

# Testing Hormone Metabolites and Neurotransmitters: Considerations for Comprehensive Patient Support

Lylen Ferris, ND

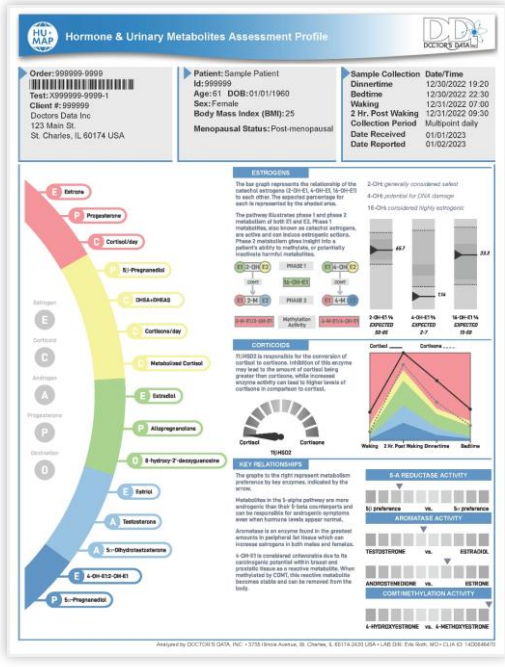


## HuMap™ + Neurotransmitter (NT) testing

- Patients are complex. Similar symptoms can be the result of many different, and individualized, neurotransmitter and hormone imbalances.
- Mental health problems are not isolated in the brain alone. There are many factors affecting our mental wellbeing and by assessing neurotransmitters and reproductive and adrenal hormones we can get a more well-rounded understanding of how nutrition, digestion, inflammation, stress, metabolism, and methylation may be contributing to the symptom picture.
- Test in order to determine which therapeutics are appropriate.

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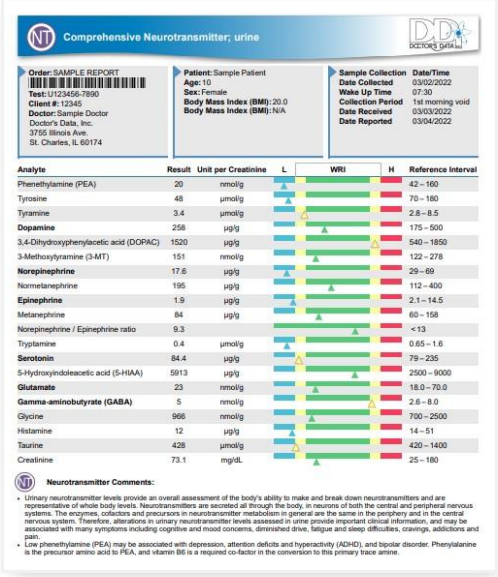
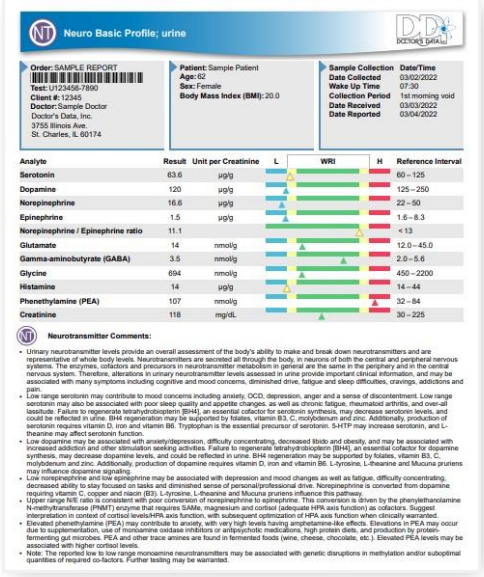
# HuMap™

## Hormone & Urinary Metabolites Assessment Profile

- Extensive estrogens and metabolites: E1, E2, E3, 2-OH-E1/E2, 4-OH-E1/E2, 16-OH-E1, 2-Methoxy-E1/E2, 4-Methoxy-E1/E2
- Comprehensive metabolites of progesterone and androgens
- Diurnal cortisol and cortisone
- Metabolized cortisol, cortisone, corticosterone
- Key enzyme activity: COMT-11βHSD2, aromatase, 5α-reductase
- Biomarker for oxidative DNA damage: 8-OHdG
- Abbreviated profiles available to monitor treatment response



# Neuro Basic or Comprehensive Neurotransmitter



## Road Map

- Introduction to the HuMap™
- What are urinary hormone and metabolites?
- What is involved in metabolism of sex hormones?
- Major hormones and metabolites to pay attention to in your practice
- 8-OhdG and its role in oxidative stress
- Influences of hormones on neurotransmitters and vice versa – the benefits of testing together

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## What is LC-MS/MS?

- Liquid chromatography (LC) tandem mass spectrometry (MS)
- LC separates hormones in a liquid urine sample, which are then injected at various times for MS analysis.
  - MS technology monitors the injected sample for specific hormones based on their molecular weights and expected injection times (commonly called retention times).
  - Tandem MS verifies each hormone identity based on fragmentation and determines its amount.
- **The combination of LC and tandem MS allows for extremely sensitive and specific hormone measurements, even in samples containing similar substances that would interfere with other methods.**

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## STEP 3: Collect Sample

BEFORE YOU BEGIN COLLECTING SAMPLE:  
Write the Patient's Name, Date of Birth and the Date Collected on each Specimen Vial.

Day 1 Collection	Midsleep	Day 2 Collection
#1 Dinnertime: collect within 1-2 hours prior to eating	Midsleep: collect only if you wake to urinate while sleeping	#3 Waking: collect within 10 mins of waking
#2 Bedtime: collect within 1 hour prior to sleep		#4 Post-waking: collect after 2-4 hours

1. Collect a midstream specimen of urine using 1 of the 5 collection cups provided. If needed, you may reuse these cups.
2. Pipette or pour urine into the properly labelled tube to the "fill line" marked on the tube label. Any remaining urine in the collection cup can be discarded in the toilet. DO NOT urinate directly into the tube, which contains an acid preservative\* that may irritate the skin.
3. Close tube and rock gently.
4. Record your NAME and the DATE and TIME OF COLLECTION on the tube, and on the Requisition Form.
5. Place the urine tube back into the original plastic bag containing the absorbent pad. Store the urine in the freezer with the frozen ice pack.

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## WHAT DAYS OF THE MONTH SHOULD I COLLECT?

Men and post-menopausal women	Women with menstrual cycles	Irregular Menstrual Cycles
Collect any day	Begin the collection between days 19 and 23 of a 28-day cycle, counting the first day of your period as day 1.	If cycles are irregular, contact your practitioner for clarity about the timing of your collection.

## DISCONTINUE THE FOLLOWING

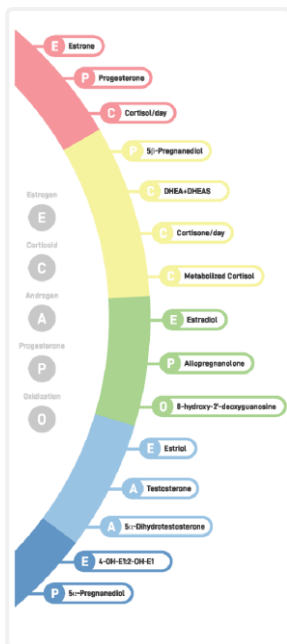
5 days before and during test	<p>Cortisol/glucocorticoid supplementation:</p> <ul style="list-style-type: none"> <li>Certain medications such as asthma inhalers and hydrocortisone contain cortisol. To evaluate natural cortisol production, it is recommended to stop cortisol containing products 5 days prior to sample collection.</li> </ul> <p>Consult your practitioner for specific instructions before stopping any medications. Never discontinue prescription medications without first consulting your doctor.</p>
3 days before and during test	<p>If using hormonal supplementation:</p> <ul style="list-style-type: none"> <li>Discontinue oral and sublingual hormones for 72 hours before and during test.</li> <li>Do not use hormones vaginally, as this may directly contaminate the urine.</li> <li>Other hormones such as topical creams and gels, injectables, and patches can be used on your regular schedule.</li> <li>Do not skip doses of oral birth control pills unless instructed by your healthcare provider.</li> </ul> <p>Note: A baseline hormone level may require supplement usage to stop for a longer period of time. Call your health practitioner for more information.</p>
24 hours before and during test	<p>Avoid alcohol, caffeine, tobacco or nicotine-containing products and strenuous exercise.</p> <p>Avoid these foods: avocados, eggplant, tomatoes, bananas, melons, pineapple, grapefruit, plums, fruit juice, nuts, nut butters, wine, cheese, rice, and chocolate.</p>
Day of Collection	<p>Avoid all supplements and medications until after all samples have been collected (including those that regulate allergy, mood, sleep, pain and inflammation).</p> <p>Consult your practitioner for specific instructions before stopping any medications. Never discontinue prescription medications without first consulting your doctor.</p>

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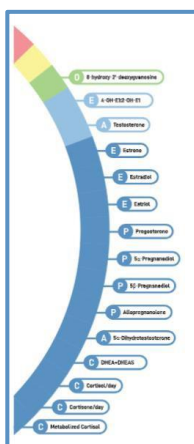






Important, clinically relevant markers are color-coded to respond to an individual's imbalances, providing an at-a-glance format on the summary page.

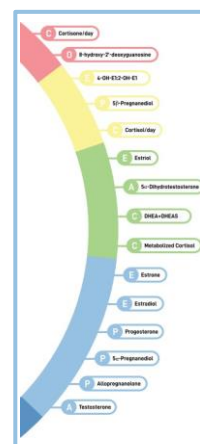
Patient #1



Patient #2



Patient #3



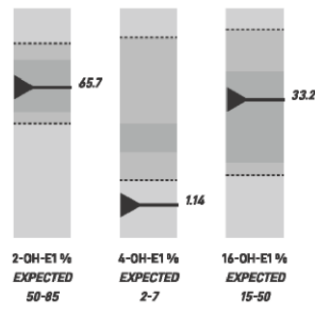
### ESTROGENS

The bar graph represents the relationship of the catechol estrogens (2-OH-E1, 4-OH-E1, 16-OH-E1) to each other. The expected percentage for each is represented by the shaded area.

The pathway illustrates phase I and phase 2 metabolism of both E1 and E2. Phase 1 metabolites, also known as catechol estrogens, are active and can induce estrogenic actions. Phase 2 metabolism gives insight into a patient's ability to methylate, or potentially inactivate harmful metabolites.



2-OH: generally considered safest  
4-OH: potential for DNA damage  
16-OH: considered highly estrogenic



Comparing Phase I vs Phase II estrogen metabolism, methylation activity, and percentage of 2-OH, 4-OH, and 16-OH



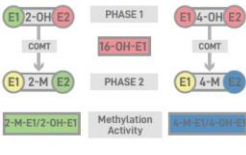
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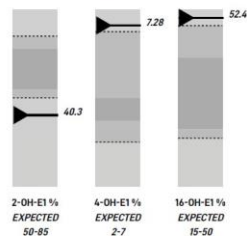
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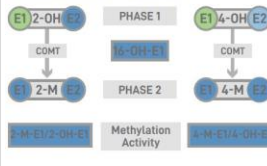
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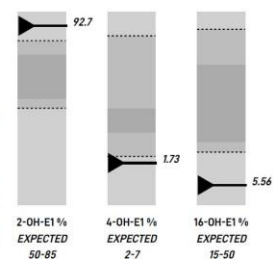
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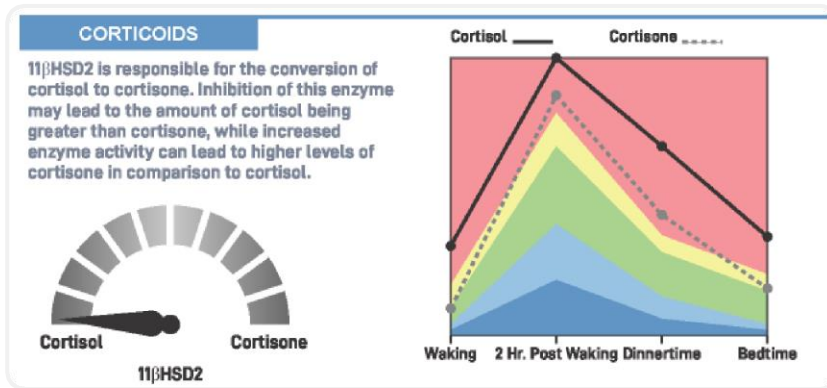
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Cortisol and cortisone are plotted on the same graph to quickly identify their relationship to each other.



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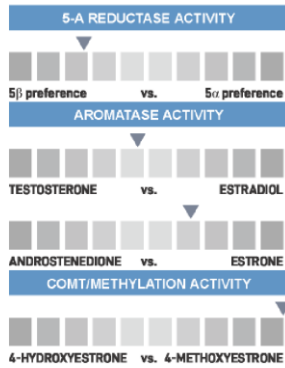
**KEY RELATIONSHIPS**

The graphs to the right represent metabolism preference by key enzymes, indicated by the arrow.

Metabolites in the 5-alpha pathway are more androgenic than their 5-beta counterparts and can be responsible for androgenic symptoms even when hormone levels appear normal.

Aromatase is an enzyme found in the greatest amounts in peripheral fat tissue which can increase estrogens in both males and females.

4-OH-E1 is considered unfavorable due to its carcinogenic potential within breast and prostatic tissue as a reactive metabolite. When methylated by COMT, this reactive metabolite becomes stable and can be removed from the body.



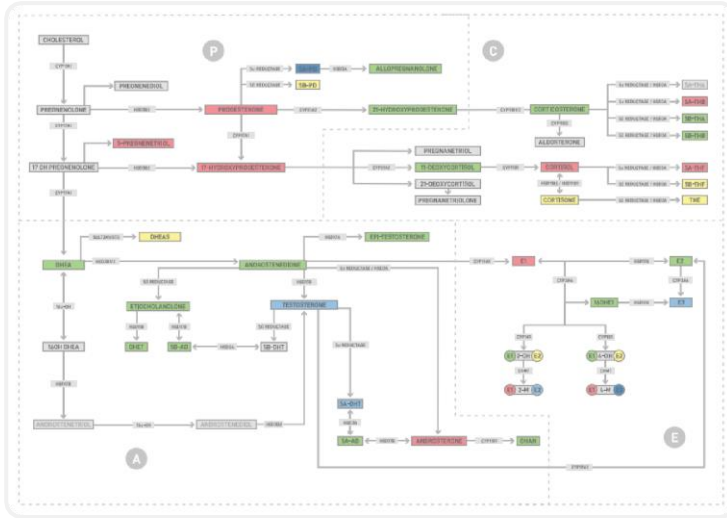
Important relationships are highlighted here, representing metabolism preference by key enzymes.

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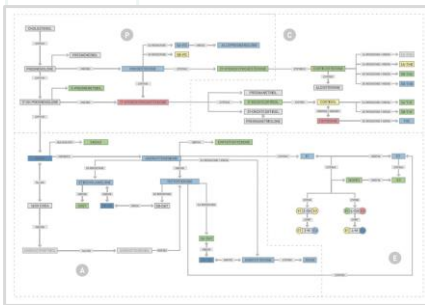
Dynamic color-coded overview of the entire hormone steroid cascade displaying actual results for your patient. Colors are used to easily identify highs and lows for each metabolite.



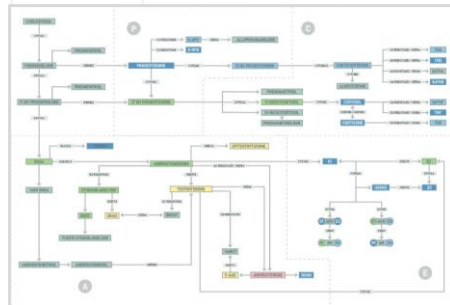
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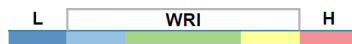
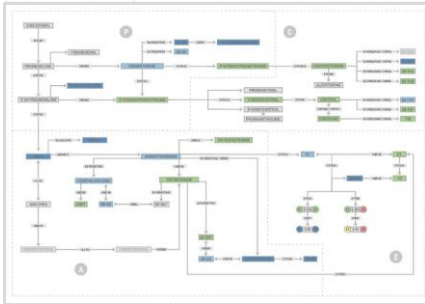
Patient #1



Patient #3



Patient #2



WRI – Within Reference Interval - represented by bracket and stated ranges on report

- Dark Blue = Below RI
- Light Blue = WRI low
- Green = Optimal
- Yellow = WRI high
- Red = Above RI

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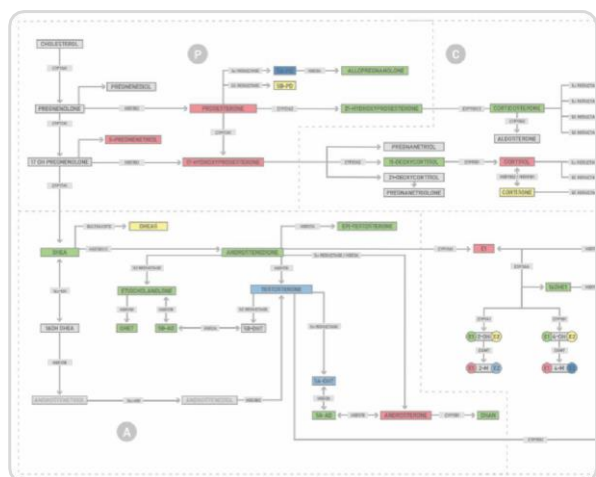
Can't see  
the forest  
for the  
trees...



- Utilizing urinary hormone testing requires the practitioner to take a step back and look at the pattern of hormone secretion
- Metabolism is complex and will be different for every individual
- Attempting to bring every analyte within range is not necessarily the goal of this type of testing

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## Postmenopausal women who are supplementing

- For postmenopausal women who are supplementing hormones, premenopausal reference ranges are also provided to assist practitioners in assessing treatment

Progesterones		Pre-menopausal Reference Interval	Progesterones	Result	Unit	L	WRI	H	Reference Interval
Progesterone <sup>†</sup>	(P4)	0.29 – 1.6	Progesterone <sup>†</sup>	(P4)	0.453	ng/mg	Creat/Day		0 – 0.22
5α-Pregnanediol <sup>‡</sup>	(5A-PD)	50 – 505	5α-Pregnanediol <sup>‡</sup>	(5A-PD)	12.1	ng/mg	Creat/Day	▲	21 – 50
5β-Pregnanediol <sup>‡</sup>	(5B-PD)	375 – 2210	5β-Pregnanediol <sup>‡</sup>	(5B-PD)	121	ng/mg	Creat/Day	▲	79 – 280
Allopregnanolone <sup>‡</sup>	(ALLOP)	3.3 – 130	Allopregnanolone <sup>‡</sup>	(ALLOP)	2.55	ng/mg	Creat/Day	▲	1.4 – 4.8
21-Hydroxyprogesterone <sup>‡</sup>	(21-OHP)	0.4 – 5.6	21-Hydroxyprogesterone <sup>‡</sup>	(21-OHP)	0.639	ng/mg	Creat/Day	▲	0.3 – 1.4
5-pregnenetriol <sup>‡</sup>	(5-PT)	70 – 245	5-pregnenetriol <sup>‡</sup>	(5-PT)	46.3	ng/mg	Creat/Day	▲	35 – 120

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## Neighborhoods

- Progesterones
- Corticoids
- Androgens
- Estrogens

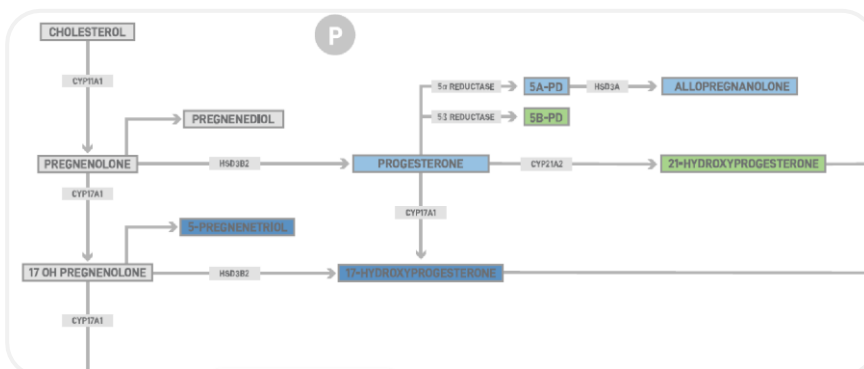


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# Progesterones



## Progesterone Neighborhood

- Progesterone
- 5-alpha pregnanediol
- 5-beta pregnanediol
- Allopregnanolone

# Functions of Progesterone

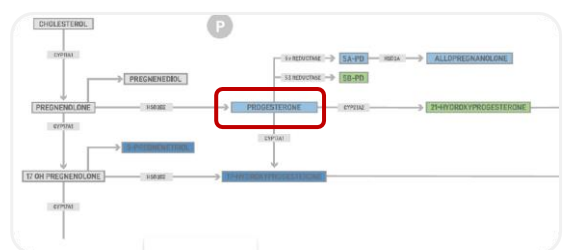
- Opposes effects of estrogen in breast, brain, and endometrial tissue
- Improves estrogen receptor sensitivity
- Essential for maintaining pregnancy
- Promotes sleep patterns (calming effect on the brain/GABA receptors)
- Reduces cholesterol
- Inhibits coronary vasospasm
- Protects against coronary hyper-reactivity
- Diuretic
- Promotes glucose utilization and improves insulin resistance
- Enhances thyroid hormone activity by decreasing thyroid binding globulin
- Increases scalp hair
- Helps to burn fat for energy
- Anti-depressant activity
- Promotes osteoblasts
- Stabilizes and induces secretory endometrial changes
- Promotes cell differentiation
- Promotes normal cell death (apoptosis)

Speroff L. *Clinical Gynecologic Endocrinology and Infertility*. Philadelphia, PA: Lippincott Williams & Wilkins; 2005.  
 Lee J. *Natural Progesterone: The Multiple Roles of a Remarkable Hormone*. 4<sup>th</sup> ed. Sebastopol, CA: BLL Publishing; 1995.



# Progesterone Metabolism

- Metabolism occurs mainly in the liver, but also in other tissues like brain and skin. PMID558037
- Major metabolites
  - 5A-PD
  - 5B-PD
- Also, an intermediate for corticosteroids
  - Corticosterone-mineral corticoid
  - Cortisone-gluocorticoid
  - Cortisol-gluocorticoid



## Progesterone Metabolites

### 5-alpha pregnanediol (5A-PD)

- 5-alpha reduction of progesterone
- Intermediate in the production of allopregnanolone
  - Research indicates 5A-PD may be a partial agonist for GABA receptor-PMID:2550257

### 5-beta pregnanediol (5B-PD)

- Major metabolite of progesterone
- Currently, research indicates this is an inactive metabolite
- Useful as an indirect measurement of progesterone in the body

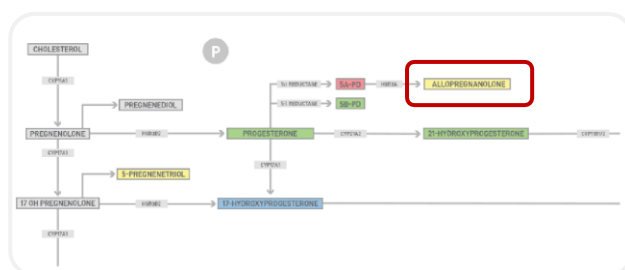
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## Allopregnenolone

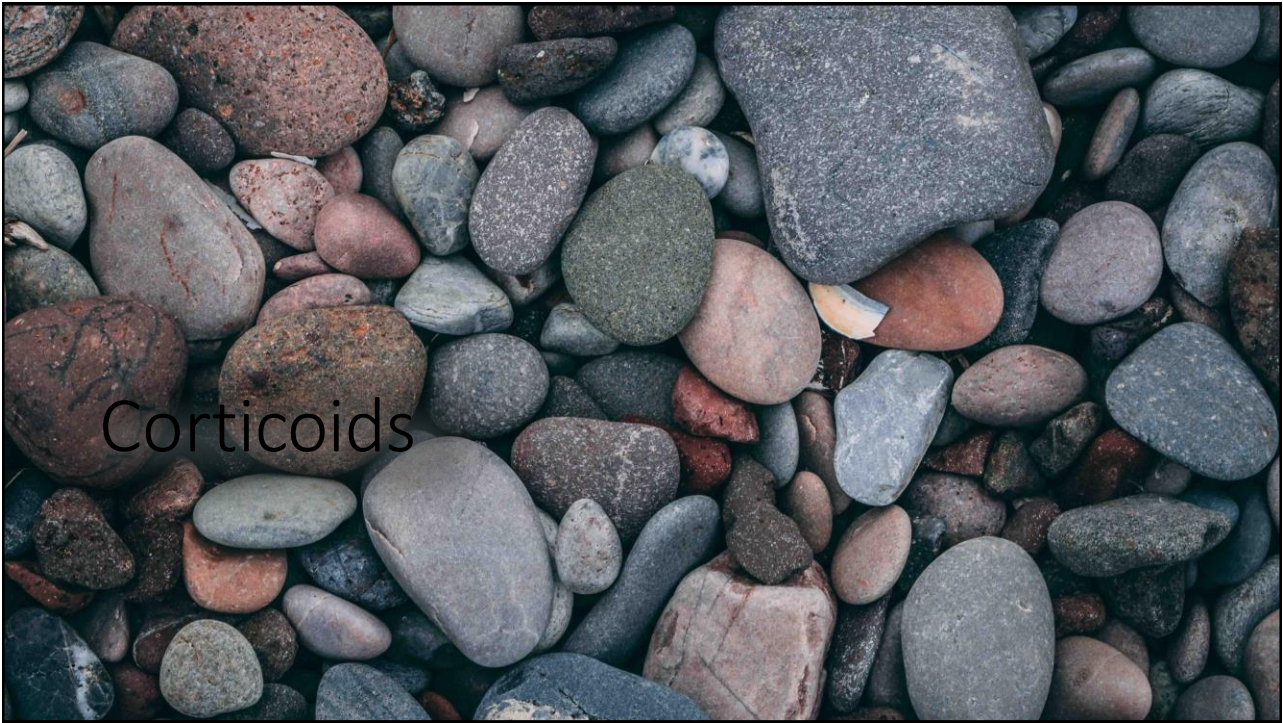
- Crosses the BBB binds the GABA receptor
- Research indicates clinical utility for mood disorders, depression, and anxiety
- Paradoxical reaction seen in PMDD-PMID 23978486
- Major metabolite from oral supplementation
- May help evaluate therapeutic oral progesterone metabolism



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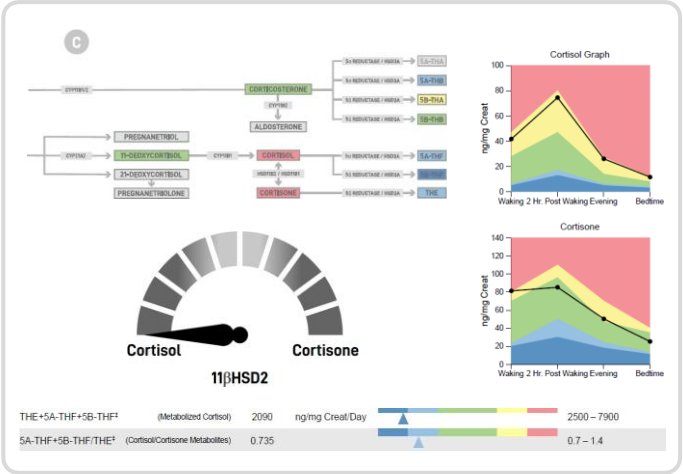




# Corticoids

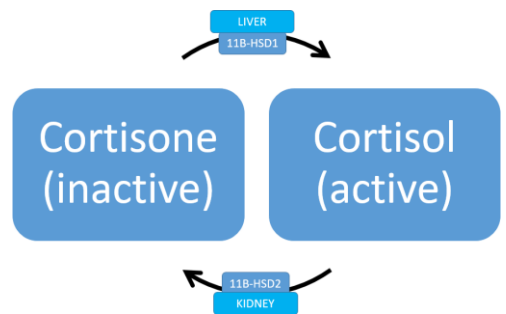
## Corticoids Neighborhood

- Cortisol vs. Cortisone
- Metabolized cortisol
- Cortisol vs. cortisone metabolites



## Cortisol vs. Cortisone

- Cortisol (active) vs. Cortisone (storage)
  - 11B-HSD enzyme: most active in the kidney
    - 11B-HSD1-pulls cortisol out of storage
      - correlated with obesity, metabolic syndrome, inflammation, hypothyroid.
    - 11B-HSD2- protects the mineral corticoid receptor from aldosterone effects
- Because we are looking at the influence of the kidney, we aren't necessarily seeing the HPA axis activity directly, rather the bodies response to stressful stimuli.



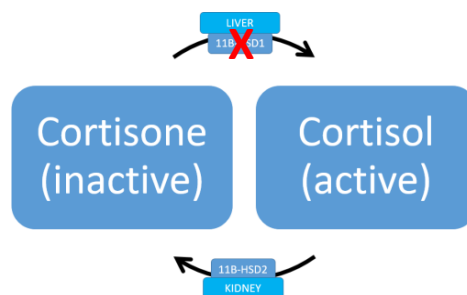
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## What downregulates 11B-HSD1 activity?

- Reduce inflammation, insulin resistance/insulin, central adiposity
- Physical activity
- Rooibos
- Holy Basil
- Curcumin
- Bitter melon
- EGCG
- Progesterone
- Coffee



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## 11B-HSD1 references

- Guilliams T. The Role of Stress and the HPA Axis in Chronic Disease Management. Point Institute; 2015.
- Chapman K, Holmes M, Seckl J. 11 $\beta$ -hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev*. 2013;93(3):1139-1206. doi:10.1152/physrev.00020.2012
- Schloms L, Smith C, Storbeck KH, Marnewick JL, Swart P, Swart AC. Rooibos influences glucocorticoid levels and steroid ratios in vivo and in vitro: a natural approach in the management of stress and metabolic disorders?. *Mol Nutr Food Res*. 2014;58(3):537-549. doi:10.1002/mnfr.201300463
- Jothie Richard E, Illuri R, Bethapudi B, et al. Anti-stress Activity of *Ocimum sanctum*: Possible Effects on Hypothalamic-Pituitary-Adrenal Axis. *Phytother Res*. 2016;30(5):805-814. doi:10.1002/ptr.5584
- Hu GX, Lin H, Lian QQ, et al. Curcumin as a potent and selective inhibitor of 11 $\beta$ -hydroxysteroid dehydrogenase 1: improving lipid profiles in high-fat- diet-treated rats. *PLoS One*. 2013;8(3):e49976. doi:10.1371/journal.pone.0049976
- Blum A, Loerz C, Martin HJ, Staab-Weijnitz CA, Maser E. *Momordica charantia* extract, a herbal remedy for type 2 diabetes, contains a specific 11 $\beta$ -hydroxysteroid dehydrogenase type 1 inhibitor. *J Steroid Biochem Mol Biol*. 2012;128(1-2):51-55. doi:10.1016/j.jsbmb.2011.09.003
- Hintzpeter J, Stapelfeld C, Loerz C, Martin HJ, Maser E. Green tea and one of its constituents, Epigallocatechine-3-gallate, are potent inhibitors of human 11 $\beta$ -hydroxysteroid dehydrogenase type 1. *PLoS One*. 2014;9(1):e84468. Published 2014 Jan 3. doi:10.1371/journal.pone.0084468
- Atanasov AG, Dzyakanchuk AA, Schweizer RA, Nashev LG, Maurer EM, Odermatt A. Coffee inhibits the reactivation of glucocorticoids by 11 $\beta$ -hydroxysteroid dehydrogenase type 1: a glucocorticoid connection in the anti-diabetic action of coffee?. *FEBS Lett*. 2006;580(17):4081-4085. doi:10.1016/j.febslet.2006.06.046

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## Additional corticoid markers

- Metabolized cortisol: (THE + 5 $\alpha$ THF + 5 $\beta$ THF)
  - (active + potential)
  - measure of what the body has utilized
- Cortisol / Cortisone metabolites: (5 $\alpha$ THF + 5 $\beta$ THF) vs. THE
  - High metabolized cortisol: increased cortisol clearance due to over production of cortisol, also influenced by; obesity, IR, inflammation, hyperthyroidism
  - Low metabolized cortisol: decreased cortisol clearance which could also be due to hypothyroidism, anorexia, decreased liver function
  - Because free levels of cortisol can be converted to cortisone in the kidney before excretion, looking at the metabolites gives clinicians a better idea of overall metabolic preference within the body

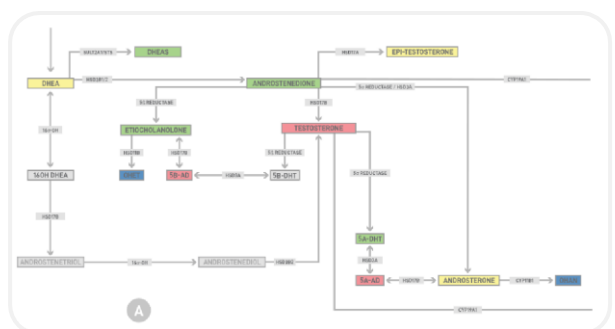
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## Androgens Neighborhood

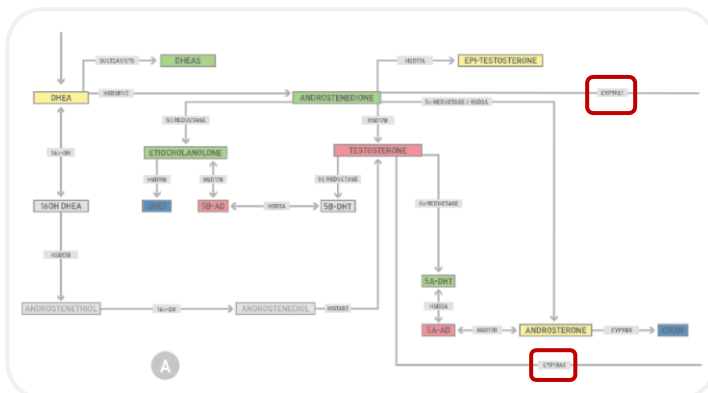
- Evaluate enzyme activity
  - (aromatase and 5 alpha reductase)
- Monitor the activity of the testosterone metabolite 5A-DHT
- 5 alpha vs 5 beta metabolism
  - (Androsterone (5 $\alpha$ ) / Etiocholanolone (5 $\beta$ ))
- DHEA + DHEAs
- Further evaluation of PCOS patients (symptoms, pathways, enzymes)



## Aromatase (CYP19A1)

Converts androstenedione to estrone and testosterone to estradiol

Active in adipose tissue



Minich, D., and R. Hodges. "Modulation of metabolic detoxification pathways using foods and food-derived components: A scientific review with clinical application." *Journal of Nutrition and Metabolism*, Vol. 2015 (2015): 760689.

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## Activation of Aromatase (CYP19A1)

- Stress/cortisol: PMID: 21878510 PMID: 23835908 (animal studies, acts in a protective manner for brain health)
- Xeno-estrogens/endocrine disruptors/toxins (pesticides, herbicides, benzene, plastic by-products, some pharmaceuticals and cosmetics, petroleum, UV filters) PMID: 2223368
- Poor dietary choices/high glycemic foods PMID: 2223368
- Excess adipose tissue/Obesity/Leptin resistance: PMID: 10349800 PMID: 3226011
- High insulin: PMID: 3322018
- Inflammatory cytokines: IL-6, TNFα, prostaglandin PGE(2) PMID: 10405348
- Alcohol/Red wine PMID: 19268535
- Major illness PMID: 16670151
- Brain injury PMID: 16498364 (animal study)
- Estrogen PMID: 2223368
- Forskolin (found in coleus plant) PMID: 14709151
- Free-fatty acids (increased in metabolic syndrome): PMID: 2223368

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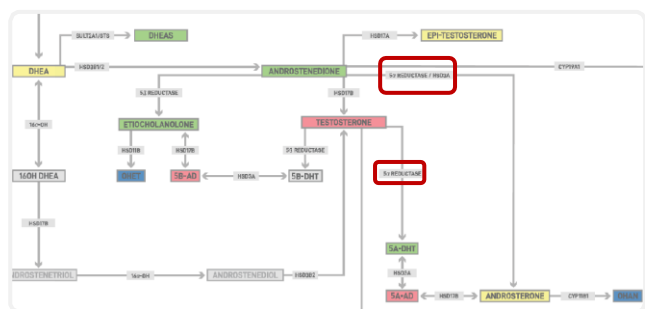


# Down regulate Aromatase (CYP19A1)

- Chrysin (honey and propolis): PMID: 32181408
- Zinc PMID 8613886
- Damiana PMID: 18948180
- Resveratrol PMID16611627
- Plant flavonoids
  - Apigenin PMID 18690828 PMID: 20635310
  - Catechins (EGCG in green tea): PMID 12065214
  - Eriodictyol (Yerba santa) PMID18690828
  - Hesperetin (citrus)
- Anti-Mullerian hormone PMID: 2726747
- Glyphosate PMID: 19539684
- Grape seed extract PMID: 16740737
- Nettles: PMID: 17509841
- Ketoconazole PMID: 2004042
- Prolactin
- Metformin PMID: 20300828
- Plant phenols:
  - Chalcones: PMID: 11205867
  - Isoliquiritigenin (licorice) PMID 18690828
- Mangostin PMID 18690828
- Myosime (alkaloid from tobacco and other plants/related to nicotine)
- Nicotine PMID 20188349
- Vitamin E PMID 15084515
- White button mushrooms PMID 17178902
- Aromatase inhibitors – (both steroidal and nonsteroidal) i.e. Formestane, Anastrozole
- Things that reduce inflammation:
  - Paclitaxel – breast cancer treatment, downregulates TNF-receptors stimulated by aromatase: PMID: 10405348
  - 2-MeOE2- downregulates TNF-receptors stimulated by aromatase: PMID: 10405348

# 5 $\alpha$ -reductase

- Enzyme used in the conversion of testosterone to its more potent form; dihydrotestosterone (DHT)
  - DHT has 2-3 times the affinity for the androgen receptor than testosterone
- May lead to increase in androgenic symptoms in females (acne, scalp hair loss, hirsutism, mood instability) and in males, hair loss and prostate issues.

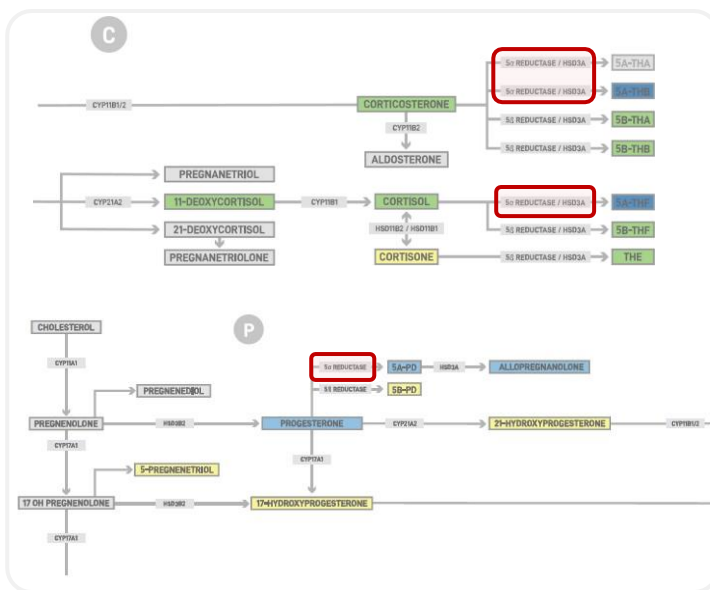


## Downregulate 5a reductase

- Polyunsaturated fatty acids
- Berberine
- Saw palmetto
- Nettles
- Pygeum
- Green tea/EGCG
- Progesterone
- Zinc

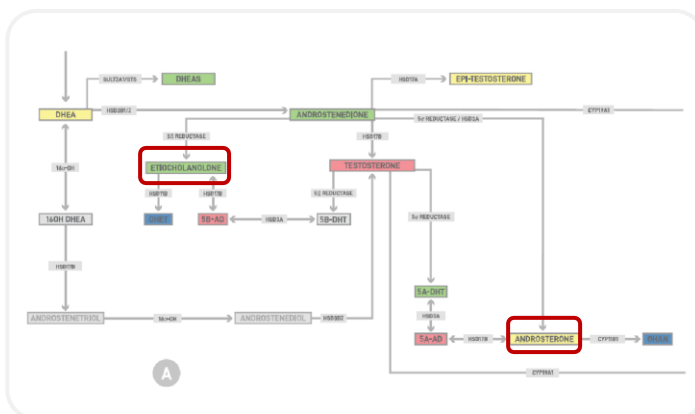
\*Will also be inhibiting:

- Progesterone to 5-APD
- Corticosterone to 5aTHA, 5aTHB
- Cortisol to 5aTHF



## $\alpha$ vs. $\beta$ : Metabolic Preference of Androgens

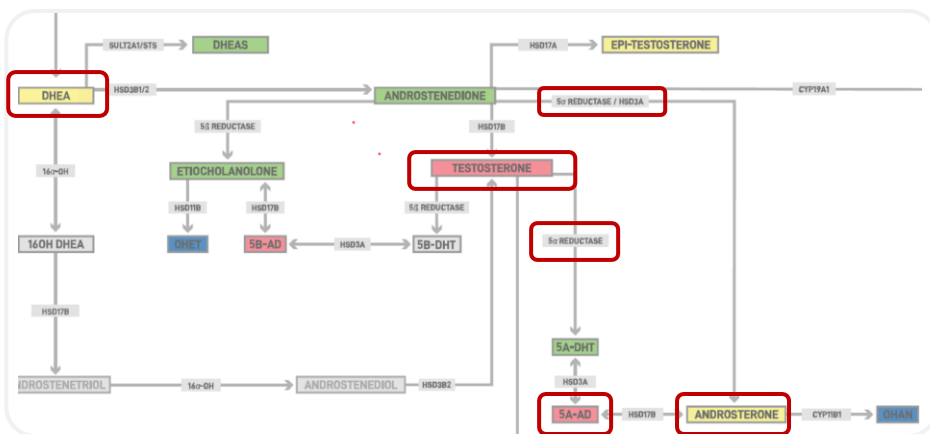
- Androsterone ( $5\alpha$ ) / Etiocholanolone ( $5\beta$ )
- Alpha ( $\alpha$ ) metabolism: More potent forms of metabolites
  - May produce more androgenic symptoms
- Beta ( $\beta$ ) metabolism: Less potent forms of metabolites
  - May produce less androgenic symptoms



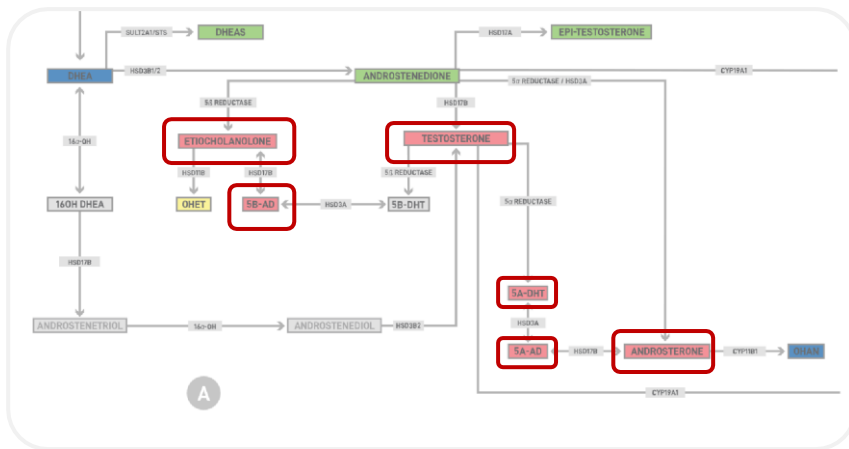
# DHEA + DHEAs

- DHEA is a prohormone with the potential to increase androgens as well as estrogens
- DHEA is the active form
- DHEAs is the inactive form
- Adding DHEA and DHEAs together may be a better representation of the total level of DHEA than measuring DHEA alone.

# PCOS case



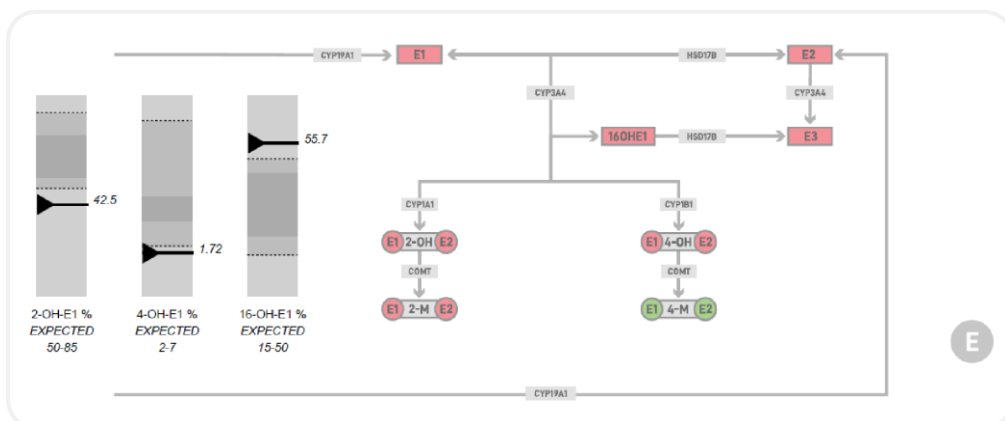
# Male on very high dose topical testosterone



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# Male on very high dose topical testosterone continued



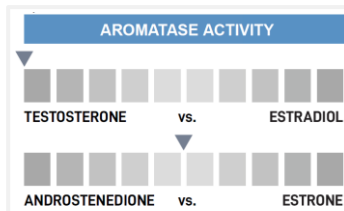
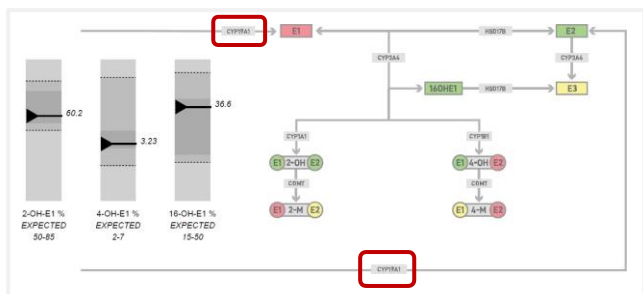
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## Aromatase (CYP19A1)

- Converts androstenedione to estrone and testosterone to estradiol
- Might help explain elevated endogenous estrogens
- Upregulated in peripheral fat in both men and women



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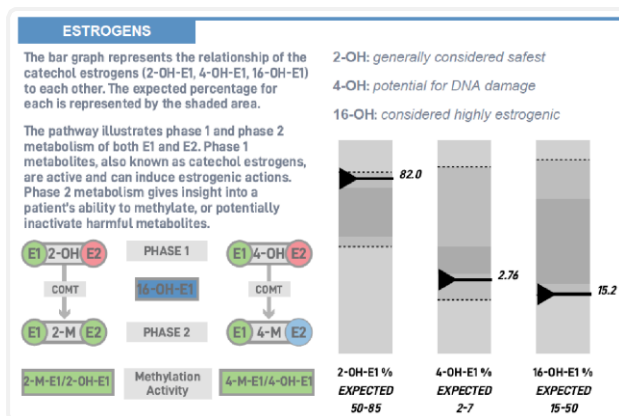




## Phase 1 Detox-Estrogen Metabolism

### “Catechol Estrogens”

- 2-OH E1,E2- “safe” estrogen (CYP1A1)
- 4-OH E1,E2- DNA damage potential (CYP1B1)
- 16-OH E1-DNA damage potential (less so)(CYP3A4)



Fuhrman BJ, Schairer C, Gail M, et al., Estrogen Metabolism and Risk of Breast Cancer in Postmenopausal Women, *JNCI: Journal of the National Cancer Institute*, Volume 104, Issue 4, 22 February 2012, Pages 326–339, <https://doi.org/10.1093/jnci/djr531>

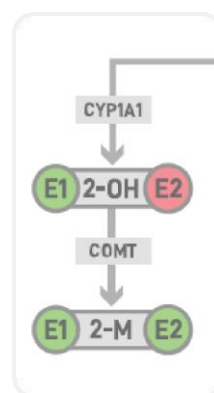
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## CYP1A1

- Healthy pathway of phase 1 (\*\*pushes the 2 pathway\*\*)
  - Pushes E1 to 2-OH E1
  - Pushes E2 to 2-OH E2
- Also, metabolizes polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene, released from burning coal, oil, gasoline, trash, tobacco, wood, or charcoal-broiled meat.
- Mainly involved in detoxification, though can sometimes activate cancer-promoting substances such as PAHs: PMID: 19531241 PMID: 28074113
- Can also activate aflatoxin B1 (cancer promoting) and tobacco-related carcinogens PMID: 19531241



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# CYP1A1

## Upregulate ☺

- DIM/cruciferous veggies PMID: 27261275
- 13C (requires stomach acid to convert to DIM) PMID: 12147290
- Coffee: PMID: 26063478
- Rosemary PMID: 9806165
- Resveratrol PMID: 20716633 PMID: 26167297
- Green and black tea PMID: 24815822
- Fish oil and garlic oil PMID: 12575903
- Hops PMID: 32986415
- Andrographolide, from the *Andrographis paniculata* plant PMID: 17825862
- Astaxanthin ( shrimps and some algae) PMID: 21414371

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# CYP1A1

## Downregulate ☹

- PAHs, PCBs, xenoestrogens, phalates
- BPA PMID: 31374317
- Smoking PMID: 27106177
- Charred meats
- Grapefruit juice (bergamottin) PMID: 22705772
- Berries (ellagic acid) can reduce overactivity PMID: 26167297
- Green tea extracts PMID: 11064004
- Sulforaphane found in broccoli PMID: 23566952
- St. John's Wort PMID: 16271822
- Lycopene, a red pigment found in tomatoes, carrots, and watermelon PMID: 20400267
- Naringenin and 6',7'-dihydroxybergamottin (from grapefruit juice) PMID: 27444380
- Galangin, found in some plants (*Alpinia officinarum*, *Alpinia galanga*, and *Helichrysum aureonitens*) and propolis PMID: 10188874
- A widely used herbal formulation produced from the extracts of ten common herbs (rosemary, turmeric, ginger, holy basil, green tea, hu zhang, Chinese goldthread, barberry, oregano, and Baikal skullcap) PMID: 22374940

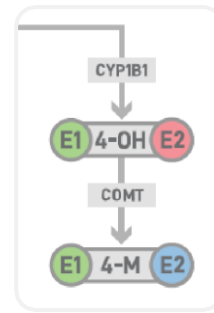
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# CYP1B1

- Takes estrogen down the 4 OH pathway
  - Pushes E1 to 4-OH E1 and E2 to 4-OH E2
- **CYP1B1 is more readily found in tumor tissue compared to normal.**
- Can activate cancer promoting compounds
- Accumulating evidence indicates that modulation of CYP1B1 can decrease adipogenesis and tumorigenesis, and prevent obesity, hypertension, atherosclerosis, and cancer. PMID: 28322972
- Given the role of CYP1B1 in pro-carcinogen and estrogen metabolism, polymorphisms in CYP1B1 could result in modifications in its enzyme activity and subsequently lead to hormone-mediated carcinogenesis.
- Also metabolizes: cortisol, aldosterone, FAs, fat-soluble vitamins, melatonin, retinol, plant flavonoids, many environmental toxins.
- Increases fat uptake and can lead to factors of metabolic syndrome



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# CYP1B1

## Upregulate

- Leptin resistance/inflammation/insulin resistance: PMID: 25433128 and DOI: 10.20892/j.issn.2095-3941.2016.0079
- Tetrahydrocannabinol (THC), found in cannabis PMID: 21867498
- UV exposure PMID: 11858729
- Estrogen: PMID 15126349
- PAHs, PCBs: PMID: 28322972
- Diesel exhaust particles (DEP) PMID: 21867498

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# CYP1B1

## Downregulate ☺

- Apeacea family: carrots, cumin, anise, celery, caraway PMID: 26381237
- Grapefruit PMID: 16338240
- Resveratrol PMID: 32533462
- Quercetin PMID: 22469840 PMID: 16271822
- Apigenin and amentoflavone (St. John's wort) PMID: 16271822
- Ginseng PMID: 11901090
- Lycopene, a red pigment found in tomatoes, carrots, and watermelon PMID: 20400267
- Chrysoeriol, present in rooibos tea and celery PMID: 26167297
- Naringenin (grapefruit juice) PMID: 22935222
- A polyherbal formulation produced from the extracts of ten common herbs (rosemary, turmeric, ginger, holy basil, green tea, hu zhang, Chinese goldthread, barberry, oregano, and Baikal skullcap) PMID: 22374940
- Many natural flavonoids and synthetic stilbenes show inhibitory activity toward CYP1B1 expression and function, notably isorhamnetin and 2,4,3',5'-tetramethoxystilbene. PMID: 28322972
- Genetics:
  - 150 gene polymorphism have been reported

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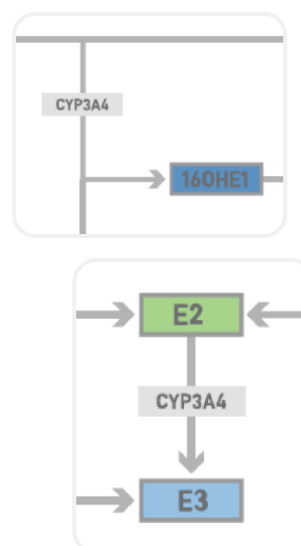
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# CYP3A4

## Moves E2 to E3 and E1 to 16OHE1

- It has been estimated that CYP3A4 metabolizes about half of all drugs on the market
- Mostly found in the liver, but most active in the gut
- The activity of this enzyme varies and is affected by health, environment (smoking, diet, and co-medication), hormones, and genetics
- Metabolizes many internal compounds such as cholesterol, fatty acids, prostaglandins, leukotrienes, retinoids and biogenic amines PMID: 25332983.
- Detoxifies bile acids PMID: 25332983
- Partially degrades vitamin D PMID: 22985909



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# CYP3A4

## Upregulate

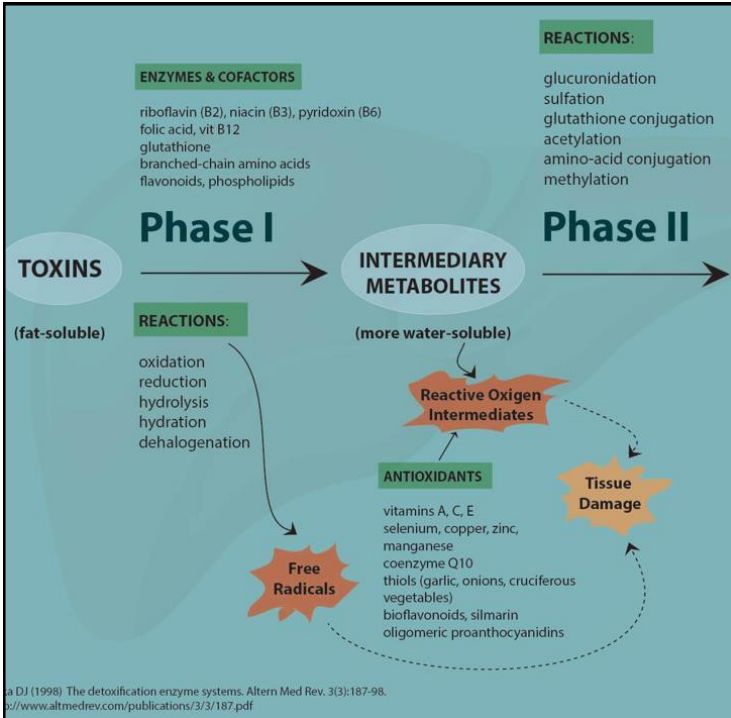
- CYP3A4 medication inducers
  - Carbamazepine
  - Dexamethasone PMID: 22370628
  - Modafinil
  - Phenobarbital
    - Phenytoin: PMID 10901705
- St.John's wort PMID: 17010103 PMID:17214607
- Capsaicin PMID: 22648626
- Valerian PMID: 17214607
- Gingko Bilboa PMID: 17214607
- Vitamin D/UV exposure PMID:22985909
- Being female PMID: 2333322
- Diabetes PMID: 24739263
- Fatty acids PMID: 24739263
- Polycyclic aromatic hydrocarbons (PAH) found in cigarettes PMID: 23845848
- Aflatoxin B1 PMID: 21641981
- Healthy levels of iron (CYP enzymes are heme dependent)



# CYP3A4

## Downregulate

- Polyphenols
  - Flavonoids:
    - Kaempferol (kale, beans, tea, spinach, broccoli) PMID: 25684704
    - Quercetin PMID: 25196644 PMID:25684704
    - Apigenin PMID: 26180597
    - Chrysin
    - Luteolin
    - Genestein
    - Green tea flavonols EGCG andepicatechin gallate PMID: 26180597
  - Phytoestrogen
    - Coumestrol (soybeans, brussels sprouts, spinach and a variety of legumes)
  - Phenolic acids
    - Caffeic acid PMID: 25196644
  - Tannic acid PMID: 26180597
  - Gallic acid PMID: 26180597
- Other Polyphenols
  - Licochalcone A from licorice PMID: 26100226
  - Sesamin in sesame seeds (*Sesamum indicum*) PMID: 22645625
  - Resveratrol PMID: 25341566 PMID: 26180597
  - Sulforaphane PMID: 17028159
  - Berberine PMID: 21870106
  - Allyl isothiocyanate (creates the pungent taste of mustard, radish, horseradish, and wasabi) PMID: 25069801
  - Ginsenoside Rd derived from Ginseng PMID: 15133536
  - Gomisin C and gomisin G found in *Schisandra Chinensis* PMID: 28344076



## Phase 2 Detoxification

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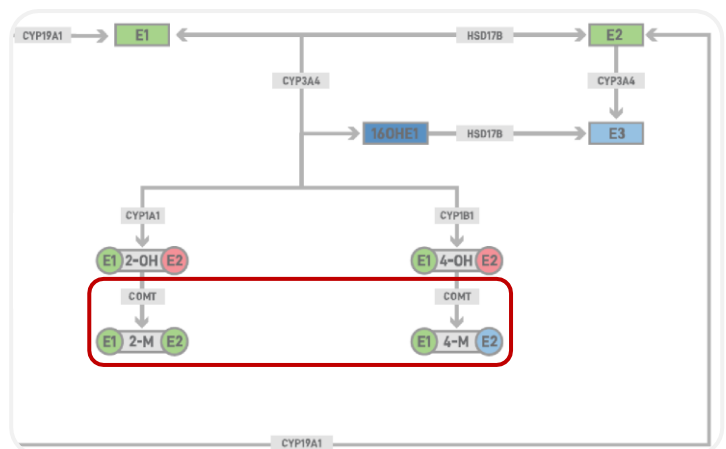
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## Estrogen Metabolism: Phase 2 Detox

### Oxidation or Methylation?

- Methylation (COMT)
  - Inactivates catechol estrogens
  - Increases solubility/prepare intermediates for renal and biliary excretion
  - methoxy metabolites → are stable and safely leave the body
- Oxidation
  - CYP peroxidase → quinone and semiquinone formation
    - Glutathionization (GST)
    - Glucuronidation (UGT)
    - Sulfonation (SOD2)
    - Acetylation



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## Phase 2: Methylation Activity (COMT)

- Metabolites can demonstrate methylation activity

- The ratio of **4-M E1/E2 to 4-OH E1/2** as well as **2-M E1/E2 to 2-OH E1/E2** is evaluated to determine if adequate methylation of catechol estrogens is occurring.

- The higher the ratio of methylation potential, the higher the likelihood of metabolizing more of the catechol estrogens toward the less harmful pathway of methylation and therefore less reactive quinone formation.

2-M-E1:2-OH-E1 <sup>2</sup>	(COMT/Methylation activity)	0.111		0.1 – 0.36
2-M-E2:2-OH-E2 <sup>2</sup>	(COMT/Methylation activity)	0.037		0.07 – 0.37
4-M-E1:4-OH-E1 <sup>2</sup>	(COMT/Methylation activity)	0.212		0.09 – 0.54
4-M-E2:4-OH-E2 <sup>2</sup>	(COMT/Methylation activity)	0.051		0.04 – 0.54

R. S. Lord, B. Bongiovanni, and J. A. Bralley, "Estrogen metabolism and the diet-cancer connection: rationale for assessing the ratio of urinary hydroxylated estrogen metabolites," *Alternative Medicine Review*, vol. 7, no. 2, pp. 112–129, 2002.  
 J. D. Yager, "Mechanisms of estrogen carcinogenesis: the role of E2/E1-quinone metabolites suggests new approaches to preventive intervention— a review," *Steroids*, 2014.

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## Phase 2: Methylation (COMT) Factors

### Upregulate ☺

- **Food Support:**

- Cruciferous vegetables
- Soy foods (ex. genestein)
- Resveratrol
- Citrus foods
- Teas (rooibos, dandelion)
- Spices (rosemary, curcumin)

PMID: 26167297

- **Cofactors and Methyl donors:**

- Methionine
- Vitamin B12
- Vitamin B6
- Betaine
- Folate
- Magnesium

PMID: 26167297

Romilly E. Hodges, Deanna M. Minich, "Modulation of Metabolic Detoxification Pathways Using Foods and Food-Derived Components: A Scientific Review with Clinical Application", *Journal of Nutrition and Metabolism*, vol. 2015, Article ID 760689, 23 pages, 2015. <https://doi.org/10.1155/2015/760689>

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## Phase 2: Methylation (COMT) Factors

### Downregulate □

- High sucrose diet may inhibit methylation PMID: 26167297
- Leptin resistance: DOI: 10.20892/j.issn.2095-3941.2016.0079
- Estrogen PMID: 10385681 PMID: 30684530
- TNFalpha, present in inflammatory states
- Nuclear factor-kappa B (NF-κB) PMID: 26187567
- Serotonin (competes with the methyl donor S-adenosyl-L-methionine (SAM), thus competing with methylation of COMT) PMID: 22500608
- Anything that affects the methionine cycle (homocysteine to cysteine):  
B6 insufficiency

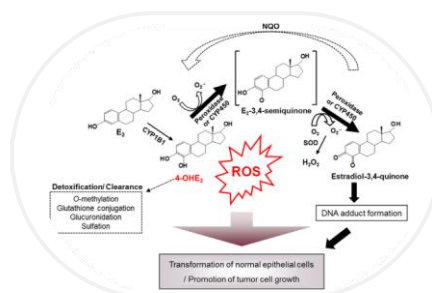
## Phase 2: Methylation (COMT) Factors

### Downregulate continued . . .

- Bisphenol and PCBs (PMID: 20945454)
- Having too little SAM (s-adenosylmethionine) and too much SAH (s-adenosylhomocysteine) from undermethylation
- Entacapone, tolcapone, opicapone and nitecapone
- PPIs, antibiotics
- Genetics polymorphisms PMID: 24593143
  - V158M or rs4680: The A allele results in 3 to 4-fold decrease

# Estrogen Quinones and Breast Cancer

- Un-methylated 2-OH and 4-OH metabolites can form quinones / semi-quinones, resulting in DNA adduct formation/DNA damage
- Estrogen quinones are highly carcinogenic
- Potentiated by oxidative damage
- Genotoxic: DNA breaks/adducts
- Mutagenic: Depurinated DNA
- Carcinogenic: high levels in BC and HR breast tissue
  - Induction and propagation of BC
  - Increased risk of hormone responsive BC
- “Increased amounts of estrogen-DNA adducts are found not only in people with several different types of cancer but also in women at high risk for breast cancer, indicating that the formation of adducts is on the pathway to cancer initiation.”



Yager JD. Mechanisms of estrogen carcinogenesis: The role of E2/E1-quinone metabolites suggests new approaches to preventive intervention—A Review. *Steroids*. 2015;99:56-60. doi:10.1016/j.steroids.2014.08.006  
 Cavalleri E, Rogan E. The 3,4-quinones of estrone and estradiol are the initiators of cancer whereas resveratrol and N-acetylcysteine are the preventers. *International Journal of Molecular Sciences*. 2021;22(15):8238. doi:10.3390/ijms22158238  
 Park, S-A. Catechol Estrogen 4-Hydroxyestradiol is an Ultimate Carcinogen in Breast Cancer. *Biomedical Science Letters* 2018, 24(3): 143-149 <https://doi.org/10.15616/BSL.2018.24.3.143>

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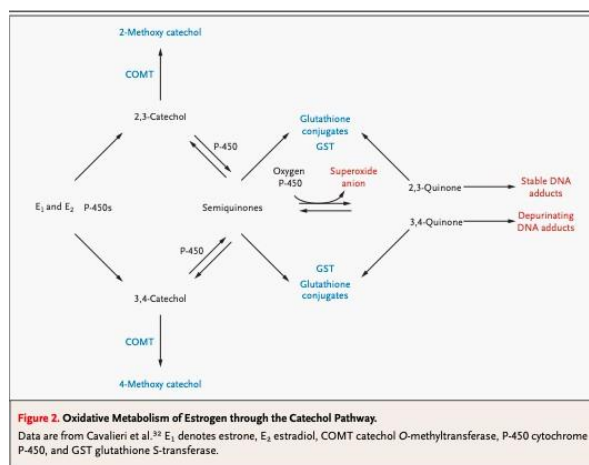
## Quinone reduction

### NAC

- Reduce estrogen semiquinones back to catechol estrogens
- Primary effect is to react with quinones to form conjugates preventing the formation of estrogen-DNA adducts.

### Resveratrol

- Reduce catechol estrogen semiquinones back to catechol estrogens
- Induce the estrogen-protective enzyme quinone reductase
- Modulates CYP1B1, thereby reducing its activity and thus the formation of 4-OH-E<sub>2</sub>
- Shrivastava N, Parikh A, Dewangan RP, et al. Solid Self-Nano Emulsifying Nanopatform Loaded with Tamoxifen and Resveratrol for Treatment of Breast Cancer. *Pharmaceutics*. 2022;14(7):1486. Published 2022 Jul 18. doi:10.3390/pharmaceutics14071486



**Figure 2. Oxidative Metabolism of Estrogen through the Catechol Pathway.**  
 Data are from Cavalleri et al.<sup>32</sup> E<sub>1</sub> denotes estrone, E<sub>2</sub> estradiol, COMT catechol O-methyltransferase, P-450 cytochrome P-450, and GST glutathione S-transferase.

Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. *N Engl J Med*. 2006;354(3):270-282. doi:10.1056/NEJMra050776  
 Cavalleri E, Rogan E. The 3,4-quinones of estrone and estradiol are the initiators of cancer whereas resveratrol and N-acetylcysteine are the preventers. *International Journal of Molecular Sciences*. 2021;22(15):8238. doi:10.3390/ijms22158238

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## 8-hydroxy-2-deoxyguanosine (8OH2dG)

- **Not an estrogen metabolite, but pairs nicely with metabolite information**
  - Marker for DNA damage due to oxidative stress in general
  - May be helpful in identifying or confirming DNA damage from harmful metabolites (quinones and DNA adducts)
- In nuclear and mitochondrial DNA, 8-OHdG is one of the predominant forms of free radical-induced oxidative lesions and widely used as a biomarker for oxidative stress and carcinogenesis.

Oxidative Stress Metabolite	Result	Unit	L	WRI	H	Reference Interval
8-hydroxy-2'-deoxyguanosine <sup>†</sup>	(8-OHdG) 9.08	ng/mg Creat/Day				0 – 7.5

VALAVANIDIS ATHANASIOS, VLACHOGIANNI THOMASIS, FIOTAKIS CONSTANTINOS. 8-hydroxy-2'-deoxyguanosine (8-ohdg): A critical biomarker of oxidative stress and carcinogenesis. *Journal of Environmental Science and Health, Part C*. 2009;27(2):120-139. doi:10.1080/10590500902885684

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## Oxidative stress and disease states

- Chronic stress
- Cortisol elevation
- Inflammation
- Insomnia in postmenopausal women
- Degenerative diseases (rheumatoid arthritis, Parkinson's disease, Huntington's disease, Alzheimer's disease)
- Chronic fatigue syndrome
- Major depression
- Hypertension
- Cardiovascular disease
- Diabetes type II
- Cystic fibrosis
- Psoriasis
- Chronic hepatitis
- Gastritis
- Irritable bowel disease
- Pancreatitis
- Cancer
- Acute viral infection
- Copper implants
- Toxic exposures (tobacco smoke, methamphetamines, asbestos, heavy metals, polycyclic hydrocarbons)

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# Treatment for elevated 8-OHdG

## Address the cause of oxidative stress ☺

- Glutathione
- NAC
- Green tea
- CoQ10
- Onion
- Garlic
- Alpha lipoic acid
- Vitamins C and E
- Melatonin
- Folate
- Berberine
- EPA/DHA
- Fermented papaya powder
- Increase fruit and veggies
- Yoga

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# 8-OHdG:treatment references

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- Dai P, Wang J, Lin L, Zhang Y, Wang Z. Renoprotective effects of berberine as adjuvant therapy for hypertensive patients with type 2 diabetes mellitus: Evaluation via biochemical markers and color doppler ultrasonography. *Exp Ther Med* 2015, Sep;10(3):869-76.
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- Franke AA, Cooney RV, Henning SM, Custer L. Bioavailability and antioxidant effects of orange juice components in humans. *J Agric Food Chem.* 2005;53(13):5170-5178. doi:10.1021/jf050054y
- Nirwan M, Halder K, Saha M, Pathak A, Balakrishnan R, Ganju L. Improvement in resilience and stress-related blood markers following ten months yoga practice in Antarctica. *J Complement Integr Med.* 2020;18(1):201-207. Published 2020 Jun 19. doi:10.1515/jcim-2019-0240

# What are the advantages of HuMap™ ?



## Additional Analytes

- Progesterone itself
- DHEA
- Allopregnanolone
- Androstenedione
- (2-OH E2 and 4-OH E2) included in our ratios
- (2-M E2 and 4-M E2) included in our ratios

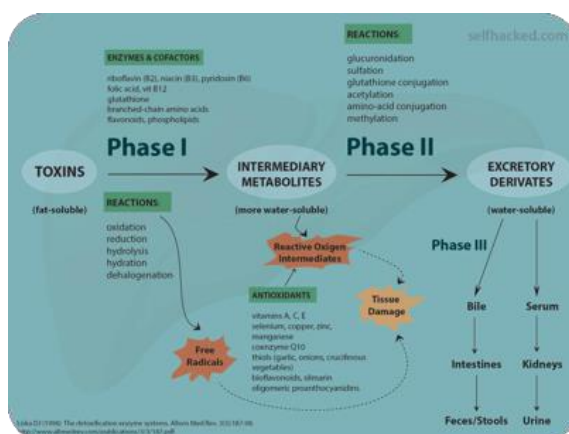
## Liquid Collection

- The main advantage of liquid urine collection is enhanced sensitivity, especially for low concentration metabolites. Dried urine must be reconstituted from the filter paper once the sample arrives. This reconstitution can lead to loss of polar steroid metabolites or creatinine for some patient samples.
- With liquid urine, samples can be shipped after being frozen for 4-6 hours, can be processed faster, and concentrated further to enhance the detection of low-level analytes. Steroid hormones and metabolites are also quite stable in liquid urine if the correct preservative is used.

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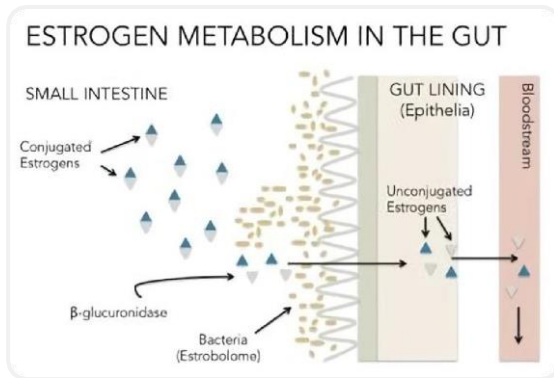
# Phase 3 Detox



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# Elimination or Recirculation



- Conjugated estrogens pass into the gut for elimination
- Beta-glucuronidase can deconjugate estrogens
- Unconjugated estrogens reabsorbed into circulation
- High levels of beta-glucuronidase could mean more recirculating of estrogens
- **Beta-glucuronidase increases with: low fruits and veggies, low fiber, high sugar, processed foods, SAD diet, alcohol, toxicants, antibiotics**

Baker, J. M., Al-Nakkash, L., & Herbst-Kralovetz, M. M. (2017). Estrogen-gut microbiome axis: Physiological and clinical implications. *Maturitas* 103, 45-53. doi:10.1016/j.maturitas.2017.06.025

75



## Stool testing: beta glucuronidase

Digestion / Absorption	Result	Unit	L	WRI	H	Reference Interval
Elastase	>500	µg/mL				> 200
Fat Stain	None					None - Few
Carbohydrates <sup>1</sup>	Negative					Negative
Inflammation	Result	Unit	L	WRI	H	Reference Interval
Lactoferrin	1.6	µg/mL				<7.3
Lysozyme <sup>2</sup>	369	ng/mL				≤500
Calprotectin	<10	µg/g				≤50
Immunology	Result	Unit	L	WRI	H	Reference Interval
Secretory IgA <sup>3</sup>	34.8	mg/dL				30-275
Short Chain Fatty Acids	Result	Unit	L	WRI	H	Reference Interval
% Acetate <sup>4</sup>	62					50-72
% Propionate <sup>4</sup>	17					11-25
% Butyrate <sup>4</sup>	17					11-32
% Valerate <sup>4</sup>	3.7					0.8-5.0
Butyrate <sup>4</sup>	2.4	mg/mL				0.8-4.0
Total SCFA's <sup>4</sup>	14	mg/mL				5.0-16.0
Intestinal Health Markers	Result	Unit	L	WRI	H	Reference Interval
pH	6.1					5.8-7.0
β-glucuronidase <sup>5</sup>	1508	U/L				100-1200
Occult Blood	Negative					Negative

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## Consider treating phase 3 (elimination) first

- Hydration PMID: 20646222
- Fiber PMID: 26026145
- Herbs to prevent/treat constipation (aloe, ginger, Avipattikar, Triphala) PMID: 30680163 PMID: 28696777
- Magnesium
- Movement
- Calcium D-Glucarate PMID: 2346674

Genius SJ. Elimination of persistent toxicants from the human body. Hum Exp Toxicol. 2011 Jan;30(1):3-18. doi: 10.1177/0960327110368417. Epub 2010 Apr 16. PMID: 20400489.



## Urinary Hormone and Metabolites Summary

- HuMap™ gives a comprehensive assessment of unconjugated hormones and their metabolites
- Allows the practitioner to examine what the body has utilized
- Can give greater insight into
  - Alpha vs. Beta metabolism (progesterones, androgens, and corticoids)
  - Phase I and Phase II detoxification (COMT /methylation activity)
    - With additional estradiol metabolites for 2-OH and 4-OH to give a greater picture of metabolism
  - Potential risk assessment for breast health and other disease processes
  - Utilization of hormones both endogenous and exogenous
  - Can assist with complex cases where BHRT and salivary testing appear to be normal, but the patient may still have symptoms.
- Remember, elimination is key to any treatment case
  - Ensure proper elimination first before addressing phase 1 or phase 2 metabolism



# Why test neurotransmitters and urinary metabolites together?

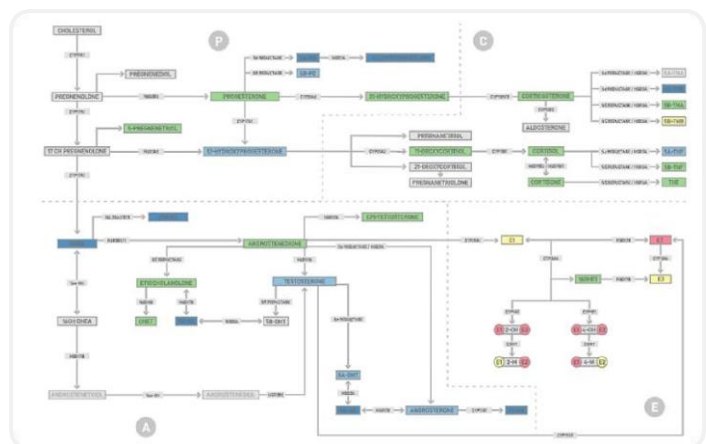
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It could be especially important to combine HuMap™ and NT testing in cases involving mental health symptoms that came on during a time of hormonal transition:

- For example, an aging male with depression might have low testosterone.
- Mood symptoms that happen at certain times of the menstrual cycle can involve NT imbalance and also estrogen dominance.



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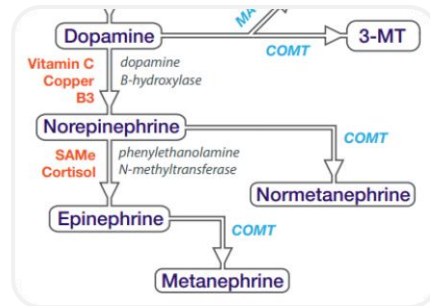
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## COMT

- An obvious connection between HuMap™ and Neurotransmitter testing is COMT activity, which is important with estrogen and catecholamine metabolism.



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## COMT Genetic variants

- Slow COMT (+/+), increases catecholamines
  - a.k.a. A/A or Met/Met, “worrier”
  - Tendency towards lower stress resiliency
  - Possible advantage in memory and attention
- Fast COMT (-/-), depletes catecholamines
  - a.k.a. G/G or Val/Val, “warrior”
  - Tendency towards issues with focus, memory, low mood, motivation
  - Possible advantage in processing aversive stimuli and better performance under stressful conditions
- 80% of the population may have at least one COMT gene SNP

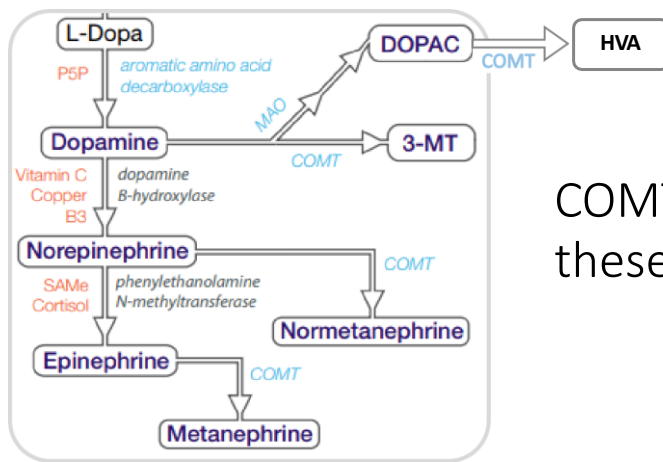


Sleep Med Rev 2015 Aug;22:43 PMID 25466290  
 Davis T. What is the COMT gene? And how does it affect your health? Are your genes responsible for your unhappiness? (Part 2) : The COMT gene. Jan 15 2020. Available at <https://www.psychologytoday.com/us/blog/click-here-happiness/202001/what-is-the-comt-gene-and-how-does-it-affect-your-health>  
 Stein DJ, Newman TK, Savitz J, Ramesar R. Warriors versus worriers: the role of COMT gene variants. *CNS Spectr*. 2006;11(10):745-748.  
 doi:10.1017/s1092852900014863

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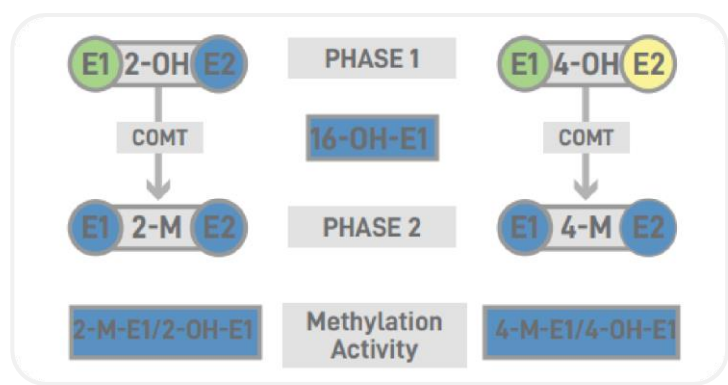
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COMT activity affects these markers

COMT is also involved in estrogen metabolism.  
Low 2/4-M Metabolites



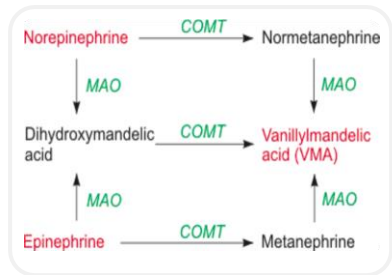
# COMT Support

## COMT sluggish

- SAmE: 100-500 mg
- Mg: 150-500 mg
- MTHF: 400-5000 mcg
- Methylcobalamin: 1000-5000 mcg
- Foods/herbs: cruciferous vegetables, soy foods (ex. genestein), resveratrol, citrus, rooibos, dandelion, rosemary, curcumin
- Limit caffeine, alcohol, tobacco smoke

## COMT over active

- Support MAO enzyme...why?
  - Vitamin B2: 50 mg (riboflavin 5 phosphate)
  - Vitamin B3: 100 mg
  - Iron: 25-50 mg



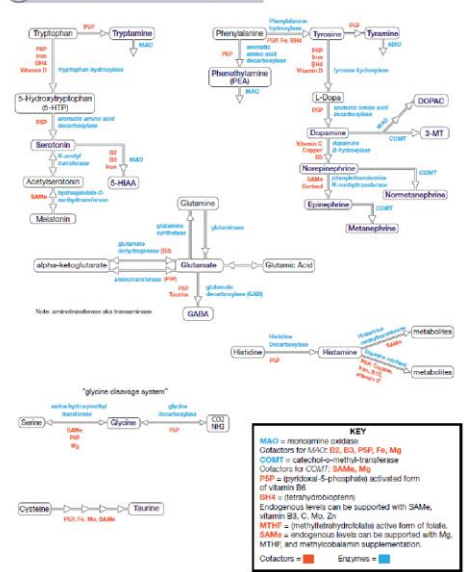
Minich, D., and R. Hodges. "Modulation of metabolic detoxification pathways using foods and food-derived components: A scientific review with clinical application." *Journal of Nutrition and Metabolism*, Vol. 2015 (2015): 760689.  
 Davis T. What is the COMT gene? And how does it affect your health? Are your genes responsible for your unhappiness? (Part 2): The COMT gene. Jan 15 2020. Available at <https://www.psychologytoday.com/us/blog/click-here-happiness/202001/what-is-the-comt-gene-and-how-does-it-affect-your-health>  
 Klabunde RE. Cardiovascular Pharmacology Concepts. Available at <https://www.cupharmacology.com/norepinephrine>

LOE: C

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## Neurotransmitter Pathways



# Methylation variants

- People who have MTHFR gene mutations make faulty MTHFR enzymes which have difficulty processing dietary folate causing trouble with NT synthesis
- Commonly see low/suboptimal levels of **serotonin, dopamine, norepinephrine, epinephrine, glycine**
- If a MTHFR defect is suspected, consider genetic testing. The most common mutations are at position 677 and position 1298 on the MTHFR gene.

[www.mthfr.net](http://www.mthfr.net). Accessibility verified 3/8/22.

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## Other factors that affect enzyme activity

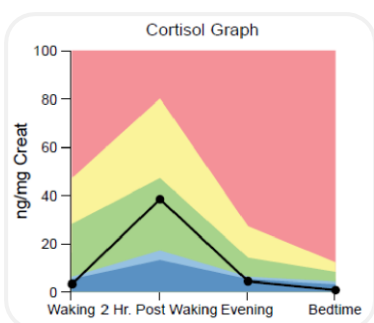
- Inflammatory cytokines can influence the activity of both MAO and COMT.
- Insulin resistance may increase MAO levels
- Excess estradiol slows COMT and MAO activity, potentially resulting in higher levels of neurotransmitters
- Low estradiol increases MAO activity, lowering serotonin
- Progesterone downregulates COMT gene expression

Salama, Salama A et al. "Progesterone regulates catechol-O-methyl transferase gene expression in breast cancer cells: distinct effect of progesterone receptor isoforms." *The Journal of steroid biochemistry and molecular biology* vol. 107,3-5 (2007): 253-61. doi:10.1016/j.jsbmb.2007.03.049  
 Kleinridders A, Cai W, Cappellucci L, et al. Insulin resistance in brain alters dopamine turnover and causes behavioral disorders. *Proc Natl Acad Sci U S A*. 2015;112(11):3463-3468. doi:10.1073/pnas.1500877112

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## Stress



- The stress response involves the coordination of epinephrine and norepinephrine (aka adrenaline and noradrenaline) as well as cortisol
- Stress is inflammatory
- Inflammation hijacks neurotransmission

Norepinephrine	17.2	µg/g		22 – 50
Epinephrine	1.5	µg/g		1.6 – 8.3
Norepinephrine / Epinephrine ratio	11.5			< 13

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## Coordinated fight or flight response

- Norepinephrine and epinephrine are released from the brain as well as storage vesicles in the adrenal medulla in response to stress: fright, exercise, cold, low blood glucose.
- They increase degradation of glycogen and triacylglycerol, as well as increase blood pressure and cardiac output.
- Cortisol is then released to continue the stress response and continues to be released until the threat is over.



Champe P, Harvey R, Ferrier D. *Biochemistry*. 3<sup>rd</sup> ed. Philadelphia: Lippincott Williams & Wilkins; 2005.

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## Stress and the Brain

- Stress literally shrinks the brain and degrades the BBB
  - Stressors: Environmental stress, but also smoking, food intolerances, blood sugar imbalances, anemia, bacterial gut infections, gut parasites, autoimmune dz, joint pain and inflammation, poor digestion, etc.
- Excess fat is pro-inflammatory and a chronic stressor for body and brain.
- Studies show that high cortisol in response to high stress damages the hippocampus, which regulates our circadian rhythm.
  - Eventually the adrenals become less responsive to ACTH.

Esposito P, et al. Acute stress increases permeability of the blood-brain-barrier through activation of brain mast cells. *Brain Res*. 2001 Jan 5;888(1):117-127.  
Tavanti M et al. Evidence of diffuse damage in frontal and occipital cortex in the brain of patients with post-traumatic stress disorder. *NeuroSci*. 2012 Feb;33(1):59-68.

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# Stress response and serotonin

## Stress response

- The response of the CNS to stress is predominantly through activation of the locus coeruleus/sympathetic nervous system, with the consequent release of **norepinephrine and epinephrine**, and the limbic/HPA system, with the consecutive release of **serotonin**, CRH, ACTH, and **cortisol**.

Tafet GE, Idoyaga-Vargas VP, Abulafia DP, et al. Correlation between cortisol level and serotonin uptake in patients with chronic stress and depression. *Cogn Affect Behav Neurosci*. 2001 Dec; 1(4): 388-93.

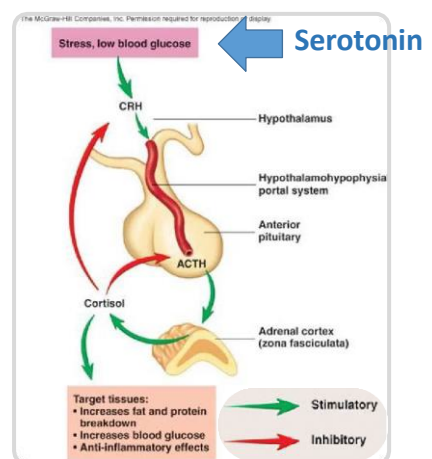
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# Biochemistry of serotonin's influence on cortisol secretion

- Cerebrocortical signals to the midbrain are initiated in the cerebral cortex by stress.
- These stresses elicit the production of acetylcholine and **serotonin** which stimulate the release of CRH.
- CRH stimulates ACTH which stimulates cortisol secretion.



Heisler LJ, et al. Serotonin activates the hypothalamic-pituitary-adrenal axis via serotonin 2C receptor stimulation. *J Neurosci*. 2007 Jun 27;27(26):6956-64.  
Lieberman M, PhD; Marks A, MD. *Basic Medical Biochemistry: A Clinical Approach*. Philadelphia: Wolters Kluwer/Lippincott Williams & Wilkins. 2009. Print.

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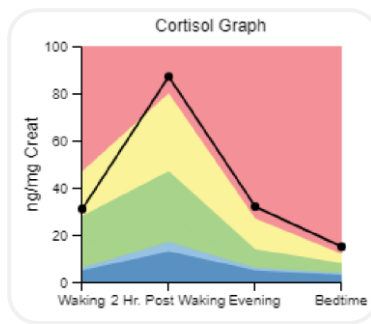
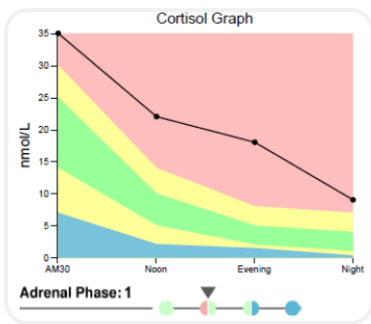


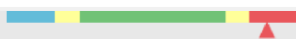

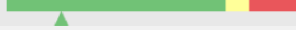
# Stress response and catecholamines

- Within seconds of an acutely stressful event, NE and epinephrine are released into the blood stream, resulting in the fight or flight response.
- **Chronic stress depletes the brain reserves of epi and norepi** (and their precursor dopamine).
- Depletion of catecholamines are closely related to stress induced performance decline.
  - Issues with focus, energy, motivation, mood

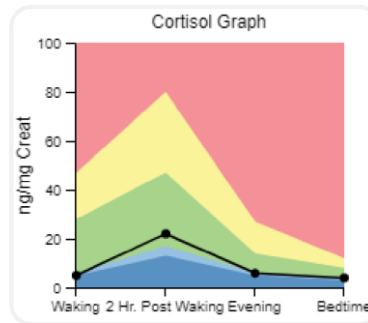
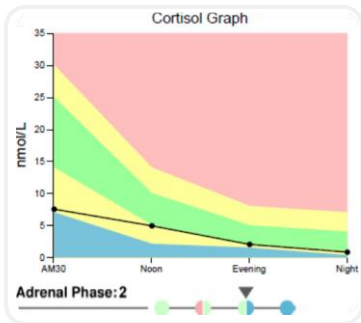
Head KA, Kelly GS. Nutrients and botanicals for treatment of stress: adrenal fatigue, neurotransmitter imbalance, anxiety, and restless sleep. *Alt Med Review*. Vol 14, No 2. 2009.

# Three phases of stress response: Phase I/Early Stage



Norepinephrine	68	µg/g		22-50
Epinephrine	25	µg/g		1.6-8.3
Norepinephrine / Epinephrine ratio	2.72			< 13

## Phase II/Mid Stage



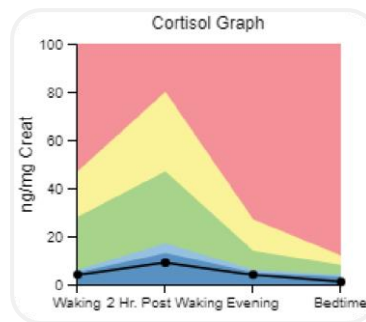
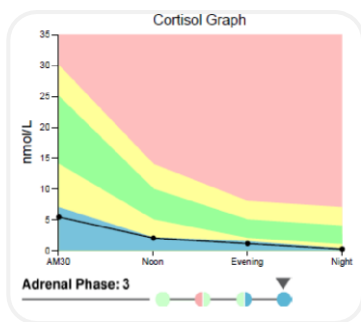
Norepinephrine	32	µg/g		22–50
Epinephrine	25	µg/g		1.6–8.3
Norepinephrine / Epinephrine ratio	1.28			< 13

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## Phase III/Late Stage



Norepinephrine	22	µg/g		22–50
Epinephrine	0.6	µg/g		1.6–8.3
Norepinephrine / Epinephrine ratio	36.6			< 13

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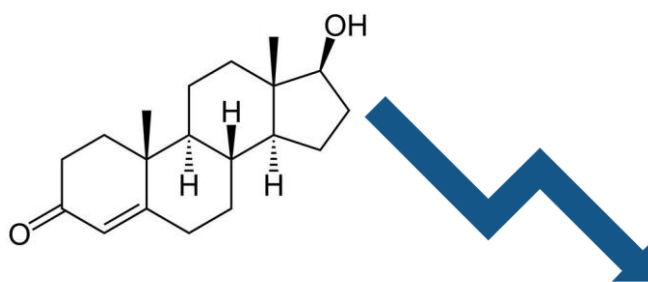
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## Watch out for stress!

- Dopamine and serotonin can acutely stimulate testosterone production, while epinephrine can inhibit T production.



Levin J, et al. The effect of epinephrine on testosterone production. *Eur J Endocrin.* Vol 55 (1):184-192.  
Elman I, Goldstein DS, Adler CM, Shoaf SE, Breier A. Inverse relationship between plasma epinephrine and testosterone levels during acute glucoprivation in healthy men. *Life Sci.* 2001 Mar 9;68(16):1889-98.

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## Inflammation

- The HuMap™ may suggest inflammation when
  - Cortisol is favored over cortisone (increased 11BHSD1 activity)
  - Increased cortisol metabolites (THE+THF)
  - When aromatase activity is elevated
  - Androgens: Elevated DHEA and Testosterone in a female (inflammation related to insulin resistance) or low Testosterone in a male
  - 5 $\alpha$  reductase metabolites
  - Elevated 8OH2dg

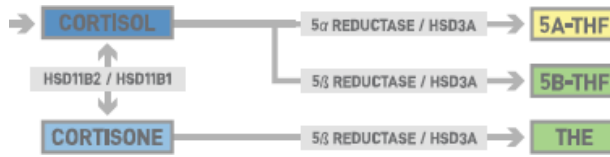
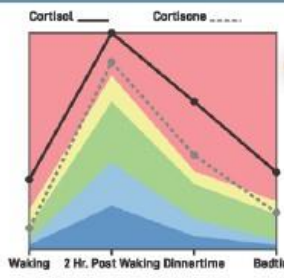
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### CORTICOIDS

11 $\beta$ HSD2 is responsible for the conversion of cortisol to cortisone. Inhibition of this enzyme may lead to the amount of cortisol being greater than cortisone, while increased enzyme activity can lead to higher levels of cortisone in comparison to cortisol.



5A-THF+5B-THF/THE<sup>+</sup> (Cortisol/Cortisone Metabolites) 1.34 0.7 - 1.4

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### AROMATASE ACTIVITY



TESTOSTERONE vs. ESTRADIOL



ANDROSTENEDIONE vs. ESTRONE

### AROMATASE ACTIVITY



TESTOSTERONE vs. ESTRADIOL



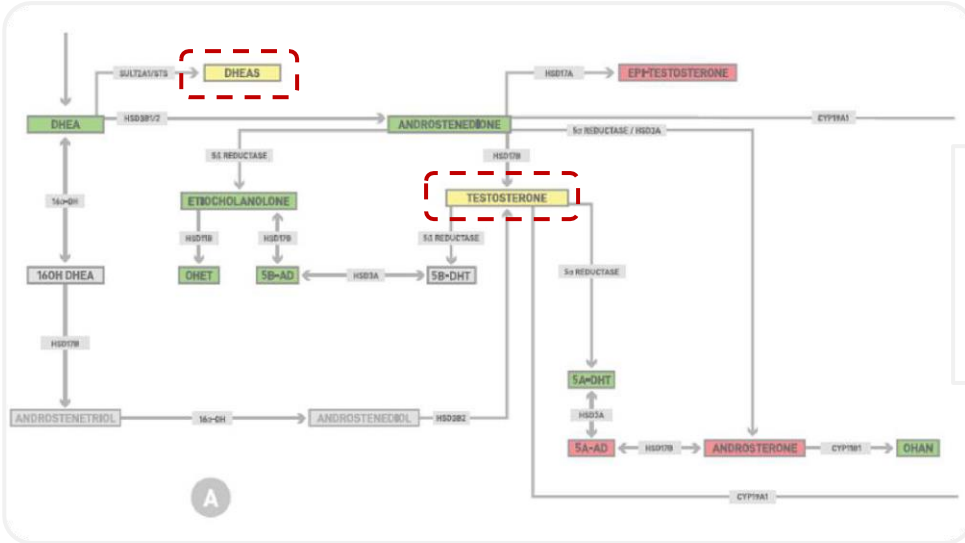
ANDROSTENEDIONE vs. ESTRONE

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## Female



L WRI H

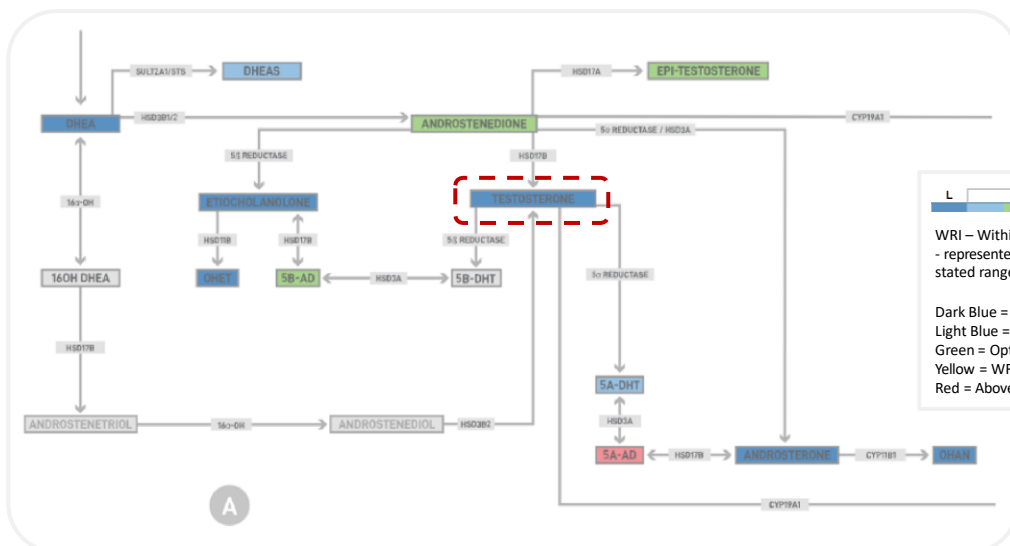
WRI – Within Reference Interval - represented by bracket and stated ranges on report

Dark Blue = Below RI  
Light Blue = WRI low  
Green = Optimal  
Yellow = WRI high  
Red = Above RI

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## Male



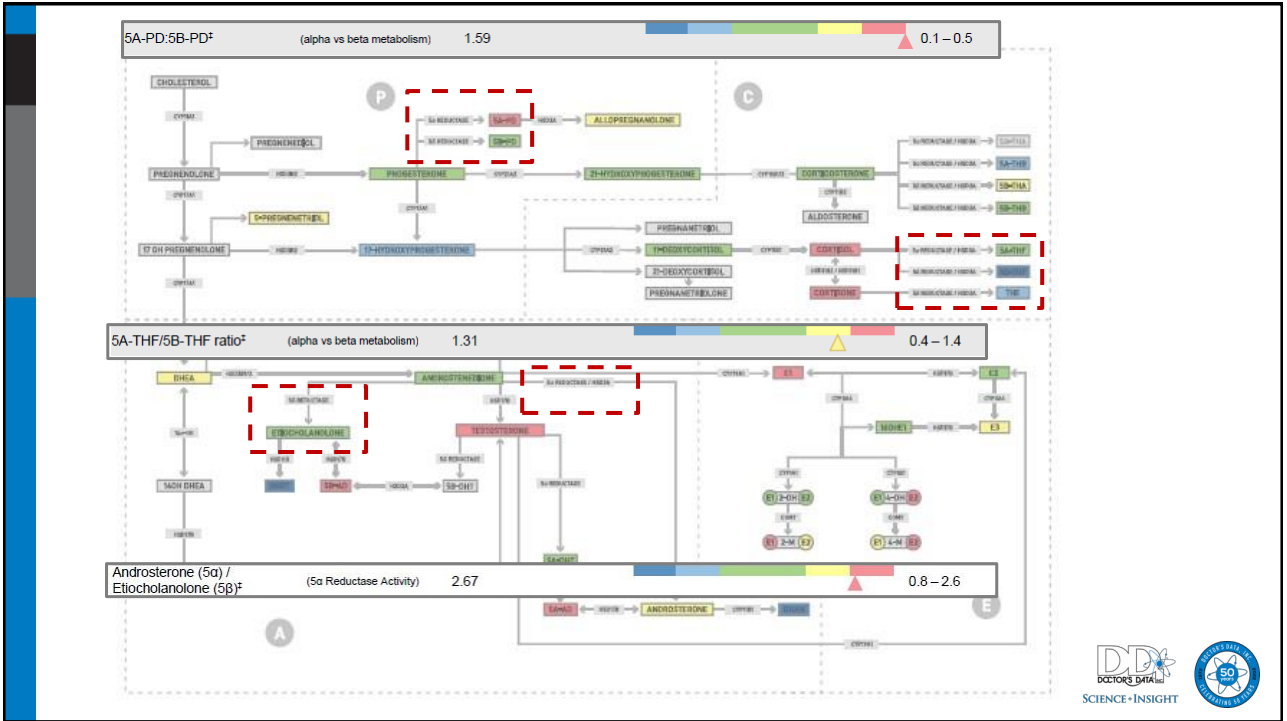
L WRI H

WRI – Within Reference Interval - represented by bracket and stated ranges on report

Dark Blue = Below RI  
Light Blue = WRI low  
Green = Optimal  
Yellow = WRI high  
Red = Above RI

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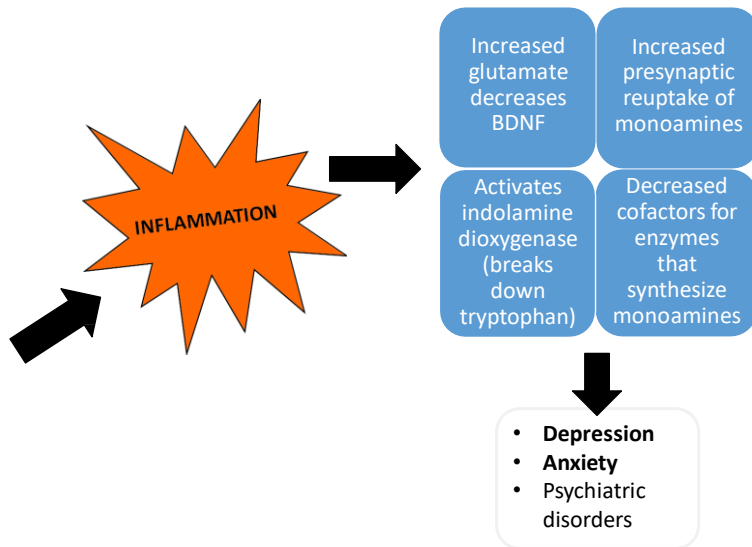




Ratios and Calculations	Result	Unit	L	WRI	H	Reference Interval
2-OH-E1 % <sup>2</sup>	(2-OH-E1 %)	60.2	%			50 – 85
4-OH-E1 % <sup>2</sup>	(4-OH-E1 %)	3.23	%			2 – 7
16-OH-E1 % <sup>2</sup>	(16-OH-E1 %)	36.6	%			15 – 50
2-M-E1:2-OH-E1 <sup>2</sup>	(COMT/Methylation activity)	0.395				0.1 – 0.36
2-M-E2:2-OH-E2 <sup>2</sup>	(COMT/Methylation activity)	0.348				0.07 – 0.37
4-M-E1:4-OH-E1 <sup>2</sup>	(COMT/Methylation activity)	0.195				0.09 – 0.54
4-M-E2:4-OH-E2 <sup>2</sup>	(COMT/Methylation activity)	0.095				0.04 – 0.54
2-OH-E1:16-OH-E1 <sup>2</sup>		1.64				1.6 – 5.1
4-OH-E1:2-OH-E1 <sup>2</sup>		0.054				0.02 – 0.07
<b>Oxidative Stress Metabolite</b>						
8-hydroxy-2'-deoxyguanosine <sup>2</sup>	(8-OHdG)	9.20	ng/mg Creat/Day			0 – 7.5

**Table 1. Factors associated with increased inflammation**

Obesity
Sedentary lifestyle
Disordered sleep
Childhood maltreatment
Emotional and physical trauma
Medical illnesses (eg, cardiovascular disease, diabetes, cancer, autoimmune and inflammatory disorders)
Bacterial or viral infection (including exposure to a high pathogen load [eg, unsanitary living conditions, poor dentition, poor hygiene])
Medical treatments (eg, surgery, radiation, chemotherapy)
Antidepressant treatment resistance



Miller AH, Five Things to Know About Inflammation and Depression. *Psychiatric Times*, Vol 35 No 4, Volume 35, Issue 4. 30 April 2018.

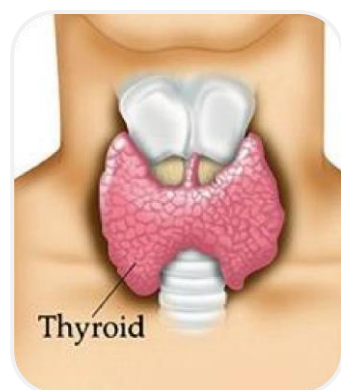
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## Thyroid

- Thyroid issues can affect both NT secretion and urinary metabolite secretion.
- HuMap™ Hypothyroid could result in - higher cortisol, low cortisol metabolites.
  - Lower metabolites in general, across the report (slower metabolism)
  - Hyperthyroid could result in - high cortisol metabolites, elevated cortisone.
- NT testing
  - Low tyrosine could impact thyroid.
  - Hypothyroid = low dopamine, low serotonin, low GABA, and could increase toxicity of elevated glutamate.

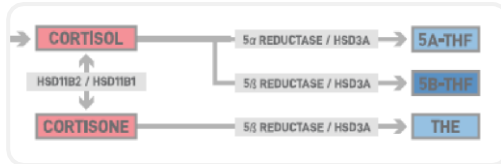


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5A-THF+5B-THF/THE <sup>‡</sup>	(Cortisol/Cortisone Metabolites)	0.832		0.7 – 1.4
Cortisol/Cortisone <sup>‡</sup>	(11B HSD activity)	0.638		0.24 – 0.45

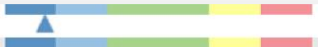
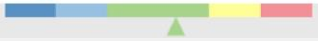


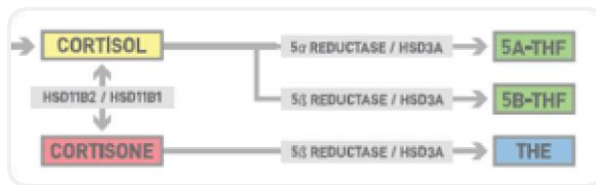
Possibly associated with hypothyroidism

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Cortisol/Cortisone <sup>‡</sup>	(11B HSD activity)	0.184		0.24 – 0.45
5A-THF/5B-THF ratio <sup>‡</sup>	(alpha vs beta metabolism)	0.902		0.4 – 1.4



Possibly associated with hyperthyroidism

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Analyte	Result	Unit per Creatinine	L	WRI	H	Reference Interval
Phenethylamine (PEA)	20	nmol/g	▲	■	■	42 – 160
Tyrosine	48	μmol/g	▲	■	■	70 – 180
Tyramine	3.4	μmol/g	▲	▲	■	2.8 – 8.5
Dopamine	258	μg/g	▲	▲	■	175 – 500
3,4-Dihydroxyphenylacetic acid (DOPAC)	1520	μg/g	▲	▲	▲	540 – 1850
3-Methoxytyramine (3-MT)	151	nmol/g	▲	▲	■	122 – 278
Norepinephrine	17.6	μg/g	▲	■	■	29 – 69
Normetanephrine	195	μg/g	▲	▲	■	112 – 400
Epinephrine	1.9	μg/g	▲	■	■	2.1 – 14.5
Metanephrine	84	μg/g	▲	▲	■	60 – 158
Norepinephrine / Epinephrine ratio	9.3			▲	■	< 13
Tryptamine	0.4	μmol/g	▲	■	■	0.65 – 1.6
Serotonin	84.4	μg/g	▲	▲	■	79 – 235
5-Hydroxyindoleacetic acid (5-HIAA)	5913	μg/g	▲	▲	■	2500 – 9000
Glutamate	23	nmol/g	▲	▲	■	18.0 – 70.0
Gamma-aminobutyrate (GABA)	5	nmol/g	▲	▲	▲	2.6 – 8.0
Glycine	966	nmol/g	▲	▲	■	700 – 2500
Histamine	12	μg/g	▲	■	■	14 – 51
Taurine	428	μmol/g	▲	▲	■	420 – 1400
Creatinine	73.1	mg/dL	▲	▲	■	25 – 180

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## Sex Hormones affect NT secretion and vice versa

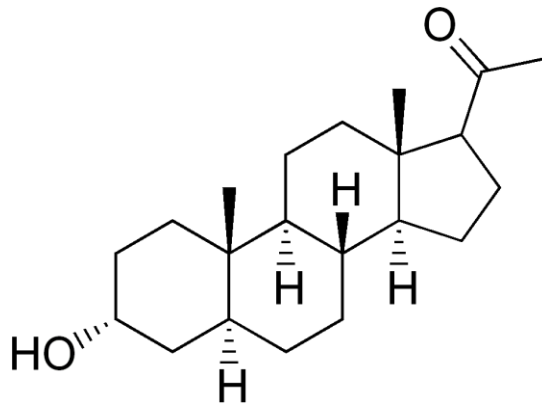
- Estradiol enhances serotonin and dopamine levels in males and females
- Progesterone (allopregnanolone) stimulates GABA receptors in males and females
- Dopamine enhances testosterone secretion in males (bidirectional)
- NE and insulin are increased in metabolic syndrome. Insulin drives the metabolic changes and NE drives the hypertension and impaired glucose metabolism.

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## Allopregnanolone and GABA

- Anxiolytic effects of allopregnanolone mediated by GABA A receptor



Bitran D, Hilvers RJ, Kellogg CK. Anxiolytic effects of 3 alpha-hydroxy-5 alpha[beta]-pregnan-20-one: endogenous metabolites of progesterone that are active at the GABA A receptor. *Brain Res.* 1991; 561: 157-61.

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## Estradiol therapy may raise serotonin levels.

- Amenorrheic, natural menopausal and ovariectomized women had lower blood serotonin levels than regularly menstruating women.
- **Treatment of menopausal women with estrogen increased the serotonin levels to values observed in regularly menstruating women.**

Gonzales GF, Carrillo C. Blood serotonin levels in postmenopausal women: effects of age and serum oestradiol levels. *Maturitas.* 1993; 17: 23-29

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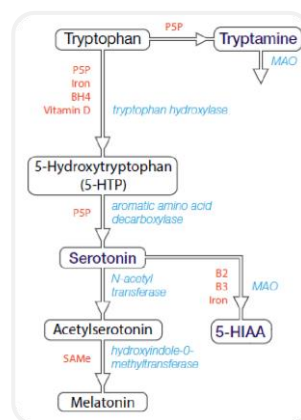
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## Estradiol increases serotonin synthesis and decreases its breakdown

- Higher levels of estradiol inhibit MAO leading to more serotonin available in the synapse.
- Estradiol supplementation increases tryptophan hydroxylase
- Increased serotonin activity improves cognitive performance and mood.



Amin Z, Canli T, Epperson CN. Effect of estrogen-serotonin interactions on mood and cognition. *Behav Cogn Neurosci Rev.* 2005; 4: 43-58.  
 Epperson CN, Amin Z, Ruparel K, et al. Interactive effects of estrogen and serotonin on brain activation during working memory and affective processing in menopausal women. *Psychoneuroendocrinology.* 2012; 37(3): 372-382  
 Klaiber EL, Broverman DM, Vogel W, et al. Effects of estrogen therapy on plasma MAO activity and EEG driving responses of depressed women. *Am J Psychiatry.* 1972 Jun;128(12):1492-8.  
 Paredes S, Cantillo S, Candido KD, Knezevic NN. An Association of Serotonin with Pain Disorders and Its Modulation by Estrogens. *Int J Mol Sci.* 2019;20(22):5729. Published 2019 Nov 15.

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## Testosterone as well as estrogen increases serotonin receptor mRNA and binding site densities in the male rat brain

- In a substantial number of men, there are significant changes in the H-P-testicular axis with age, resulting in a significant decline in free plasma testosterone.
- These **hormonal changes are accompanied by changes in mood and mental state.**
- **Estradiol and testosterone increase serotonin receptor binding sites in areas of the brain that are associated with cognition, mood and mental state.**
- The lack of effect of DHT, a potent androgen which cannot be converted to estrogen, suggests the action of testosterone depends upon its conversion to estrogen by aromatase.

Sumner BE, Fink G. Testosterone as well as estrogen increases serotonin<sub>2A</sub> receptor mRNA and binding site densities in the male rat brain. *Brain Res Mol Brain Res.* 1998 Aug 31;59(2):205-14.

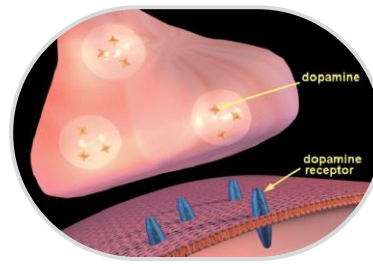
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## Estradiol and dopamine secretion

- Minor fluctuations in dopamine can profoundly alter memory.
- Estradiol has been shown to enhance dopamine activity and thus affect memory and mood.



Jacobs E, D'Esposito M. Estrogen shapes dopamine-dependent cognitive processes: Implications for women's health. *J Neurosci*. 2011 April 6;31(14):5286-5293.

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## Dopamine increases testosterone secretion

- The production of testosterone is controlled via a specific feedback loop called the hypothalamic-pituitary-gonadal axis.
- The hypothalamus secretes a gonadotropin-releasing hormone (GnRH), which signals the pituitary gland to secrete luteinizing hormone (LH), which in turn signals the gonads to produce testosterone.
- Researchers found that the administration of a dopamine agonist in rat brains increased the amount of GnRH mRNA by 67%, which is involved in the DNA replication of GnRH

Li S, Pelletier G. Role of dopamine in the regulation of gonadotropin-releasing hormone in the male rat brain as studied by in situ hybridization. *Endocrinology* 1992;131(1):395-399.

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## Dopamine and testosterone in male libido

- **Relationship is bidirectional**; dopamine can influence testosterone and testosterone can influence dopamine. In males, an important area of the brain for sexual function is the medial preoptic area (MPA).
- One study found that microinjecting dopamine agonists in the MPA of rats resulted in an increase of sexual activity
- Another study observed that castrated male rats showed no interest in fornicating and no dopamine release in the medial preoptic area. After testosterone injections, the castrated rats were able to engage in sexual intercourse and showed an increase in dopamine release in the MPA.

Dominguez JM, Hull EM. Dopamine, the medial preoptic area, and male sexual behavior. *Physiol Behav* 2005;86: 356-368  
Putnam SK, Du J, Sato S, Hull EM. Testosterone Restoration of Copulatory Behavior Correlates with Medial Preoptic Dopamine Release in Castrated Male Rats. *Hormones and Behavior* 2001;39(3):216-224.

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## Sex Hormone and NTs Summary

- Estradiol enhances serotonin and dopamine levels in males and females
- Progesterone (allopregnanolone) stimulates GABA receptors in males and females
- Dopamine enhances testosterone secretion in males (bidirectional)

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## Independent Influence of Insulin and Catecholamines on Metabolic Syndrome

- Metabolic syndrome is a state of sympathetic nerve hyperactivity.
  - Associated with elevations in NE and epi, but mostly NE.
  - Insulin and NE levels increased in Met Synd.
- This study suggests that insulin and NE cooperate independently to the development of metabolic syndrome.

De Pergola G, Giorgino F, Benigno R, et al. Independent influence of insulin, catecholamines, and thyroid hormones on metabolic syndrome. *Obesity (Silver Spring)*. 2008 Nov; 16(11): 2405-11.

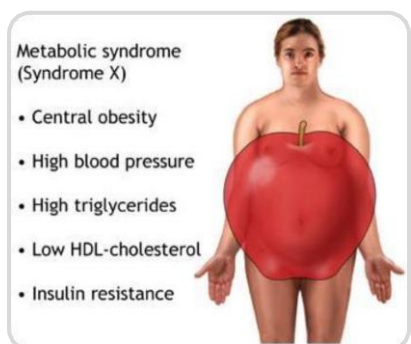
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## Independent Influence of Insulin and Catecholamines on Metabolic Syndrome

- Insulin and catecholamines likely cooperate to the development and clinical features of metabolic syndrome.
- Insulin seems to be the determinant for all the negative metabolic aspects, whereas catecholamines seem to be important for the development of hypertension and impaired glucose metabolism.



De Pergola G, Giorgino F, Benigno R, et al. Independent influence of insulin, catecholamines, and thyroid hormones on metabolic syndrome. *Obesity (Silver Spring)*. 2008 Nov; 16(11): 2405-11.

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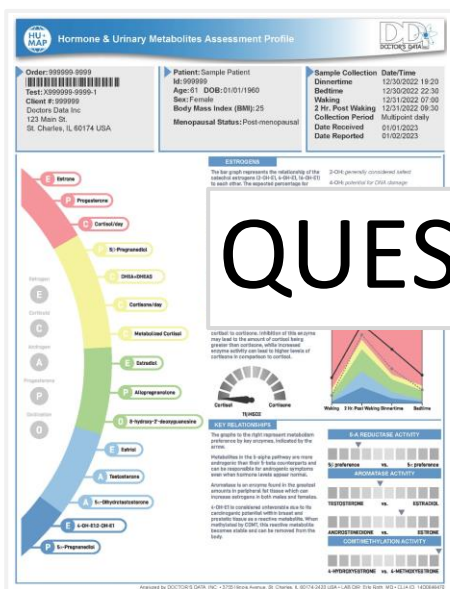
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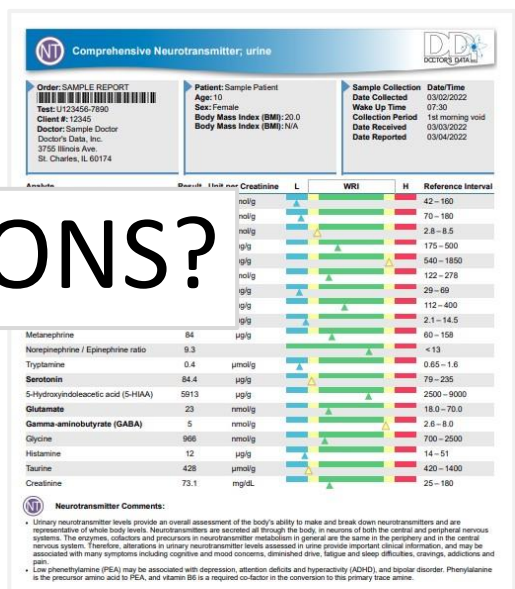
# Symptomology

- And if we just think about symptomology, working up hot flashes would benefit from both tests, as they are associated with estrogen, progesterone and serotonin imbalance.
- Anxiety, depression, and cognitive symptoms are associated with estrogen dominance, low progesterone, low estradiol, as well as various NT imbalances.
- Not all symptoms result in predictable patterns, so if there is the possibility of there being a neuroendocrine component to a symptom or pathology, test both!

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# QUESTIONS?



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