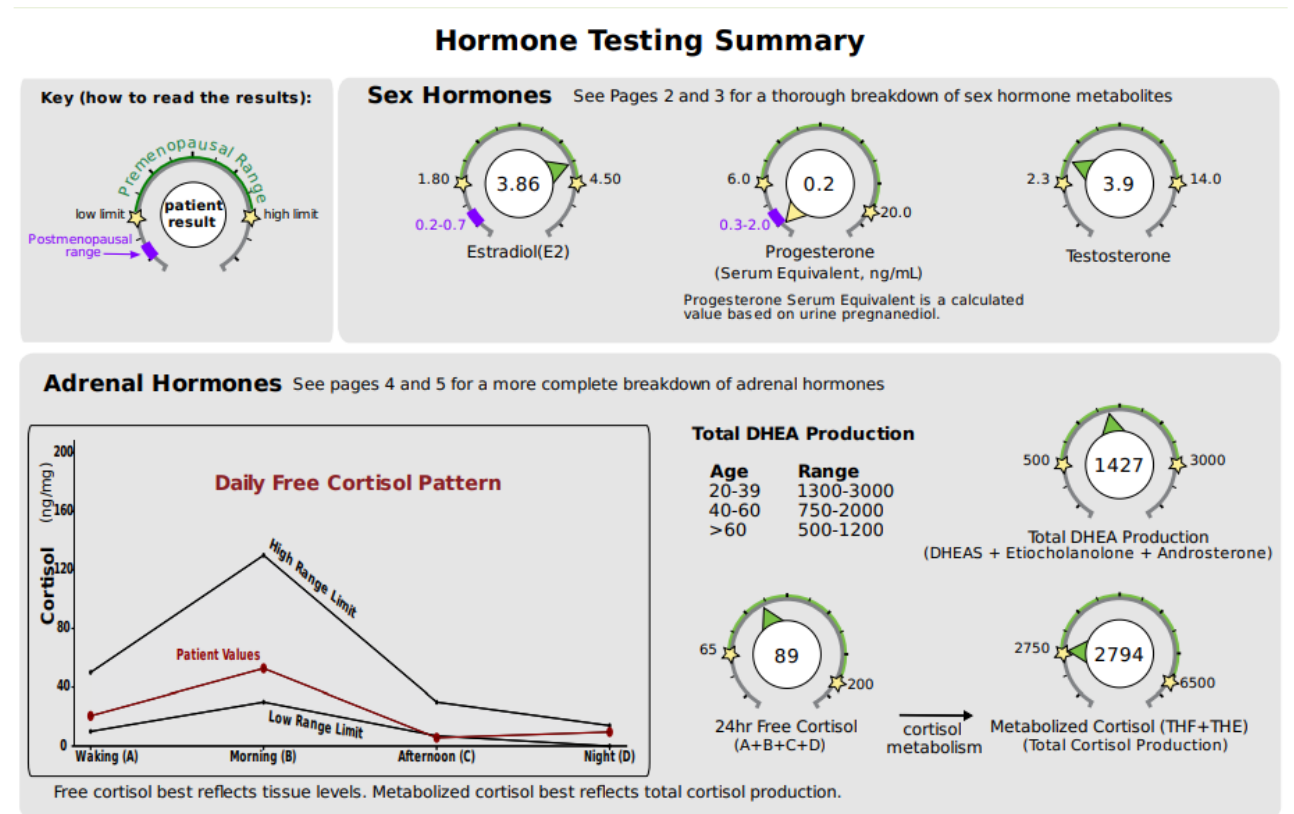
[DUTCH Complete Overview](#) [Estrogen Tutorial](#) [Female Androgen Tutorial](#) [Cortisol Tutorial](#)

**PLEASE BE SURE TO READ BELOW FOR ANY SPECIFIC LAB COMMENTS. More detailed comments can be found on page 7.**

# Value of testing

This data at baseline offers good information to understand the hormone levels – as a one day check in, we can see she is:

- Estrogen dominant relative to her progesterone levels
- Low-normal Testosterone
- Within range DHEA
- Low Cortisol output (HPA function)



The following videos (which can also be found on the website under the listed names along with others) may aid your understanding:

[DUTCH Complete Overview](#) [Estrogen Tutorial](#) [Female Androgen Tutorial](#) [Cortisol Tutorial](#)

**PLEASE BE SURE TO READ BELOW FOR ANY SPECIFIC LAB COMMENTS. More detailed comments can be found on page 7.**

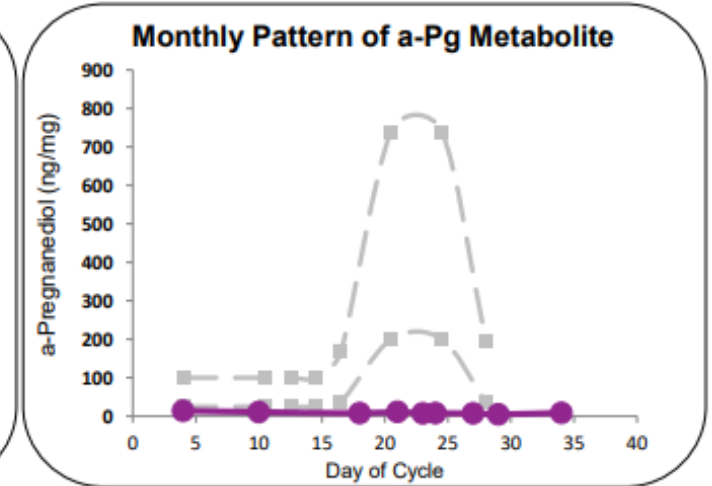
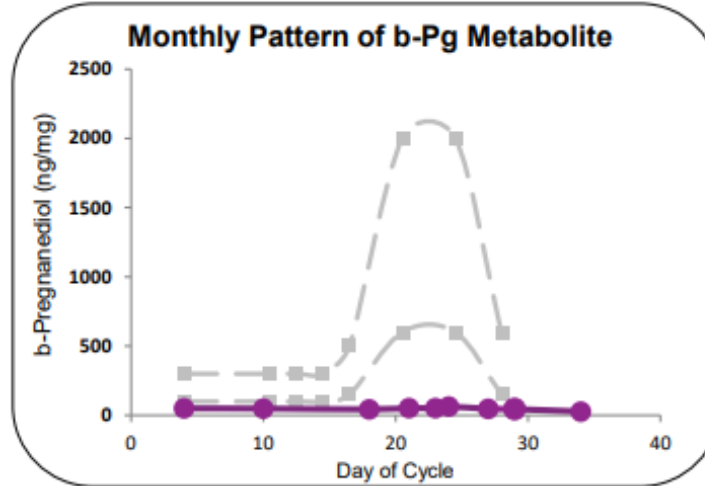
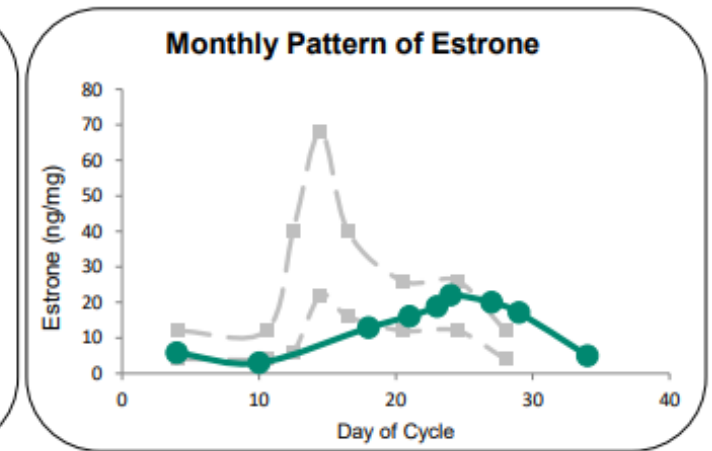
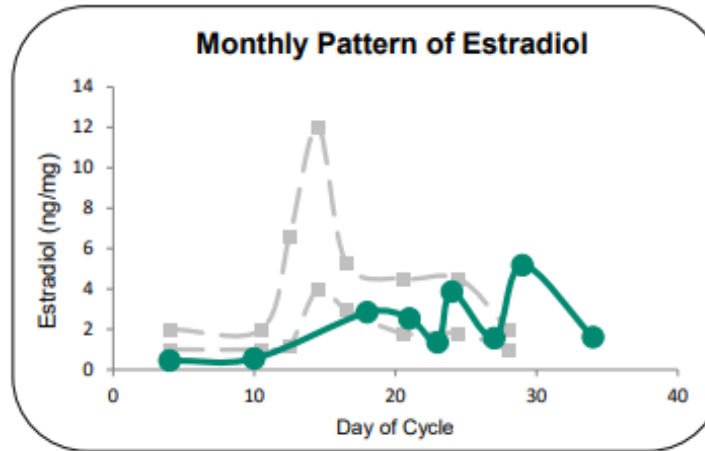


# The value of testing

Addition of a Cycle Mapping for this patient expands upon the variability of her estrogen at this stage in perimenopause.

We can see she has aberrant estrogen signaling, though she is trying to get her E2 to spike for ovulation; this is not achieved, and she ends up with no ovulation, therefore no rise in progesterone 😞

Is it overkill to do a Cycle Mapping test for a perimenopausal female? ***It depends on the patient!*** In this case, you could go either way – the CM confirms her Dutch Complete, but the DUTCH Complete offered valuable insight on its own as well



# PMP F with E2 and Progesterone HRT

NEXT CASE

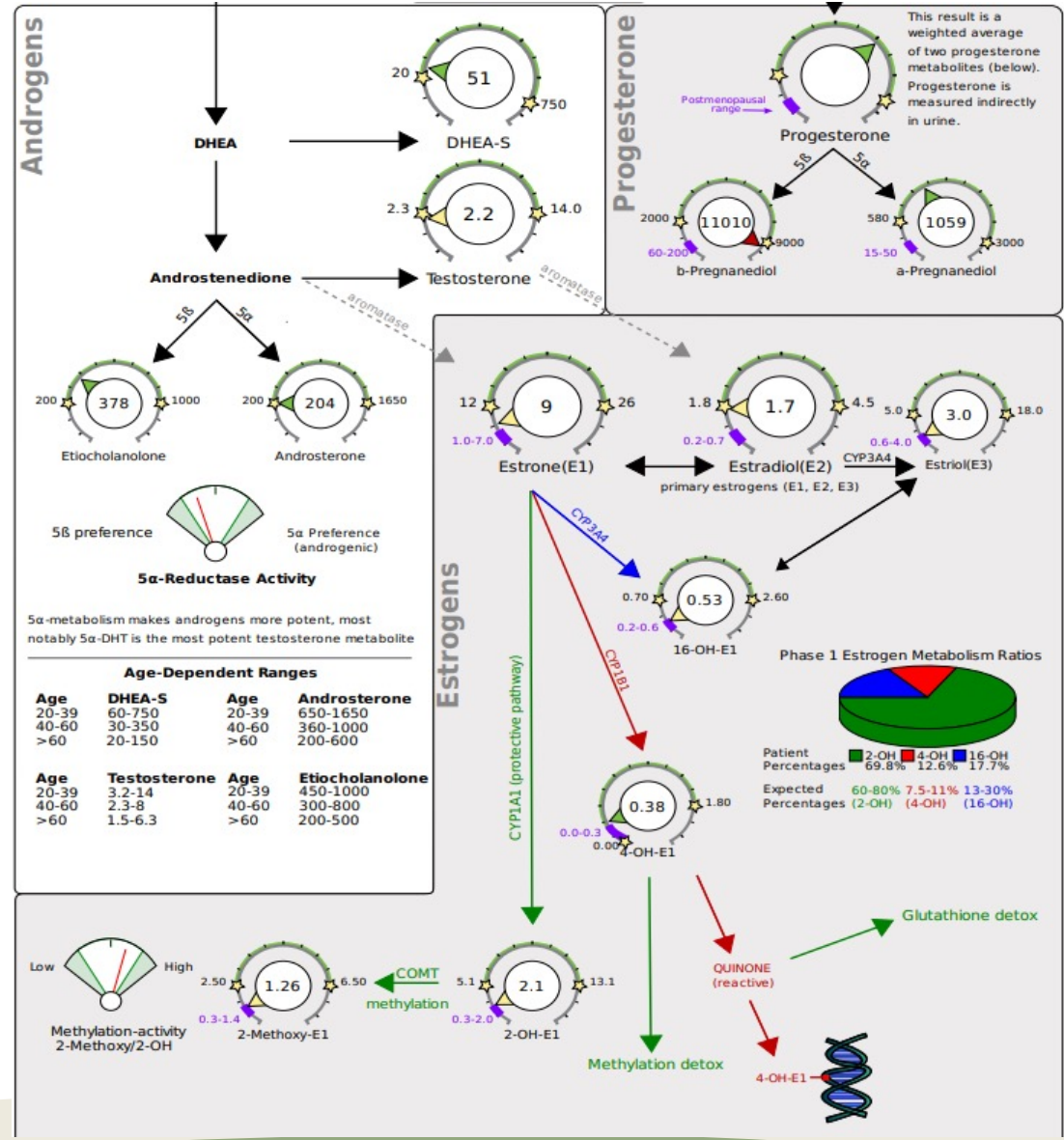
# Case Study and Examples of HRT on DUTCH Testing

Postmenopausal (PMP), uterus intact, 63 yo  
Supplementing:

- Progesterone 200 mg
- Estradiol TD 0.075 mg patch

## We can glean:

- E2 is at **1.7** (Goal is 0.7-1.8)
- Estrogen metabolism shows 4-OH-E1 slightly elevated
- Research supports 200 mg Progesterone for endometrial protection
- We can also see the value of progesterone metabolites here given her b-preg preference



# Case Study and Examples of HRT on DUTCH Testing

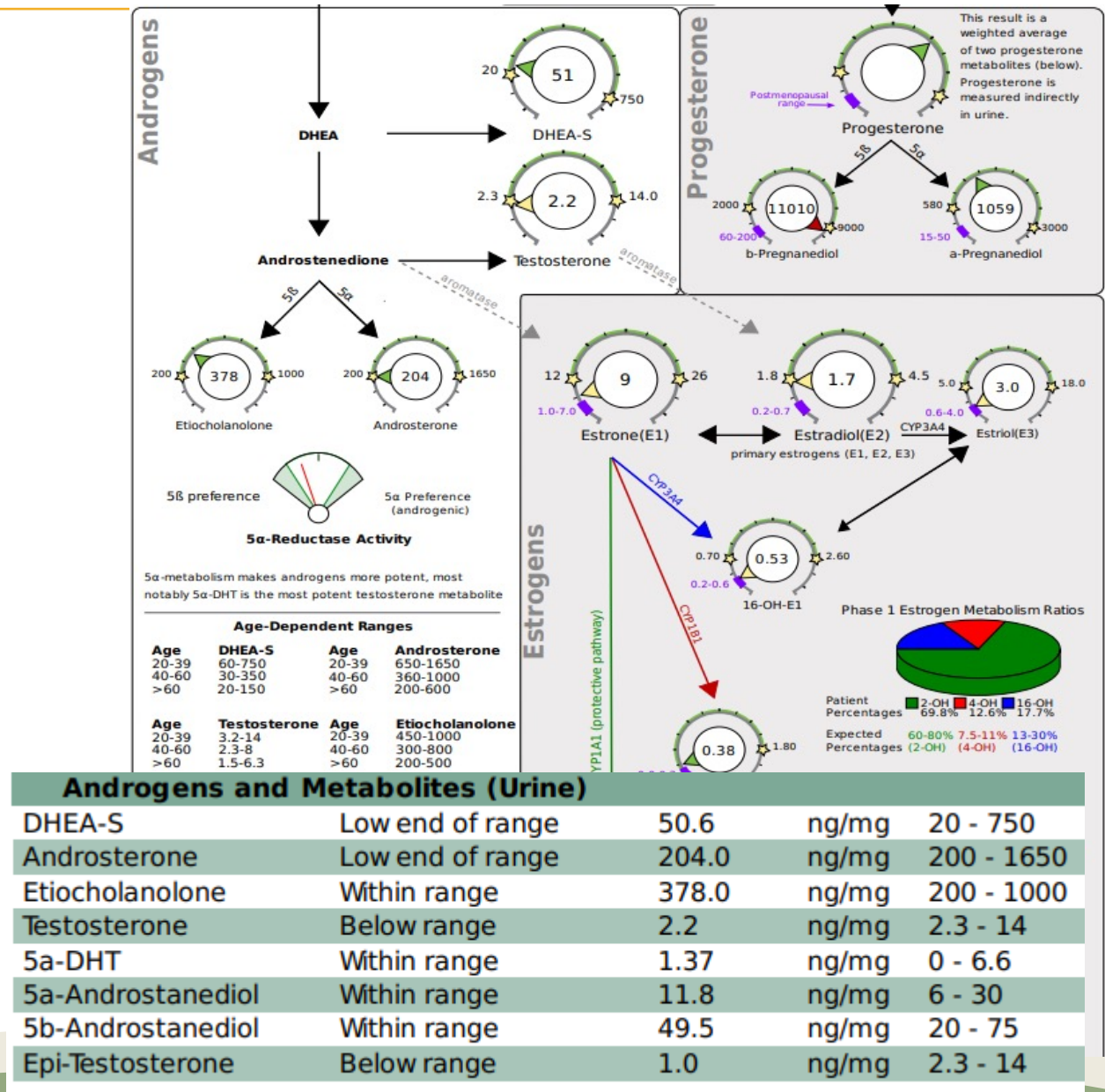
Postmenopausal (PMP)

Supplementing:

- Progesterone 200 mg
- Estradiol TD 0.075 mg patch

We can glean:

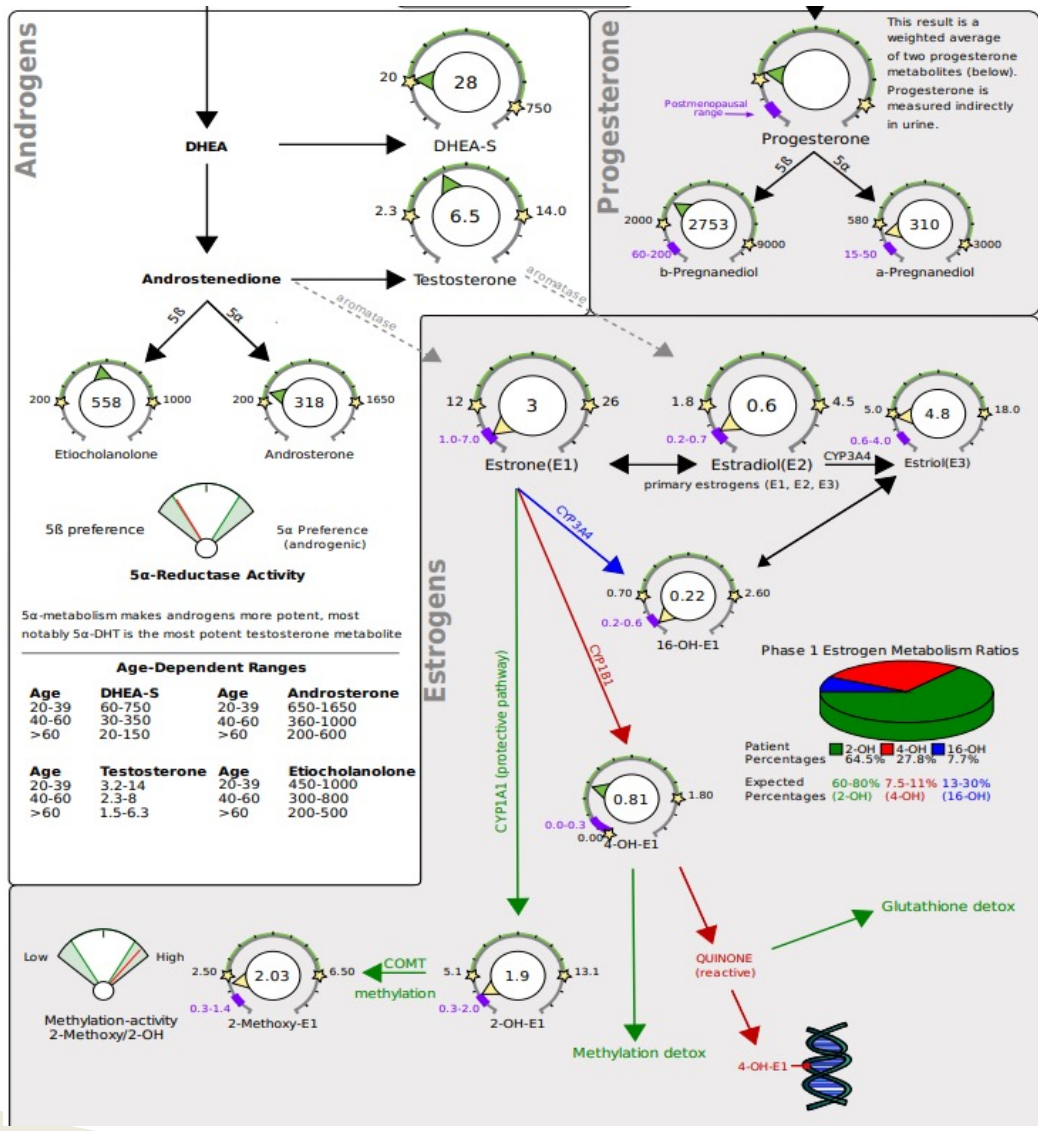
- Testosterone and DHEA (androgens) are low/low normal
- Beta-metabolizer
- Androgen metabolites are low normal



# PMP F with Biest TD and Progesterone HRT

NEXT CASE

# Case Studies and Examples of HRT on DUTCH



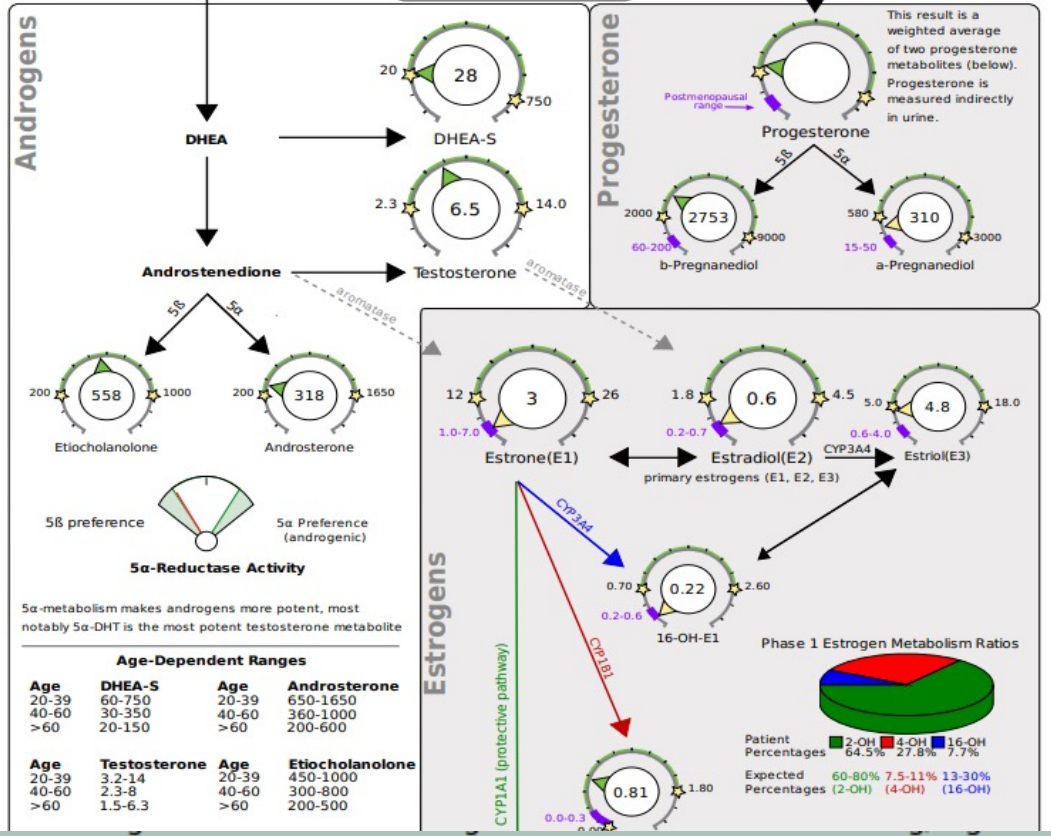
PMP F, uterus intact, 76 yo  
Supplementing:

- Biest – E3/E2 topical (1.5/0.5mg)
- Progesterone 100 mg

## Key Points:

- Progesterone: b-preg preference
- E2 is at 0.6 (Goal is 0.7-1.8)
- E3 just below luteal range
- 4-OH-E1 is HIGH
- \*not getting effective E2 dosing for research supported bone health
- \*not achieving appropriate progesterone dose for endometrial protection

# Case Studies and Examples of HRT on DUTCH



PMP F, uterus intact, 76 yo  
 Supplementing:

- Biest – E3/E2 topical (1.5/0.5mg)
- Progesterone 100 mg

## Key Points:

- Androgens:
- Robust Testosterone
- Low/Normal DHEA

Above range 5a/5b androstenediol!

- Beta preference for androgens

Androgens and Metabolites (Urine)				
DHEA-S	Low end of range	27.7	ng/mg	20 - 750
Androsterone	Low end of range	318.0	ng/mg	200 - 1650
Etiocholanolone	Within range	558.0	ng/mg	200 - 1000
Testosterone	Within range	6.5	ng/mg	2.3 - 14
5a-DHT	Within range	4.67	ng/mg	0 - 6.6
5a-Androstenediol	Above range	31.8	ng/mg	6 - 30
5b-Androstenediol	Above range	177.3	ng/mg	20 - 75
Epi-Testosterone	Below range	1.2	ng/mg	2.3 - 14

# PMP F with Biest (?) and Progesterone HRT

NEXT CASE



# Case Studies and Examples of HRT on DUTCH

PMP F, no ovaries, unsure if she has a uterus, 71 yo

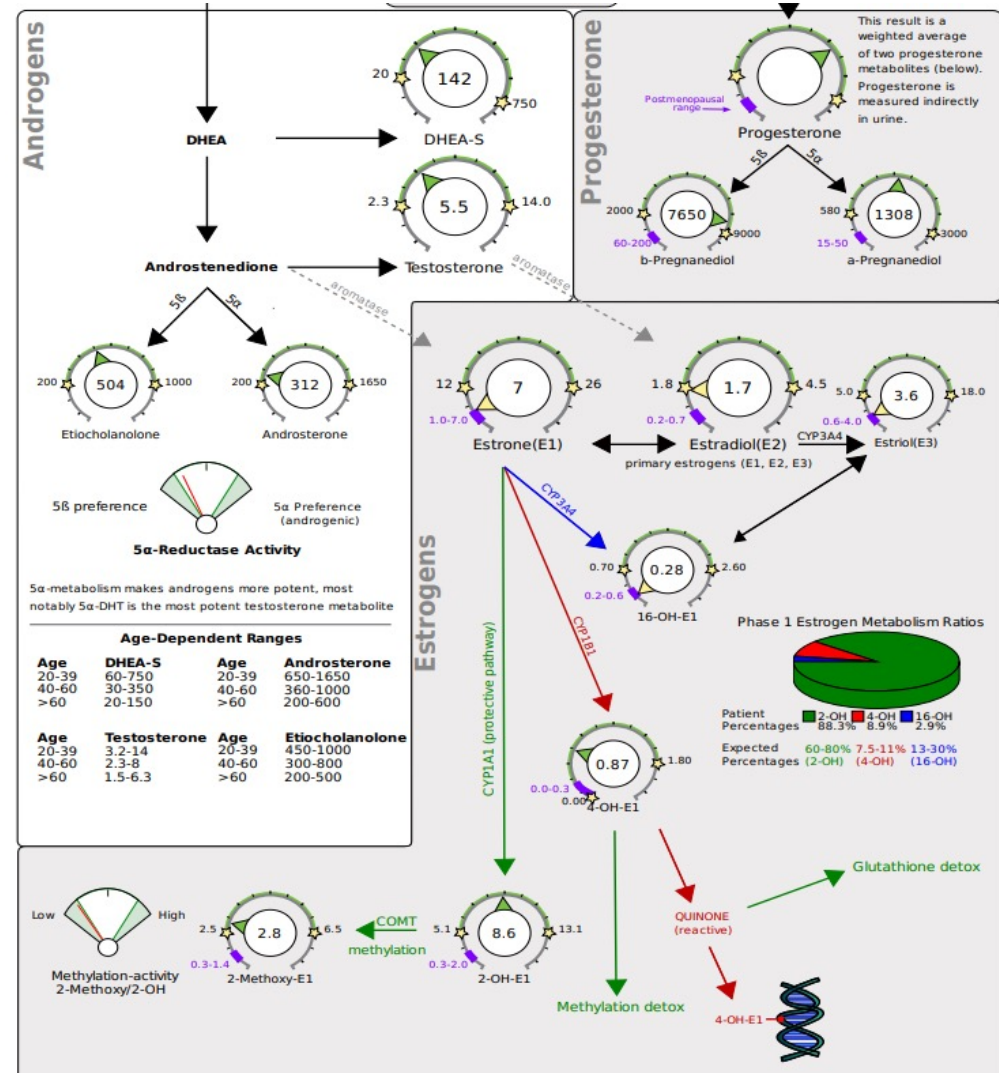
Supplementing:

- Biest: E3:E2 (2:0.5): 2 mg
  - Unsure of ROA (likely TD)
- Progesterone 200 mg



## Key Points:

- E2 is at 1.7 (Goal: 0.7-1.8)
- Progesterone: b-preg preference
- E metabolism: phase I is great, phase II is low/poor



# PMP F E2 Pellet, T pellet

NEXT CASE

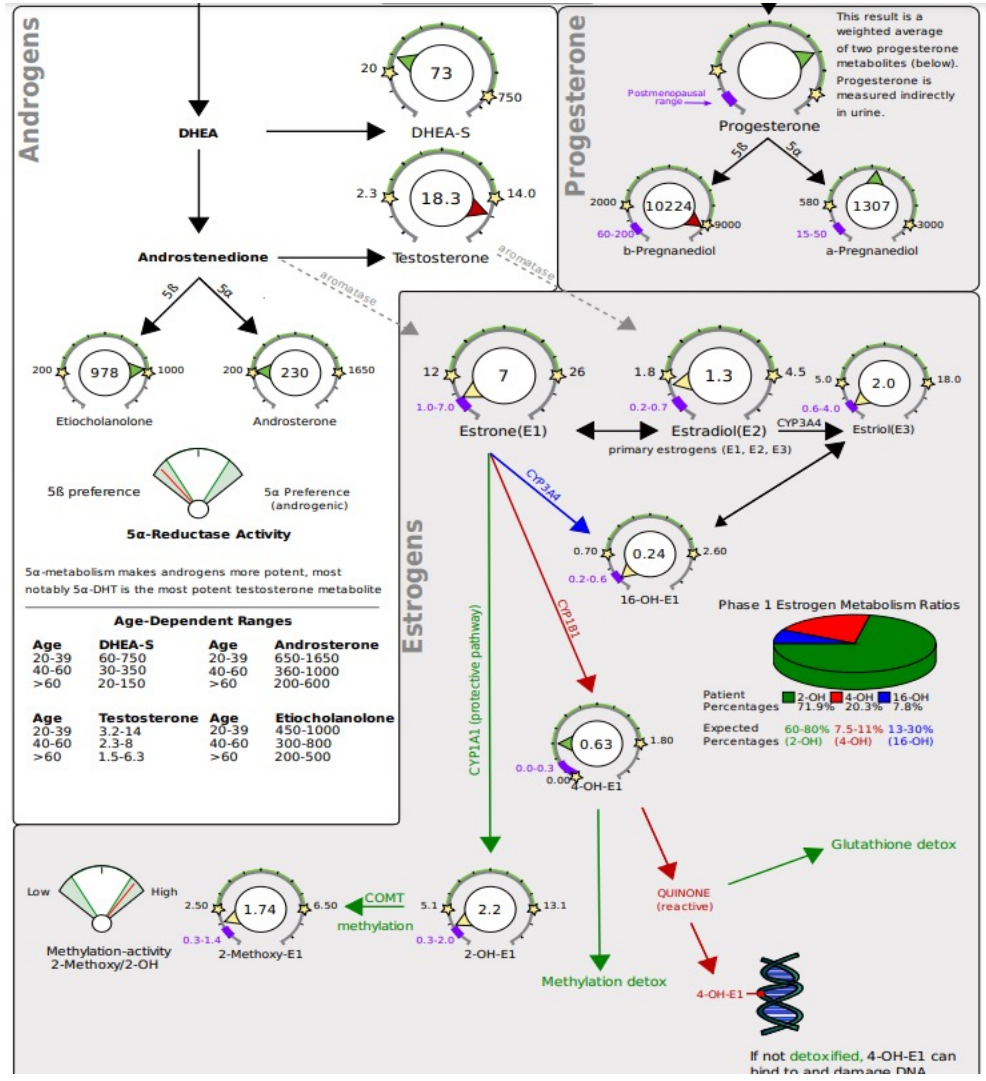
# Case Studies and Examples of HRT on DUTCH

PMP F, 53 yo, uterus intact  
 Supplementing:

- E2 Pellet
- T Pellet
- Progesterone 100 mg

## Key Points:

- E2: is at **1.3** (Goal 0.7-1.8)
- T: 18.3
- Progesterone: beta preference
- E metabolism: **HIGH 4-OH**



# Case Studies and Examples of HRT on DUTCH

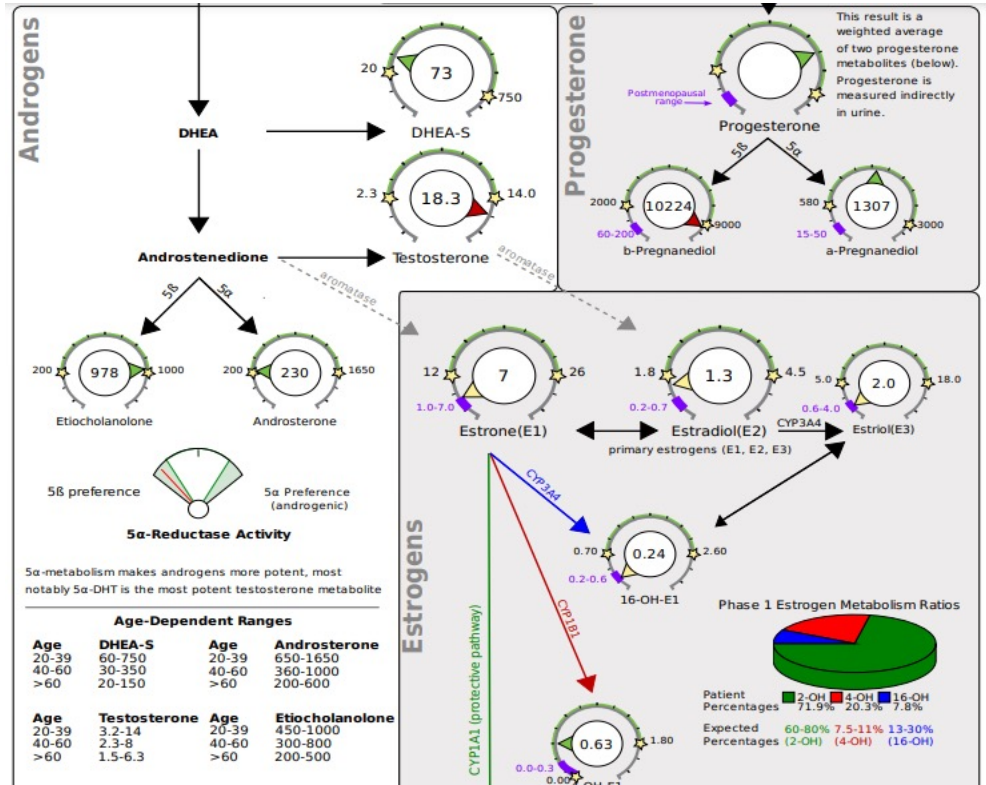
PMP F, 53 yo, uterus intact

Supplementing:

- E2 Pellet
- T Pellet
- Progesterone 100 mg

## Key Points:

- Androgens:
- Testosterone above range
- Epi-T below range
- DHEA-s/Androsterone low end
- Beta metabolizer



Androgens and Metabolites (Urine)				
DHEA-S	Low end of range	73.0	ng/mg	20 - 750
Androsterone	Low end of range	230.0	ng/mg	200 - 1650
Etiocholanolone	High end of range	978.0	ng/mg	200 - 1000
Testosterone	Above range	18.3	ng/mg	2.3 - 14
5a-DHT	Within range	1.51	ng/mg	0 - 6.6
5a-Androstanediol	Within range	23.6	ng/mg	6 - 30
5b-Androstanediol	Within range	45.8	ng/mg	20 - 75
Epi-Testosterone	Below range	0.7	ng/mg	2.3 - 14

# Case Studies and Examples of HRT on DUTCH

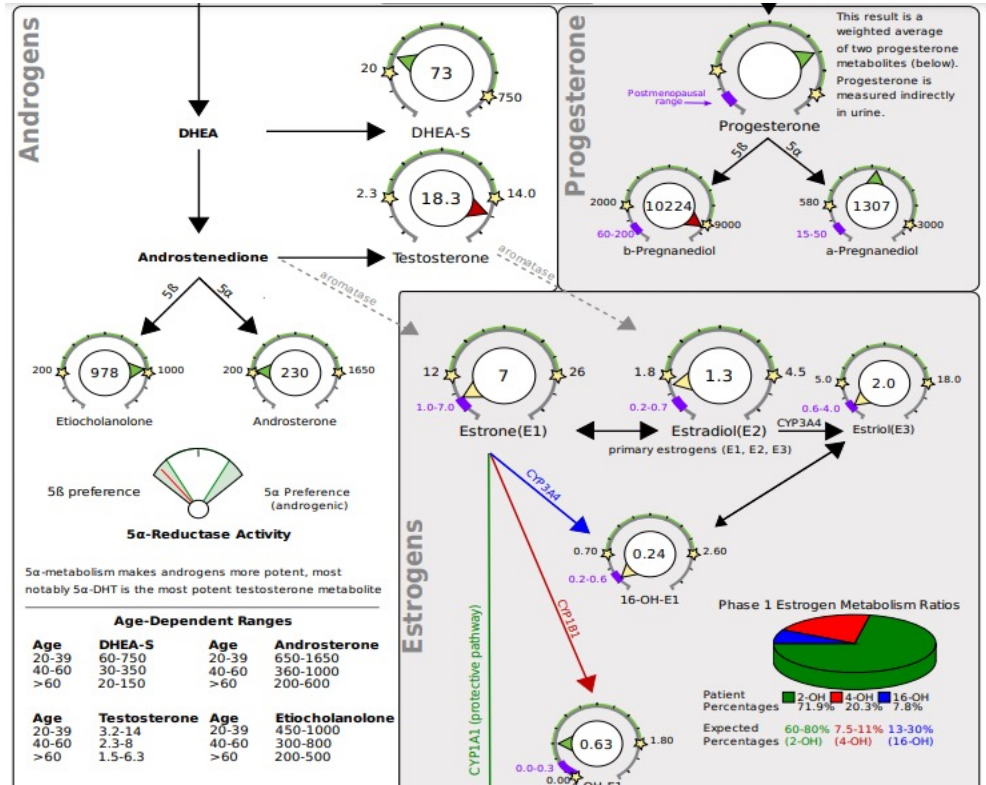
PMP F, 53 yo, uterus intact

Supplementing:

- E2 Pellet
- T Pellet
- Progesterone 100 mg

## Key Points:

- Testosterone:
- It is preferable to utilize serum testing in combination with DUTCH testing for Testosterone
- DUTCH testing augments information with metabolomics



Androgens and Metabolites (Urine)				
DHEA-S	Low end of range	73.0	ng/mg	20 - 750
Androsterone	Low end of range	230.0	ng/mg	200 - 1650
Etiocholanolone	High end of range	978.0	ng/mg	200 - 1000
Testosterone	Above range	18.3	ng/mg	2.3 - 14
5a-DHT	Within range	1.51	ng/mg	0 - 6.6
5a-Androstanediol	Within range	23.6	ng/mg	6 - 30
5b-Androstanediol	Within range	45.8	ng/mg	20 - 75
Epi-Testosterone	Below range	0.7	ng/mg	2.3 - 14

# DUTCH, HRT, and Testosterone

## MONITORING (B)HRT WITH LAB TESTING



Can serum or DUTCH, as a standalone test, effectively monitor HRT? ✓ Yes ✗ No ? Maybe

Urine testosterone does not correlate as reliably to T serum values, compared to E2 and other tests. Urine testing is best suited as a complementary test to serum for T and should not be used solely for TRT decisions

Oral Progesterone (OMP)	Estradiol (E2) Patches	E2 Gels & Creams (Skin)	Vaginal E2 & Testosterone (T)	Vaginal Progesterone (Pg)	Transdermal (TD) Testosterone	Testosterone Injections & Pellets
✓ DRIED URINE	✓ DRIED URINE	✓ DRIED URINE	✓ DRIED URINE	✗ DRIED URINE	? DRIED URINE	? DRIED URINE
<p>The DUTCH Test® provides useful feedback when using OMP in women with PMP sleep disturbances. 5a (more active) and 5b metabolites are measured to individualize OMP dosing. OMP's sleep effects are via its 5a metabolites, predominately allopregnanolone binding to the GABA receptor.</p> <p>No lab test reflects OMP's effect on the endometrium.</p>	<p>Values between the top of the postmenopausal range and the lower limit 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 because in addition to metabolites, dried urine averages out the daily up and down E2 patterns. This is particularly helpful with gels and creams that may have serum values that change rapidly over time.</p> <p>The aggregate clinical data suggests that a serum (LC-MS/MS) E2 level of ~20-40pg/mL improves clinical outcomes (VMS, VVA, BMD). This approximates a DUTCH value of ~ 0.7-1.8ng/mg.</p>	<p>The DUTCH Test® is unique in that it removes potential contamination, and monitoring is helpful with E2 and T.</p> <p>Very low doses may impact local tissue without increasing lab values. For local (not systemic) E2 therapy, keep urine E2 in PMP range.</p>	<p>The DUTCH Test® is unique in that it removes potential contamination, and monitoring is helpful with E2 and T.</p> <p>Very low doses may impact local tissue without increasing lab values. For local (not systemic) E2 therapy, keep urine E2 in PMP range.</p>	<p>Pg is measured indirectly in urine by measuring pregnenediols. These metabolites may be underrepresented when Pg is taken vaginally. Serum Pg seems to increase to a higher degree than urine metabolites with vaginal Pg application.</p>	<p>Levels generally parallel changes in serum and clinical outcomes (increased lean body mass, erythrocytosis, etc. in men). Epi-testosterone (Epi-T) values can be used to assess gonadal suppression due to TRT (Epi-T levels in men decrease as TRT increases and are &lt;10ng/mg with complete suppression).</p>	<p>Injections and pellets increase levels, as expected, but the increase may exceed what is seen in serum testing. DUTCH allows for monitoring both the dosing of hormones as well as metabolic patterns.</p>
✗ SERUM	✓ SERUM	? SERUM	✓ SERUM	? SERUM	✓ SERUM	✓ SERUM
<p>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.</p>	<p>Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion.</p> <p>Most recommendations are to push serum E2 levels to 20-40pg/mL for clinical impact.</p>	<p>The only published data for E2 creams shows serum results move up and down within a few hours, so serum testing can easily underestimate clinical impact. DUTCH results average out the daily up and down pattern and may be a better option.</p>	<p>Serum results rise quite dramatically with what may seem like modest doses due to the high uptake of hormones across the mucosal membrane. However, values may rise and fall quickly, so be careful with the interpretation of both low and high results.</p>	<p>Serum values increase with dosing and likely represent systemic exposure to Pg. However, the uterine first-pass effect loads the uterus with high levels of Pg (which may be desirable) and serum does not reflect uterine hormone levels.</p>	<p>A great deal of published research shows that serum levels reflect clinical changes in both men and women taking TD T. Be aware of potential up and down patterns throughout the day, but serum is the best tool for monitoring doses of TD T in both men and women.</p>	<p>Serum testing is well suited for use with these types of therapies. Results increase with increased dosing in a fairly linear fashion.</p> <p>Test injections halfway between doses or right before a dose.</p>
✗ SALIVA	<p>The literature does not support salivary testing's use for monitoring TD hormone creams. The saliva data is limited and, in fact, there are no saliva testing outcome studies using TD creams, injections, estradiol patches, oral estradiol, or vaginal hormones. While salivary testing is the gold standard for free cortisol measurement, avoiding its use for monitoring HRT is advised. For situations where saliva testing may parallel the clinical impact, DUTCH or serum testing are better options (see above).</p>					
✗ Oral Estradiol, Estradiol Pellets, or Sublingual Hormones	<p>Though not recommended, if you choose to use either oral estradiol or estradiol pellets, serum testing can monitor both, whereas urine should only be used with pellet therapy. Sublingual hormones may be used in some situations but lab monitoring is not helpful in optimizing doses.</p>					
✗ Transdermal Progesterone	<p>In PMP women, the evidence does not support TD Pg's use to protect the endometrium. When prescribed, laboratory monitoring is not helpful for TD Pg dosing.</p>					

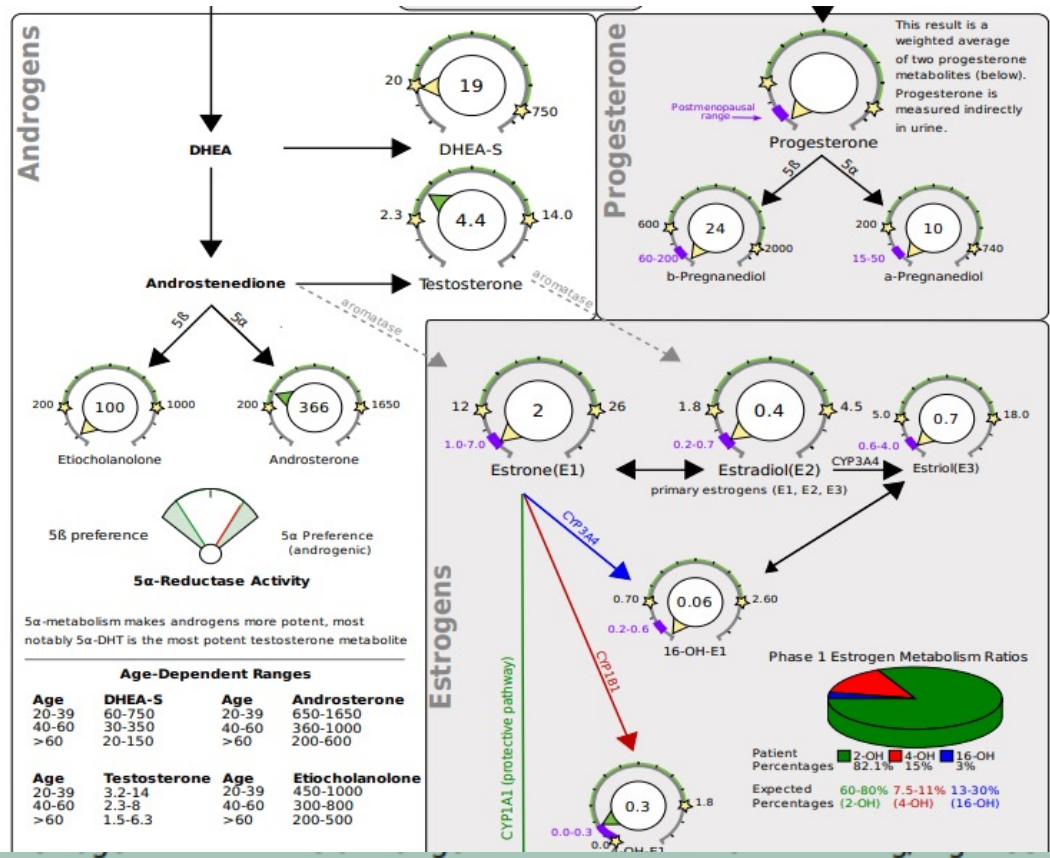
# PMP F Testosterone

NEXT CASE





# Case Studies and Examples of HRT on DUTCH



PMP F 63 yo, hysterectomy  
Supplementing:

- Testosterone TD 2.5 mg

## Key Points:

- Androgens:
- Alpha preference!
- 5a-androstanediol, 5a-DHT are within range
- All others are low or below range

Androgens and Metabolites (Urine)				
DHEA-S	Below range	19.0	ng/mg	20 - 750
Androsterone	Low end of range	366.0	ng/mg	200 - 1650
Etiocholanolone	Below range	100.0	ng/mg	200 - 1000
Testosterone	Low end of range	4.4	ng/mg	2.3 - 14
5a-DHT	Within range	2.46	ng/mg	0 - 6.6
5a-Androstanediol	Within range	23.7	ng/mg	6 - 30
5b-Androstanediol	Below range	12.2	ng/mg	20 - 75
Epi-Testosterone	Below range	0.8	ng/mg	2.3 - 14

TD Testosterone may be well reflected on DUTCH testing, but...correlate with serum values

# Non Cycling F Oral/SL Progesterone Topical E3

NEXT CASE

# Case Studies and Examples of HRT on DUTCH

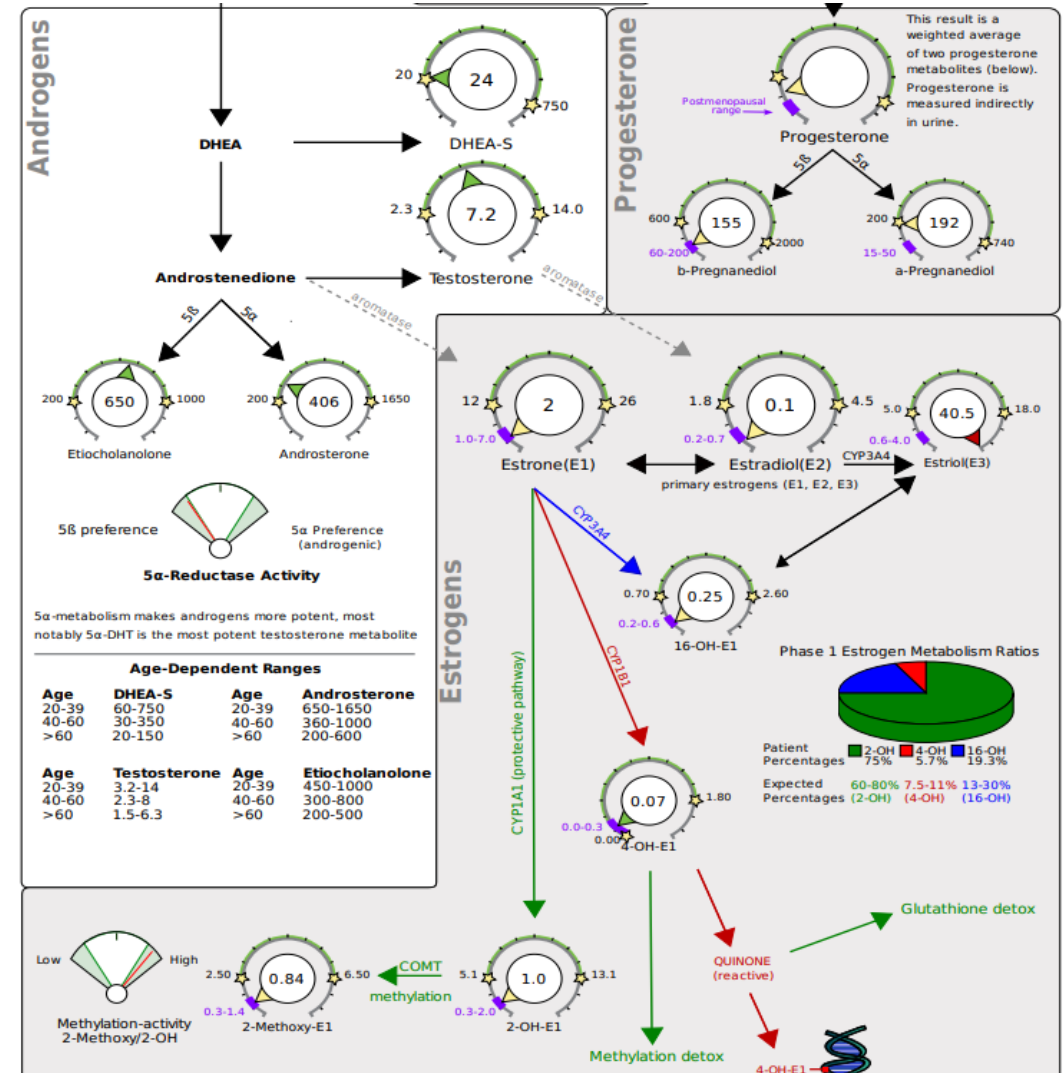
Non Cycling F 43 yo, uterus intact

Supplementing:

- Progesterone 32 mg topical
- Bio-estrogen 1 mg cream (vaginal)

## Key Points:

- Progesterone: alpha preference!
- Estrogens: E3 robust (vaginal dryness)
- E Metabolism: GOOD!



# Irregular Cycling F – all sorts of supplementation 😊

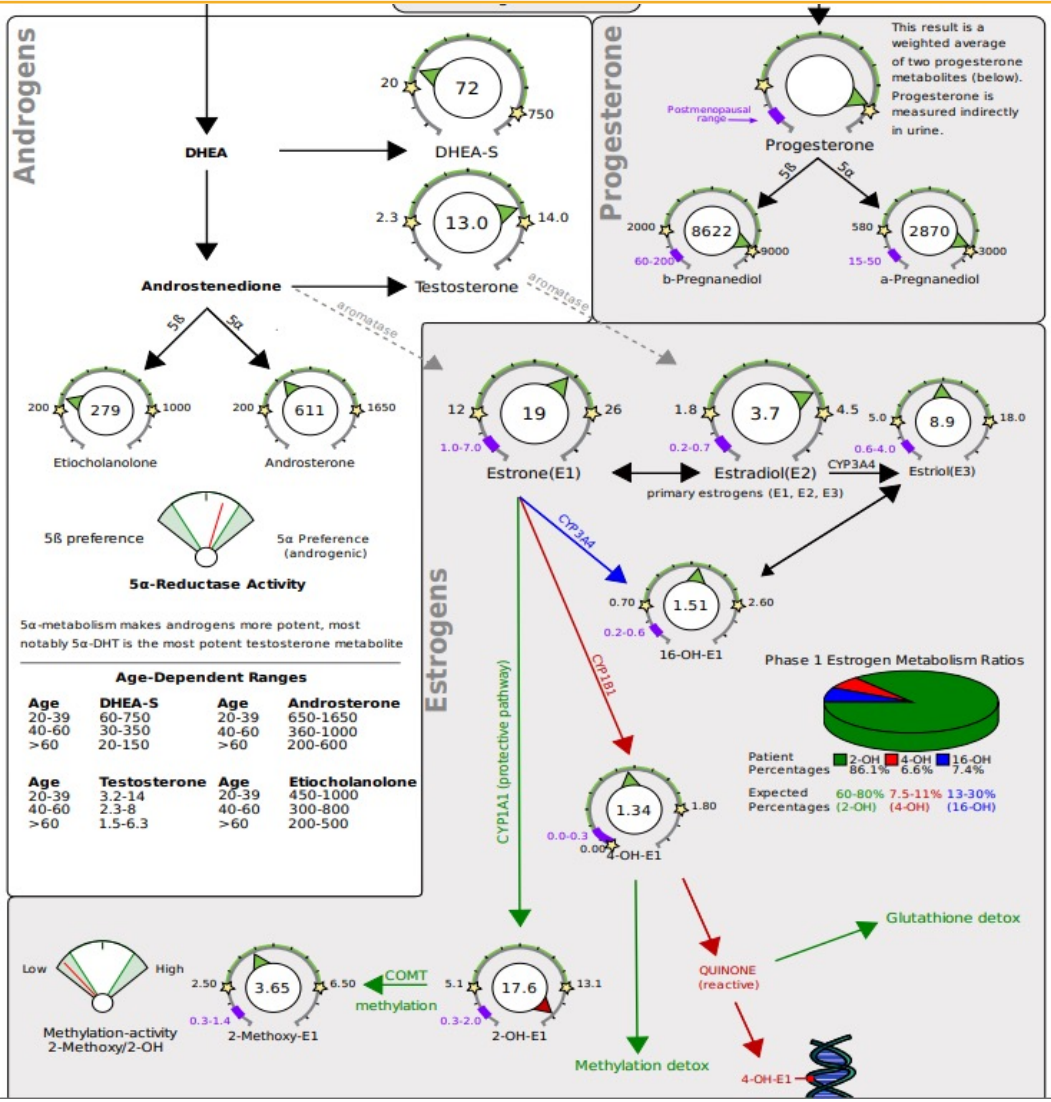
NEXT CASE

# Case Studies and Examples of HRT on DUTCH

46 yo F, irregular cycles

Supplementation:

- DHEA 5 mg oral
- Progesterone 200 mg oral
- Biest-Testosterone topical
- Biest-E2 topical
- Biest-E3 topical



# Thank You!

For questions, contact:

[info@dutchtest.com](mailto:info@dutchtest.com)

(503) 687-2050

[www.dutchtest.com](http://www.dutchtest.com)

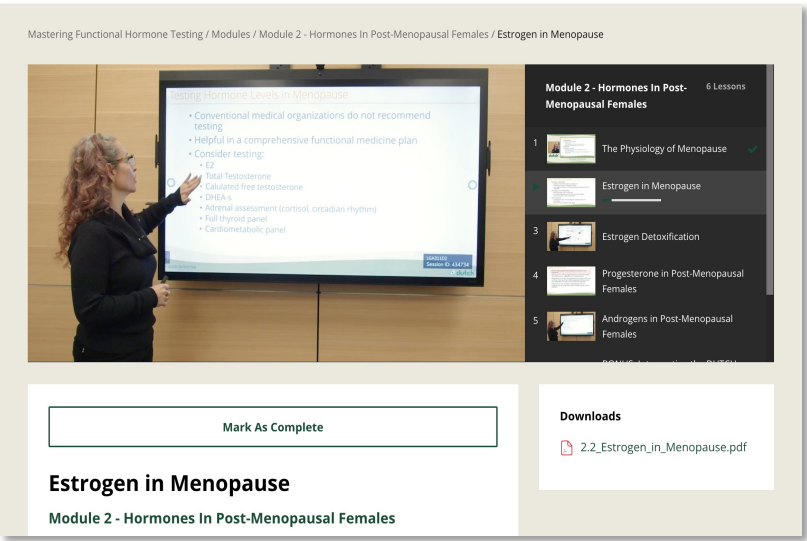


## Exclusive Hormone Education for DUTCH Providers

### DUTCH Interpretive Guide



### Mastering Functional Hormone Testing Course



### Group Mentorship Sessions



Click the Link Below to Become a DUTCH Provider Today!