VibrantWellness

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Urine Hormones Interpretive Guide

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Table of Contents

Regulatory Statement

The content within this interpretive guide is not intended to be a stand-alone medical reference guide, nor is it intended to be a substitute for medical advice from a healthcare provider. The clients who receive Vibrant Wellness Urine Hormone test results are advised to consult their physician, and/or health care provider team for diagnosis and further follow up care.

This Urinary Hormones Interpretive Guide is intended to be used in tandem with Vibrant Wellness's Urine Hormone Test and this guide is provided to users pursuant to the Terms of Use Agreement (the "Terms") on its website www.vibrant-wellness.com.

Vibrant testing does not demonstrate absolute positive and negative predictive values for any disease state or condition. Its clinical utility has not been fully established. Vibrant validates the accuracy and precision of the testing but not of its clinical or diagnostic value. The Urine Hormone Test is advised to be used for wellness and informational purposes only.

Introduction to Vibrant America's Urine Hormone Test

Vibrant America's Urine Hormone test offers a comprehensive evaluation of urinary levels of steroid hormones along with the downstream compounds resulting from metabolism, known as urinary hormone metabolites. An analysis of hormone metabolites along with parent hormones can provide valuable insight into situational factors that influence hormone pathways such as nutrition and other lifestyle factors, genetics, environmental toxicities, and inflammation.

Vibrant's Urine Hormone test also offers adrenal hormone testing at 4 points throughout the day to provide diurnal values of free cortisol as well as free cortisone. Cortisol metabolites are also measured which provide a view of systemic cortisol production, metabolism, and related enzyme functions.

Furthermore, the Urine Hormone test provides diurnal 4-point melatonin values through measurement of the MT6s metabolite. Waking to bedtime analysis of melatonin output can be helpful for assessment of circadian rhythm balance as well as melatonin replacement.

Finally, the Urine Hormone test provides two additional tests, 8-OHDG, a marker of oxidative DNA damage and Bisphenol A (BPA) an estrogenic environmental toxicant. Both factors can help further inform detoxification needs and cancer prevention strategies.

What are Hormone Metabolites?

The process of metabolism of steroid hormones generates intermediate and conjugated compounds with active, systemic effects. Conjugated hormones are forms of hormones linked to glucuronide, sulfate, or glutathione and these are important for bioavailability, storage, cellular transport, and excretion.[1] Serum testing typically measures unconjugated forms of hormones, while urine testing captures conjugated forms which are deconjugated in the lab and free portions are added to free hormone totals.

Metabolites are products of various stages of hormone metabolism. Some metabolites are linked to increased harms such as carcinogenicity or excess hormonal effects. Other metabolites are linked to potentially protective benefits. Evaluation of both the enzyme pathways and metabolites provides opportunities for greater assessment of health risks as well as therapeutic lifestyle interventions.

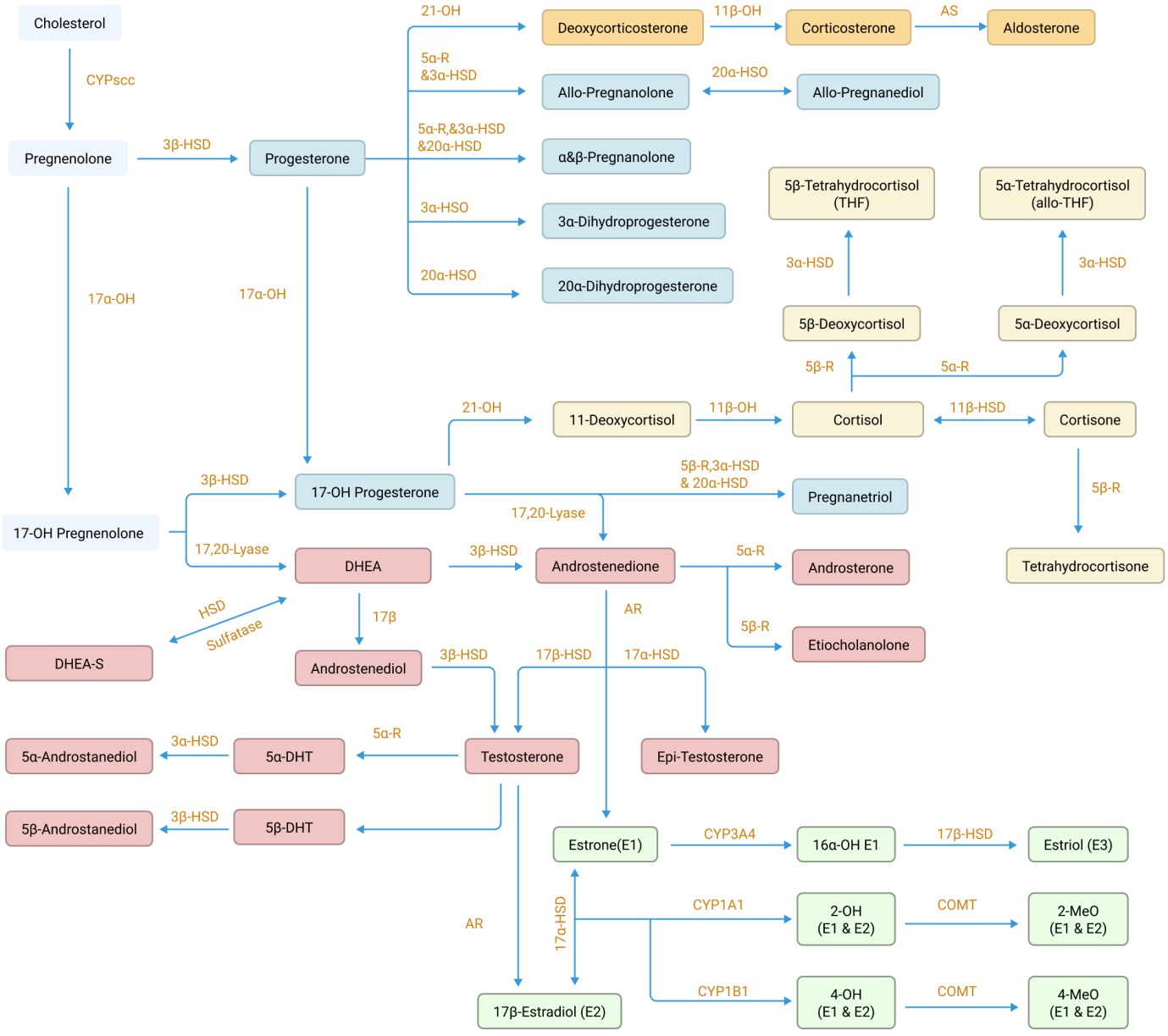
Lab Methodology of the Urine Hormones Test

Vibrant America utilizes liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure parent steroid hormones (Estradiol, Estrone, Estriol, DHEAS, Testosterone, Cortisol, Cortisone, etc.) All metabolites are measured using gas chromatography/mass-spectrometry (GC-MS). These technologies allow for simultaneous measurements of multiple analytes and have a low limit of quantification which means precision and accuracy despite low levels of hormone in the sample.

What Analytes are Included in the Urine Hormone Test?

Estrogens	Estradiol	4-OH Estrone
	Estrone	16a-OH Estrone
	Estriol	2-MeO Estradiol
	2-OH Estradiol	2-MeO Estrone
	2-OH Estrone	4-MeO Estradiol
	4-OH Estradiol	4-MeO Estrone
	4-OH Estrone	Bisphenol A
Androgens	DHEA	Etiocholanolone
	Testosterone	5α-DHT
	Epi-Testosterone	5α,3α-Androstanediol
	Androstenedione	5α-Androstanediol
	Androsterone	DHEA-S
Progestogens	Progesterone	20α-Dihydroprogesterone
	Allopregnanolone	β-Pregnanediol
	Allopregnanediol	α-Pregnanediol
	3α-Dihydroprogesterone	
Glucocorticoids	Cortisol	α-Tetrahydrocortisol (α-THF)
	Cortisone	β-Tetrahydrocortisone (β-THE)
	β-Tetrahydrocortisol (β-THF)	Corticosterone
Other	Melatonin	
	8-OHdG	

The Steroid Hormone Cascade



Androgens

Estrogens

Glucocorticoids

Mineralocorticoids

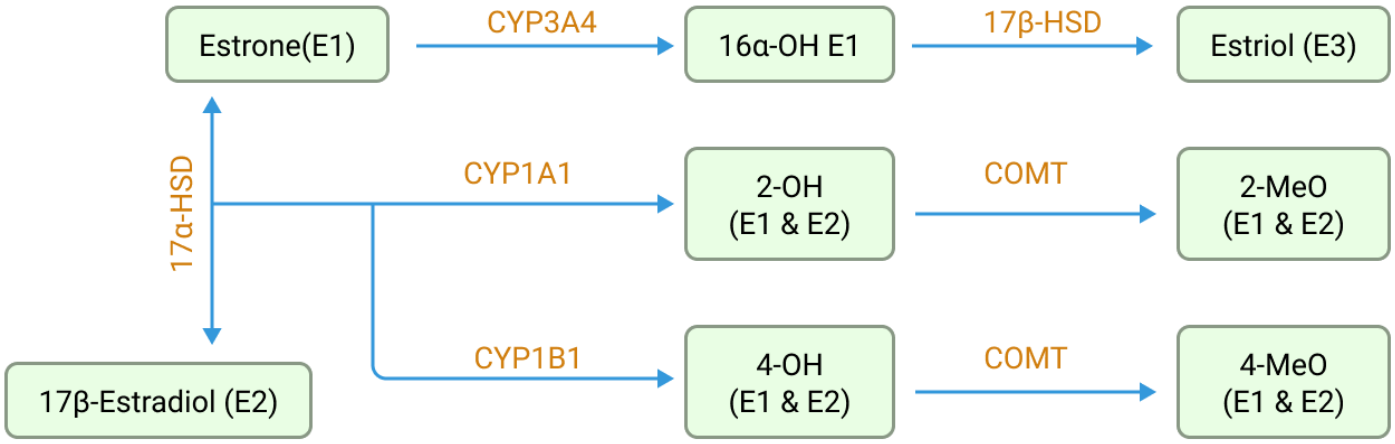
Progestogens

Enzyme Abbreviations

5α-R	*5α-Reductase*	**11β-HSD**	*11β-Hydroxysteroid dehydrogenase*
5β-R	*5β-Reductase*	**17α-HSD**	*17α-Hydroxysteroid dehydrogenase*
11β-OH	*11β-Hydroxylase*	**17β-HSD**	*17β-Hydroxysteroid dehydrogenase*
17α-OH	*17α-Hydroxylase*	**20α-HSD**	*20α-Hydroxysteroid dehydrogenase*
17,20-Lyase	*Same enzyme as 17α-OH*	**AR**	*Aromatase*
21-OH	*21-Hydroxylase*	**AS**	*Aldosterone Synthase*
3α-HSD	*3α-Hydroxysteroid dehydrogenase*	**CYP**	*Cytochrome p450 (scc, 1A1, 1B1 & 3A4)*
3β-HSD	*3β-Hydroxysteroid dehydrogenase*	**COMT**	*Catechol-O-Methyl-Transferase*

The Estrogens

Estrogens	Estradiol	4-OH Estrone
	Estrone	16a-OH Estrone
	Estriol	2-MeO Estradiol
	2-OH Estradiol	2-MeO Estrone
	2-OH Estrone	4-MeO Estradiol
	4-OH Estradiol	4-MeO Estrone
	4-OH Estrone	Bisphenol A

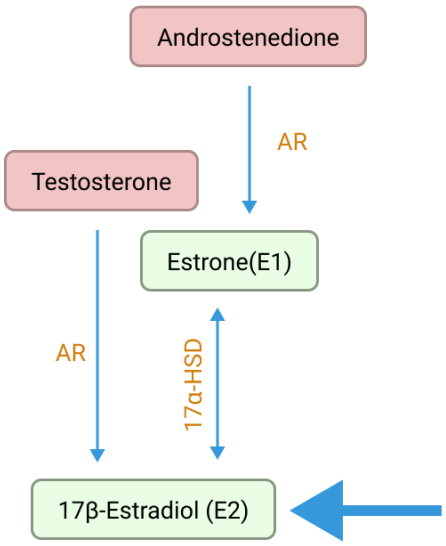


The Parent Estrogens: Estradiol (E2) and Estrone (E1)

Estradiol (E2)

Estradiol, commonly known as E2 or 17β-Estradiol, is the predominant and most biologically active estrogen in circulation in males and females. Estradiol is also the strongest estrogen and is 80 times more potent than estriol, the weakest estrogen.[2,3] Estradiol plays a key role in the development of the female reproductive system, including breast development, and has non-reproductive roles in cognition and neuroprotection, lipid and glucose homeostasis, adipose distribution, cardiovascular health, pancreatic cell function, bone maintenance and wound healing.[4] In males, E2 is predominantly produced by the testes.[4] Males and postmenopausal women have significantly lower levels of estradiol than premenopausal women.

E2 originates from cholesterol, which is converted through a cascade of progesterone and androgen intermediates, finally resulting in estradiol through aromatization from testosterone.[5] In pre-menopausal women this occurs primarily in the ovary. In postmenopausal women, estradiol is sourced directly from the peripheral tissues, predominantly from estrone, sourced from adrenal precursors, and converted to estradiol via 17β-HSD.[3,4]



Elevated E2

Known causes of increased estradiol as well as increased aromatase activity, which leads to increased estradiol, are listed in Table 1.0 below.

↑ Table 1. Causes of Increased E2

Known Causes of ↑ Estradiol	Known Causes of ↑ Aromatase Activity (which lead to ↑ E2)
Excess Aromatase Activity	Inflammation[6]
Chronic Liver Disease[7]	Age
Hyperthyroidism	Obesity
Dysregulated Gut Microbiome/Elevated Beta-Glucuronidase[6]	Insulin
Cancer Tissue	Gonadotropin Use
Exogenous Estrogen Use	Alcohol
	Rare Genetic Causes /CYP19A1 Rearrangement[8]

Conditions associated with prolonged high estradiol (E2)[4,5]

- PCOS
- Uterine Cancer
- Gastric Cancer
- Multiple Sclerosis
- Obesity
- Endometriosis
- Prostate Cancer
- Thyroid Cancer/Goiter
- Gynecomastia
- Breast Cancer
- Pituitary Cancer
- Systemic Lupus Erythematosus (SLE)
- Oligospermia
- Male Hypogonadism
- Breast Cysts
- Gallbladder Disease
- Fibroids
- Ovarian Cancer

Treatment Strategies for Increased Estrogens

Lifestyle interventions such as diet, supplements and detoxification strategies can improve overall estrogen metabolism and clearance from the body through several mechanisms.

- Reduce active estradiol, estrone and xenoestrogens through reduced exposure, increased detoxification and increased sex hormone binding globulin activity
- Target microbiome support for enhanced estrogen clearance and reduction of beta-glucuronidase
- Decrease aromatization from androgens to estrogens, especially with excess adiposity

Table 2. Lifestyle Interventions for Estrogen Reduction

Lifestyle Considerations	Reduction of Estrogens and Xenoestrogens	Support Microbiome for Estrogen Clearance	Decrease Aromatase Activity
Specific Supplements	**DIM** - up to 300mg per day[9] **Sulforaphane**[10]* **Vitamin D** Repletion in deficient women (to blood levels above 32ng/mL) ↑ SHBG and ↓ bioavailable E2 and testosterone[11]	**Lactobacillus spp**[12]* **Calcium D-glucarate** 1500mg or higher/day*	Numerous **flavonoids** show preclinical evidence of decreased aromatase activity: * **Resveratrol**[13] **Grape Seed extract**[14] **Citrus Peels/citrus flavonoids**[15] **Apigenin**[13] **Chrysin**[16]
Diet	**Brassica family vegetables** – 1.5 servings/day[11] **Wheat sprout juice** -100ml/day[17] **Broccoli sprouts**[10]*	**Fiber** from fruit/veggies - 30 g/day[18] **Flaxseed meal** – 10 -30 g/day[19], positive benefits with 50mg lignans per day **Kefir**[12]*	**White button mushrooms**[13]* **Foods with apigenin and/or resveratrol:** grapes, berries, celery, parsley, onions, oranges, chamomile, thyme, basil, oregano, red wine[20] Kale, collards
Exercise/Sauna	**Dry Sauna**- 15 minutes 3x per week* **Exercise** –100-300minutes/week treadmill/aerobic reduces estrogen[21]	**Moderate exercise** benefits microbiome diversity[22]	**150 minutes moderate activity** per week if weight loss is needed
Misc. Lifestyle Factors	Reduce or avoid exogenous HRT Avoid endocrine disruptors such as phthalates, BPA, growth hormones in meat, pesticides[23]		**Weight loss** if obesity or PCOS is present[24] Lifestyle support to reduce inflammation secondary to obesity[25]

*Dosage information for this indication is not well established, refer to empiric guidelines of safe and effective use

** Assess risk/benefit analysis for the specific individual prior to giving hormone replacement therapy

Further testing for further treatment avenues for consideration for estrogen excess:

1. **NutriPro or Methylation Panel** by Vibrant Wellness testing for genetic SNPs which may lead to estrogen metabolism issues (MTHFR, COMT)
2. **Gut Zoomer** test for beta glucuronidase, estrobolome characteristics
3. **Saliva Hormone** test for bioavailable hormone levels
4. **Environmental Toxins, Mycotoxins and/or Heavy Metals test**- for plasticizers, mold, metals and other environmental toxicant burden
5. **Vibrant America Cardiac Health and Diabetes Panel** for assessment of cardiometabolic function
6. **Neural Zoomer Plus or Neurotransmittter** test – progesterone is a neuroprotectant and neuromodulator, progesterone deficiency may affect these pathways

Decreased E2

Decreased levels of estradiol are most commonly caused by aging or ovariectomy, however there can be many causes from other mechanisms, such as decreased aromatase activity and increased prolactin levels, as outlined in Table 3.

↓ Table 3. Causes of Decreased E2

Systemic Causes of ↓ Estradiol	Causes of ↓ Aromatase Activity (which lead to ↓ E2)	Causes of ↓ Prolactin (which lead to ↓ E2)
Menopause/Aging	Aromatase Inhibitors	Antidepressants/Antipsychotics
Ovariectomy	Herbicides	Anticonvulsants
Compounds Which Cause Aromatase Inhibition	Agricultural Antifungals	Opiates
Compounds Which Increase Prolactin	Immunosuppressive Drugs	Hypothyroidism26
Hypogonadism	Antimalarials	Estrogens
Genetic Disorders/17α-hydroxy-lase/17,20-lyase deficiency[4]	Cigarette Smoke5	Anti-Androgens
	Anti-Mullerian Hormone*	Anti-Hypertensives
		H2 Receptor Antagonists

*A hormone produced by reproductive tissues, not currently measured at Vibrant Wellness

Conditions associated with prolonged low estradiol (E2)

- Alzheimer's Disease
- Osteoporosis
- Osteoarthritis
- Parkinson's Disease
- Diabetes Mellitus
- Eclampsia
- Colorectal Cancer

Treatment Strategies for Decreased Estrogens

Treatment Objectives - Consider which of the following single or combination of therapeutic approaches is the best fit for the patient with estrogen deficiency symptoms.

- Modulate and reduce vasomotor symptoms, insomnia, and mood disturbances
- Enhance quality of life during natural transitions in aging
- Prevent sequalae of severe estrogen deficiency conditions i.e., cognitive, bone, and cardiometabolic

Table 4. Lifestyle Interventions for Estrogen Deficiency

Lifestyle Considerations	Vasomotor Symptom and Mood Support
Specific Supplements	**Black Cohosh** for mood and hot flashes 40-127mg/day[27] **Soy Isoflavones** for vasomotor symptoms, bone loss, hypertension – trial doses ranged from 40-120mg isoflavones per day[28] Use organic, non-GMO isoflavones **Maca** 3.0 g/d for libido, depression in menopausal women; improvement in testosterone, but not estradiol, in women[29] **Kudzu** (Pueraria spp.)- may aid vasomotor symptoms, bone loss, vaginal symptoms – 20 mg to 2.5g/day in most human clinical studies for menopausal symptoms. Topical doses 0.5–1.0 g/day in gel[30-31] **Red Clover** for hot flashes ≥ 80mg/d[32] **Red Ginseng** 0.9-3mg/day for fatigue, memory, hot flashes[33] **Ashwagandha** 300mg 2x/day for vasomotor symptoms, increased estradiol in perimenopausal women in one study[34] **Vitamin E** 800 IU/day for hot flashes[35] **Fenugreek seed** 600mg/day de-husked seed extract for vasomotor symptoms[36]
Diet	**Mediterranean diet** to reduce menopausal symptoms[37] & cardiometabolic dysfunction[38] **Diet with increased fruits/vegetables, fiber & less sugar and fat** improves vasomotor symptoms[37] **High fiber and soy isoflavones** decrease vasomotor symptoms[37]
Exercise/Sauna	**150 minutes moderate aerobic + strength training**[39] - support for bone health and general estrogen deficiency symptoms **Yoga** variable practices aid vasomotor and psychological health[40]
Stress Management	**Cognitive Behavioral Therapy** for vasomotor symptoms and insomnia[41] **Hypnosis** for reduction in hot flash severity[42] **Aromatherapy/massage**[42] **Mindfulness Meditation** for distress from vasomotor symptoms[43] **Acupuncture** for hot flashes[44]

Miscellaneous Support	**Nutrient and Bone Support** via diet, supplements and exercise as indicated for osteoporosis prevention **Cardiometabolic** support via diet, exercise and supplements as indicated **ERT or HRT** - consider for severe symptoms or prevention of severe estrogen deficiency sequalae**

* Use of some supplements could result in specific hormone increases with use. Always research a specific product prior to use with patients, especially those at higher risk for hormone related cancer.

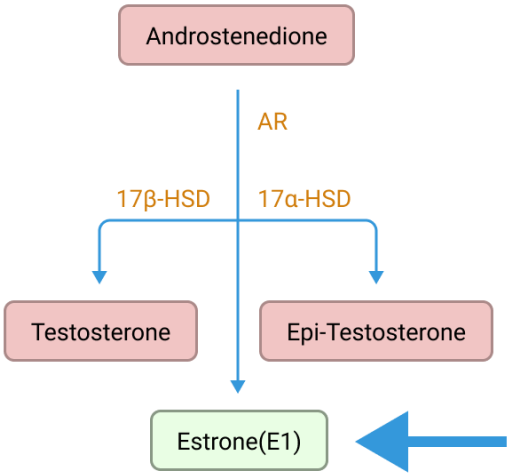
** Fully research the individual patient's risk/benefit analysis regarding hormone related cancer and other side effects or ERT or HRT prior to recommending.

Further testing considerations for estrogen deficiency:

1. **Cardiac Health Panel** and **CardiaX tests**
2. **Salivary Hormones** test for bioavailable hormone levels
3. **APOE** test for Alzheimer's Disease
4. **Colorectal cancer screening (Not available at Vibrant)**
5. **DEXA** testing for osteoporosis

Estrone (E1)

Estrone (E1) is a weaker estrogen than estradiol (E2); by some estimates estrone has less than 10% the strength of estradiol.[45] While levels of estrone do not differ significantly in pre- vs. post-menopausal women,[8] estrone is the predominant estrogen in post-menopausal women[46] by a factor of 100-fold compared to estradiol.[45] In post-menopausal women, estrone concentrations have been positively correlated with bone mineral density and breast cancer risk, and inversely correlated with colon cancer risk. Serum estrone levels are an important indicator of serum estradiol levels in post-menopausal women.[45]



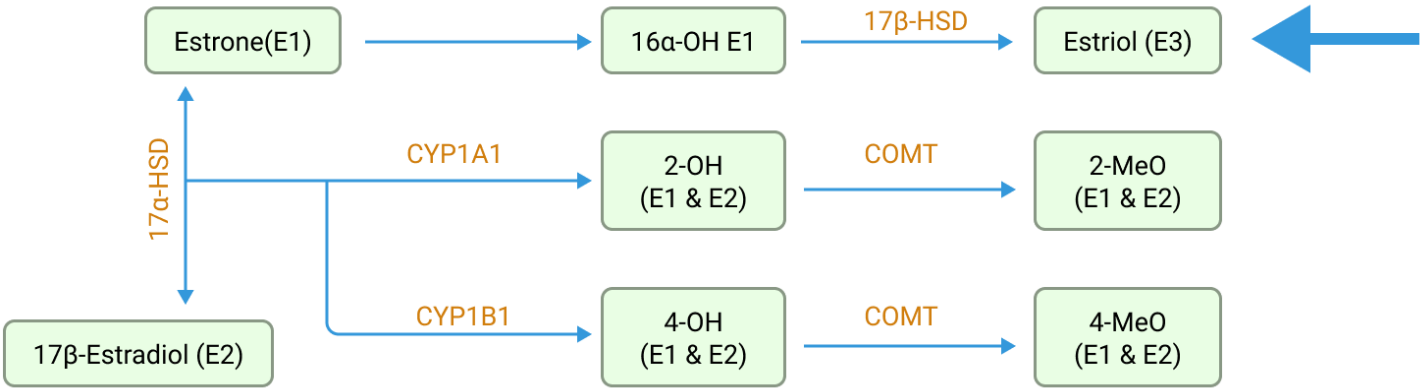
↑ Causes of Increased Estrone (E1):

- Hormone replacement therapy (HRT)
  - HRT can increase estrone, even if only estradiol is given, as estrone and estradiol can interconvert.[47]
  - Advanced age and obesity will further elevate estrone values in users of HRT.[48]
- Liver disease[49]
- Hyperthyroidism[50]
- Hormone producing tumors (ovary, adrenal gland, etc.) can also raise estrone[51,52]
- Refer to Table 2. in E2 section for lifestyle considerations to reduce excess estrogen if clinically indicated.

Causes of Decreased Estrone (E1):

- E1 levels appear to be similar in pre and postmenopausal women,[8] however there is wide variation in hormone levels in the normal range.
- Factors such as DHEA and testosterone levels, SHBG levels, genetic predisposition, adiposity, and aromatase activity affect estrone levels.
- Low estrone levels, below reference ranges, often result from decreased aromatase activity.
  - Refer to the Table 3. in E2 section of this guide for substances that can decrease aromatase activity
  - Refer to Table 4. in the E2 section for lifestyle considerations to manage low estrogen symptoms if clinically indicated.

Estriol (E3)



What is Estriol (E3)?

Estriol or E3, originates from cholesterol and androgen intermediates, notably aromatization from testosterone and androstenedione. Estradiol is reversibly oxidized to estrone and most E3 is formed from estrone via CYP3A4 metabolism and 17β-HSD as seen in the image above.[53] Both E2 and E1 can be irreversibly converted to estriol in the liver. E3 is the weakest of the three estrogens: it dissociates rapidly from estrogen receptors.54 Estriol is the predominant estrogen of pregnancy where it regulates uterine/placental blood flow and placental vascularization. Lab testing for E3 has been most used as a maternal screening for fetal anomalies.[54] E3 is used off-label for menopausal symptoms such as hot flashes, vaginal atrophy, and bone density. It has also been studied for use of immunomodulation and neuroprotection in multiple sclerosis and protection from atherosclerosis.[53]

Causes of Increased Estriol (E3):

- Pregnancy
- Use of bioidentical hormone replacement prescriptions[55]
- Conditions that create elevated estradiol may also increase estriol, refer to E2 section for further reference.

Causes of Decreased Estriol (E3):

- During pregnancy, low estriol is a marker of fetal compromise, genetic disorders, fetal adrenal deficiency, fetal growth restriction, congenital adrenal hyperplasia, aromatase deficiency and other fetal conditions.[56]
- Low estradiol conditions can contribute to a low estriol status in non-pregnant women, for further reference refer to E2 section for causes and lifestyle considerations for low estrogen.

E3/(E1+E2) Ratio

What is the Ratio of E3/E1-E2?

The ratio of estriol (E3) to the sum of estrone (E1) + estradiol (E2) is known as the EQ or estrogen quotient. This equation was popularized by Henry Lemon, MD, a breast cancer researcher. The estrogen quotient quantifies the concept that a higher ratio of estriol relative to the two stronger estrogens has value for both prevention and outcomes in breast cancer. From his research with 24-hour urine samples in female participants, he suggested that a ratio value of E3/E1+E2 of 1 or 1.5 is more optimal.[57]

Vibrant America's urine hormone test reference range for the ratio of E3/E1+E2 is based on average values found in samples from healthy women of varying ages. The high and low results from these reference ranges on the urine hormone test are not based on Dr. Lemon's findings in urinary estrogens. Thus, the ratio of E3/E1+E2 is provided for comparative use of estrogens only. Clinical application of the results should be based on the provider's discretion.

Lifestyle Considerations for Low Estriol Ratio - E3/E2+E1

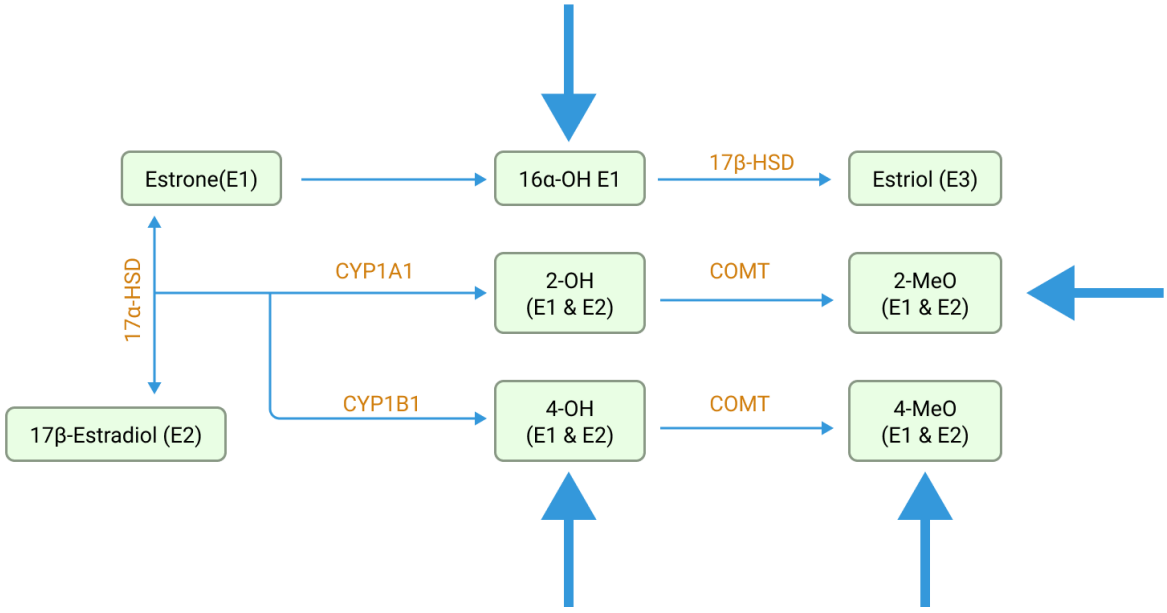
<u>Treatment objectives for a low E3/E2+E1 ratio</u>

- Minimize exposure to exogenous estrogens
- Assess and correct for any pre-existing iodine deficiency
- Refer to Table 3. Lifestyle Interventions for Estrogen Reduction and Metabolism

Integrative medicine physicians have used iodine therapy empirically to balance effects of a low E3/E2+E1 ratio and potentially reduce risk of breast disease. This has been based on combining ideas from human studies regarding estriol risk and fibrocystic breast disease and breast cancer,[57,58] as well as pre-clinical evidence that iodine/iodide can modulate estrogen metabolic pathways to favor inactivation of E2.[59,60] Further clinical trials are needed.

Iodine interventions, beyond diet suggestions for deficiency, require physician oversight including measures such as initial thyroid and iodine testing (available at Vibrant), ongoing monitoring, and careful consideration of dose, form, and length of iodine usage. Iodine excess may precipitate hyperthyroidism, hypothyroidism, goiter, and/or thyroid autoimmunity.[61]

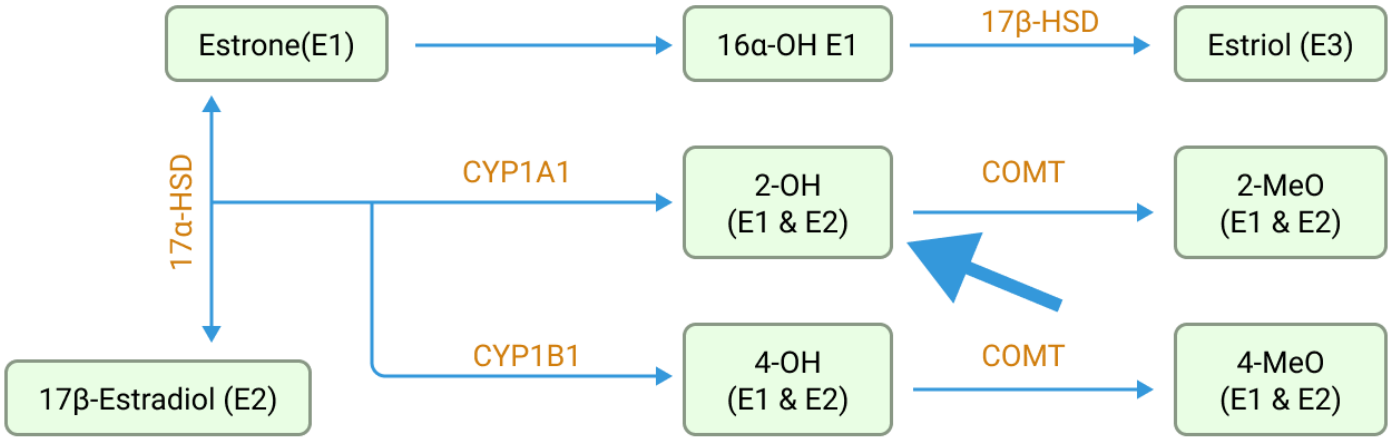
The Estrogen Metabolites



Phase I Estrogen Metabolism

While estrogen is crucial for female and some aspects of male development; the metabolites of estrogen can have negative as well as positive effects. Phase I metabolism of estrogen is an oxidative process that involves hydroxylation reactions to render the metabolites hydrophilic rather than lipophilic. The specific hydroxylation reactions that occur are either induced or inhibited by the cytochrome P-450 (CYP) enzyme system and affected by compounds that affect that system. These reactions produce estrogen metabolites. Modulation of the estrogen hydroxylation reactions is necessary, as some metabolites can promote disease through increased estrogenic effects and/or carcinogenesis.[62] Carcinogenicity of estrogens can result from the trifecta of epigenetic effects, direct estrogen receptor effects and metabolite effects. Estrogen metabolite effects cause harm through metabolites binding to estrogen receptors, which cause effects of increased cell proliferation, and production of reactive quinones which cause DNA damage. There are 13 known estrogen metabolites that can be produced from hydroxylation at the 2, 4 or 16 carbon of the steroid ring.[63] These hydroxylation pathways of estrogen metabolism are termed the 2-hydroxylation pathway (2-OH), the 4-hydroxylation pathway (4-OH), and the 16-hydroxylation pathway (16-OH).[62] The 2-hydroxyestrogen metabolites (2-OH) along with the 4-hydroxyestrogen metabolites (4-OH) are known as the catechol estrogens.

The 2-OH Metabolites: 2-OH Estradiol and 2-OH Estrone



Estrogen metabolism produces a larger amount of 2-OH and 16-OH metabolites and a lesser number of 4-OH metabolites. 2-OH metabolites are regulated by CYP1A1 enzymes.[62] 2-OH is generally considered a safer metabolic pathway regarding the potential for harmful intermediate production. There are a few reasons for this theory. First, 2-OH metabolites have a significantly reduced binding affinity for the estrogen receptor. Furthermore, some early evidence indicates that 2-OH metabolites may act as tumor suppressors to inhibit breast cancer cell growth and proliferation.[64] Finally, increased 2-hydroxylation of parent estrogens, rather than 4-or 16-hydroxylation, is associated with reduced risk of breast cancer.[65] It is, however, known that the 2-OH pathway metabolites can form increased reactive oxygen species and can also result in the formation of a low level of depurinating adducts.[66,67] Thus, though considered safer, 2-OH metabolites should be kept in balance. Prompt methylation of these intermediates in phase II metabolism via the COMT enzyme pathway is the optimal detoxification step.

Irregular levels of 2-OH intermediates suggest irregular levels of parent estrogens (E1, E2), and/or increased or decreased activity of enzyme pathways (CYP1A1 or COMT) which precede or follow 2-OH production.

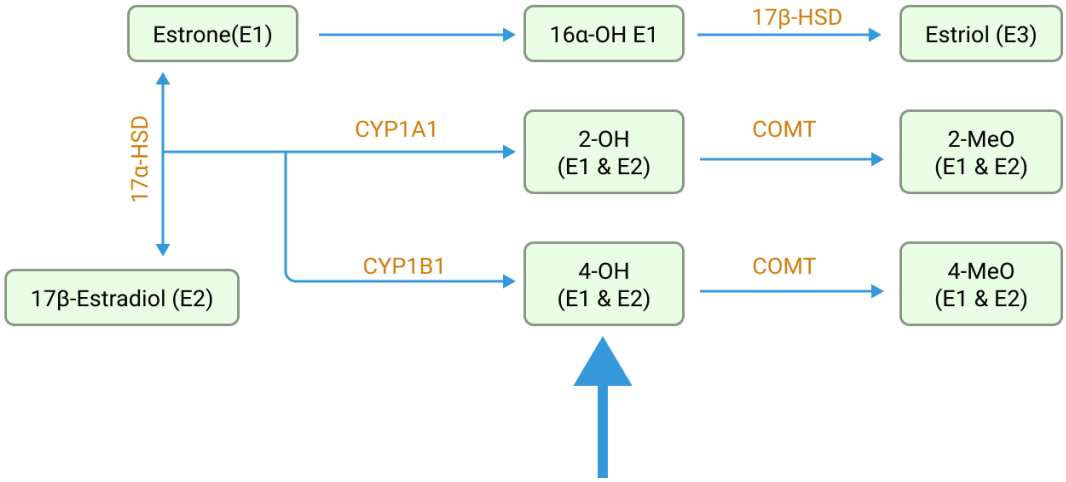
↑ Potential Causes of Increased 2-OH Estradiol and 2-OH Estrone

- Increased endogenous hormone production or exogenous hormone exposure
- Increased CYP1A1 enzyme activity relative to CYP3A4 or CYP1B1 enzyme pathways
- Decreased COMT activity and decreased production of 2-MeO metabolites
  - Consider COMT support if there are increased 2-OH metabolites and sub-optimal levels of 2-MeO metabolites
    - Support for COMT function can be gained through optimization of nutrient cofactors and methyl donors, such as increasing intake of foods high in methionine, methyl vitamin B12 (methylcobalamin), pyridoxal-5-phosphate (P5P), betaine, choline, folate (5-methyltetrahydrofolate), and magnesium.[68]
- Conditions that create elevated estrogens may also increase 2-OH metabolites
  - Refer to Table 2.0 in E2 section for lifestyle considerations to reduce excess estrogen if clinically indicated.

↓ Potential Causes of Decreased 2-OH Estradiol and 2-OH Estrone

- Decreased endogenous levels of estradiol and/or estrone
- Decreased induction of CYP1A1 enzyme activity
- Increased induction of alternate pathways of CYP3A4 or CYP1B1 enzymes
- Increased COMT activity and increased production of 2-MeO metabolites
- Are 2-OH metabolites decreased along with decreased E1 and/or E2 and estrogen deficiency symptoms?
  - Conditions that create decreased overall estrogen may also decrease 2-OH metabolites.
    - Refer to Table 4.0 in the E2 section for lifestyle considerations to manage low estrogen symptoms if clinically indicated.
- Are 2-OH metabolites decreased in combination with elevated E1 or E2, and/or increased 4-OH or 16-OH values?
  - Consider the following strategies to promote optimal estrogen metabolism and increase 2-OH pathway metabolism:
    - Refer to Table 2. in E2 section for lifestyle considerations to reduce overall estrogen burden
    - Interventions that have been studied to increase 2-OH metabolism include:
      - o Cruciferous vegetable compounds such as Indole-3-carbinol, DIM (diindolylmethane), and sulforaphane[69]
      - o Flax[70]
      - o Isoflavones (Soy, Kudzu, Clover)
      - o Resveratrol[71]
      - o Omega 3 Fatty Acids
      - o Coffee[72]
      - o Weight Loss[73]
      - o Moderate Exercise
      - o Rosemary[74]

The 4-OH Metabolites: 4-OH Estradiol and 4-OH Estrone



4-OH metabolites are the product of estrogen metabolism governed by the CYP1B1 enzyme pathway. 4-OH metabolites are considered an unfavorable estrogen intermediate. 4-OH metabolites are easily oxidized to quinone and semi-quinone conjugates, which can react with DNA and lead to cancer initiating mutations.[75] Elevated levels of depurinating estrogen-DNA adducts have been found in women with breast, ovarian and thyroid cancer. Men with prostate cancer or non-Hodgkin lymphoma also have high levels of estrogen-DNA adducts.[76]

In a recent comprehensive review, urine 4-OH metabolites were found to be significantly associated with breast cancer risk in premenopausal women. Furthermore, pre-clinical evidence links increased expression of CYP1B1, which produces 4-OH metabolites, with increased breast cancer risk.[77]

↑ Potential Causes of Increased 4-OH Estradiol and 4-OH Estrone

- Elevated 4-OH-E1 can occur more commonly in pre/peri-menopausal women transitioning to menopause.
- Increased endogenous hormone production or exogenous hormone exposure
  - Conditions that create elevated estrogens may also increase 4-OH metabolites
  - Refer to Table 2.0 in E2 section for lifestyle considerations to reduce excess estrogen if clinically indicated.
- Downregulation of CYP 3A4 or CYP1A1 pathways of enzyme metabolism
- Decreased COMT activity and/or decreased methylation to 4-MeO metabolites
  - Consider lifestyle support to increase COMT activity
    - Support for COMT function can be gained through optimization of nutrient cofactors and methyl donors, such as increasing intake of foods high in methionine, methyl vitamin B12 (methylcobalamin), pyridoxal-5-phosphate (P5P), betaine, choline, folate (5-methyltetrahydrofolate), and magnesium.[78]
- As 4-OH metabolites can react with DNA and initiate carcinogenic mutations, consider lifestyle support for harm mitigation from quinone conjugates/DNA adducts.
  - The substances below boost detoxification of quinone conjugates via increased activity of NQO1 (NAD(P)H Quinone Dehydrogenase 1 gene) and/or glutathione conjugation
    - o Resveratrol76
    - o N-Acetyl Cysteine (NAC)76
    - o Glutathione76
    - o Sulforaphane79

- Upregulation of CYP1B1 activity
  - Refer to CYP1B1 section below
    - o Weight loss, reduced pollutant exposure, and plant compounds such as stilbenes, flavonoids and more, have been shown to reduce expression of CYP1B1.

Potential Causes of Decreased 4-OH Estradiol and 4-OH Estrone

- Decreased estrone or estradiol can result in decreased 4-OH metabolites
  - Conditions that create decreased overall estrogen may also decrease 4-OH metabolites
    - Refer to Table 4.0 in the E2 section for lifestyle considerations to manage low estrogen symptoms if clinically indicated.
- In general, lower levels are preferred based on current understanding of estrogen metabolism pathways.

A Note about CYP1B1

CYP1B1 metabolizes environmental pollutants and plant flavonoids but also endogenous substances such as melatonin, retinoic acid and other fat-soluble vitamins, fatty acids, and steroid hormones.[80] It plays a role in homeostasis of glucose, weight, redox reactions, and blood pressure and supports eye development. Overexpression of CYP1B1 can have harmful effects such as increased production of genotoxic catechol estrogens, promotion of cancer and potentially other harms.81 Cancerous tumors overexpress CYP1B1, and research has been done on inhibition of this enzyme as a cancer therapeutic target.

What Causes Increased CYP1B1?

CYP1B1 can be overexpressed by increased leptins/obesity, polyaromatic hydrocarbons, cigarette smoke, inflammatory cytokines, cancerous tissue, UV exposure, biotin supplementation, environmental pollutants, and genetic polymorphisms[82-84]

What can inhibit CYP1B1?

The following compounds demonstrate an inhibitory effect on CYP1B1 in pre-clinical studies:

- Stilbene compounds such as:
  - Resveratrol and piceatannol found in grapes, berries, peanuts[85]
- Flavonoids such as:
  - Kaempferol (flavonoid found in foods such as broccoli, apples, strawberries, tea, and beans[85]
  - Quercetin[85]
  - Rutin[86]
  - Apigenin[86]– found in foods such as parsley, celery, basil, chamomile, cilantro, and oregano
  - Naringenin (flavonoid found in citrus, tomatoes, figs)[85]
- Coumarins which are found in cherries, cassia cinnamon, apricots and strawberries
- Anthraquinones which are laxative compounds found in aloe, rhubarb and senna[87]
- Melatonin[86]

What are quinone conjugates and DNA adducts and why are they important?

The catechol estrogens, 2-OHE1 or 2-OHE2 and 4-OHE1 or 4-OHE2 have 2 hydroxyl groups that are generally methylated by the enzyme COMT into 2 and 4 -methoxy forms of estrogens. However, remaining unmethylated hydroxyestrogens, if further oxidized can generate compounds called semiquinones which are further oxidized

into quinones. Semiquinones and quinones can become a part of redox cycling processes and generate reactive oxygen species, which can be neutralized, or if not, they can bind to DNA to form stable or depurinating adducts.

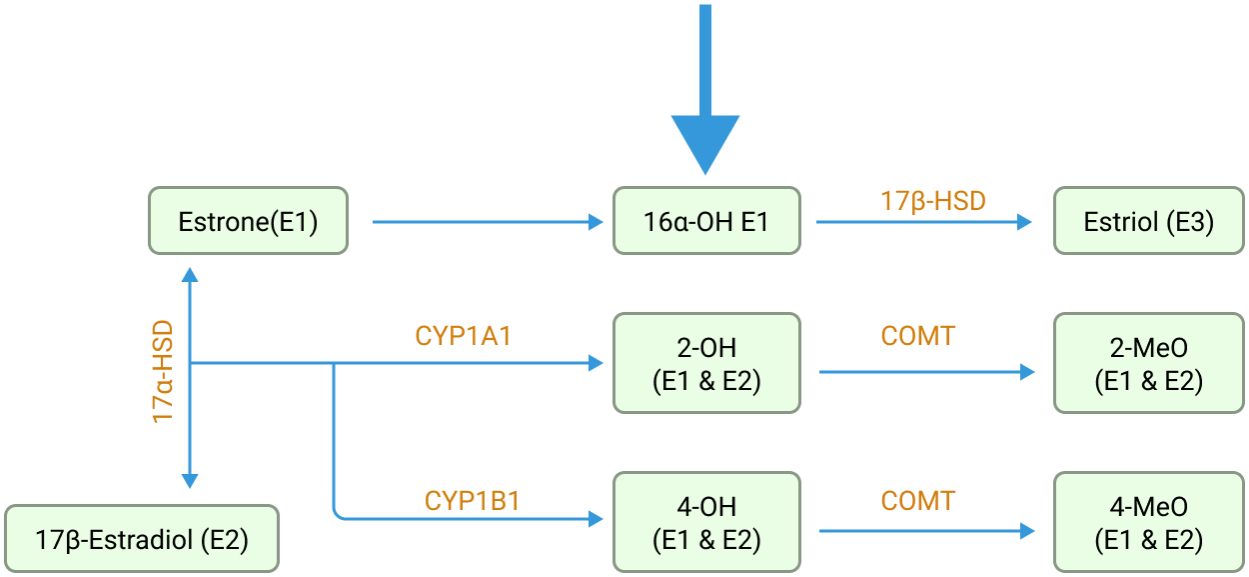
The depurinating estrogen adducts from 4-OHE1 or 4-OHE2 are called estrogen (E1 or E2) -3,4-quinones and they are known to become carcinogens. Estrogen-3,4 quinones induce DNA mutations in which errors form in the DNA repair process. Depurinating estrogen adducts can be initiators of many types of cancers, such as breast, ovarian, thyroid, non-Hodgkin lymphoma, and prostate cancer.[88]

The good news is that cells have several protective mechanisms to maintain estrogen metabolism homeostasis and avoidance of carcinogenic damage to DNA. Transformation of catechol estrogens to methyl estrogens through COMT inactivates carcinogenic potential. Also, estrogen-3,4 quinones can be reduced by NQO1 back to catechol estrogens or be conjugated with glutathione to prevent DNA adduct formation. Thus, increasing methylation, glutathione activity and/or NQO1 pathway activity are all ways to decrease carcinogenicity.[89]

Pre-clinical evidence indicates the following compounds can boost detoxification of quinone conjugates via increased activity of NQO1 and/or glutathione conjugation:

- Resveratrol[88]
- N-Acetyl Cysteine (NAC)[88]
- Glutathione[88]
- Sulforaphane[89]

16α -OH Estrone – 16α-OH-E1



The Phase I metabolism of estrone via hydroxylation at C-16 yields the product of 16α-OH-E1. Reduction of 16α-OH estrone through 17β-HSD1 enzyme activity produces estriol. Both 16α-OH-E1 and estriol are considered estrogenic. 16α-OH-E1 irreversibly and strongly binds to the estrogen receptor, which promotes estrogenic effects. It has been shown to have a mitogenic, growth enhancing, effect in breast cancer cells.[90] Pre-clinical evidence shows that 16α -OH-E1 can cause the formation of depurinating DNA adducts.[91] Similar to estrogen, 16-OH E1 appears help to protect against osteoporosis.[92]

An epidemiologic study of premenopausal women showed that an increased ratio of 16α -OH pathway metabolites to parent estrogens was associated with increased risk of breast cancer.[93] Contrary to this, more recently in postmenopausal women, higher 16α -OH levels, relative to estrone and estradiol, were found to be associated with a non-statistically significant, decreased risk of breast cancer.[94] Varying lab technologies may have confounded the issue and further research is warranted.

↑ Causes of Increased 16a-OH Estrone

- Increased Estrone production
- Increased CYP3A4 metabolism
- Reduced activity of 17B-HSD1 which converts 16-OH to estriol
- Refer to Table 2.0 in E2 section for lifestyle considerations to reduce excess estrogen if clinically indicated
- Enhance estrogen metabolism via 2-OH pathways if clinically indicated

↓ Causes of Decreased 16-OH Estrone

- Decreased levels of estrogen and/or estrone

2-OH (E1 + E2)/16a-OH E1 Ratio

This test measures the ratio of 2-hydroxy estrogens compared to 16 hydroxy estrone. Given that 2-OH estrogens are believed to be less estrogenic than 16 hydroxyestrogens, it follows that a higher 2-OH ratio would be preferred when considering the broad category of estrogen related diseases. Numerous studies have shown that a low ratio of 2-OH/16a-OH is an independent risk factor for breast cancer.[95] Numerous studies have also shown that diet and lifestyle can lower this ratio.

↓ Causes of Decreased 2-OH/16-OH Ratio

- Breast cancer risk factors
- Estrogen excess related diseases
- Alcohol[95]
- Obesity and increased BMI[95]
- Consider lifestyle interventions to increase 2-OH pathway metabolism and increase the 2-OH/16OHE1 ratio, if clinically indicated, such as:
    - o Cruciferous vegetables[96,97]
        - - Indole-3-Carbinol
        - - DIM – di-indolylmethane (studies reference doses of 100-400mg/day)
        - - Sulforaphane
    - o Flax[98] (study dose: 25grams of flax per day)
    - o Isoflavones[99] (study dose: 107mg soy isoflavones/day)
    - o Resveratrol[100] (study dose: 1gram/day)
    - o Coffee[101] (study dose: ≥4 cups/day)
    - o Weight Loss[102]
    - o Rosemary[103]

↑ Causes of Increased 2-OH/16-OH Ratio

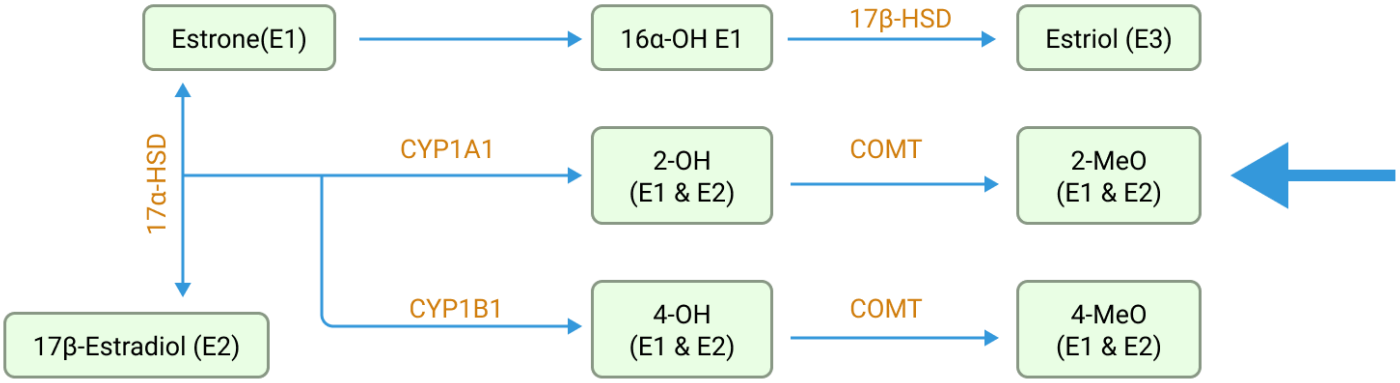
- Generally considered favorable unless estrogen deficiency related diseases, such as osteoporosis, are clinically relevant

Phase 2 Estrogen Metabolism

Metabolism of catechol estrogens in Phase 2 metabolism follows the hydroxylation step in Phase 1. Biochemical processes of methylation, sulfation, glucuronidation and glutathione conjugation help to biotransform estrogen metabolites to inactive states and into hydrophilic compounds in preparation for excretion. The actions of enzymes COMT, GST and NQ01 are notably protective in detoxification of catechol estrogens as well as protection from carcinogenic adducts.[104] COMT is present in many human tissues, notably the liver and breast, but also the kidney and red blood cells. Genetic polymorphisms in the COMT gene can result in up to a 4-fold decrease in enzyme activity. This is meaningful as it is hypothesized that there is an increased risk for breast cancer in individuals with low COMT activity, due to an increase in accumulation of harmful estrogen intermediates.[105]

Phase 2 Estrogen Metabolism

The 2 Methoxyestrogens - 2-MeO Estradiol, 2-MeO Estrone



Following CYP1A1 conversion to 2-OH metabolites, the next metabolic step for these intermediates is Phase 2 detoxification and methylation via the COMT enzyme pathway. 2-OH metabolites are converted into the 2-methyoxyestrogens, 2-MeO E2 and 2Me-O E1. 2-MeO E2 appears to have a low affinity for the estrogen eceptor and has shown anti-cancer effects in preclinical studies through promotion of apoptosis and anti-angiogenesis.[106,107] These studies have generated much interest in this molecule as a potential anti-cancer agent. 2-MeO E2 also has demonstrated protective cardiovascular effects and has been shown to have a negative feedback inhibition of CYP1B1 activity.[108]

↑ Causes of Increased 2-MeO Metabolites

- High levels of parent estrogens and/or 2-OH metabolites
- Increased COMT activity
- Generally considered favorable, however can be a signal to reduce estrogen therapy if consistent with additional estrogen elevations (E1, E2, 2-OH metabolites)

↓ Causes of Decreased 2-MeO Metabolites

- Low levels of parent estrogens or 2-OH metabolites
- Reduced COMT activity/methylation
    - o Consider addition of methyl donors (See COMT Support section)

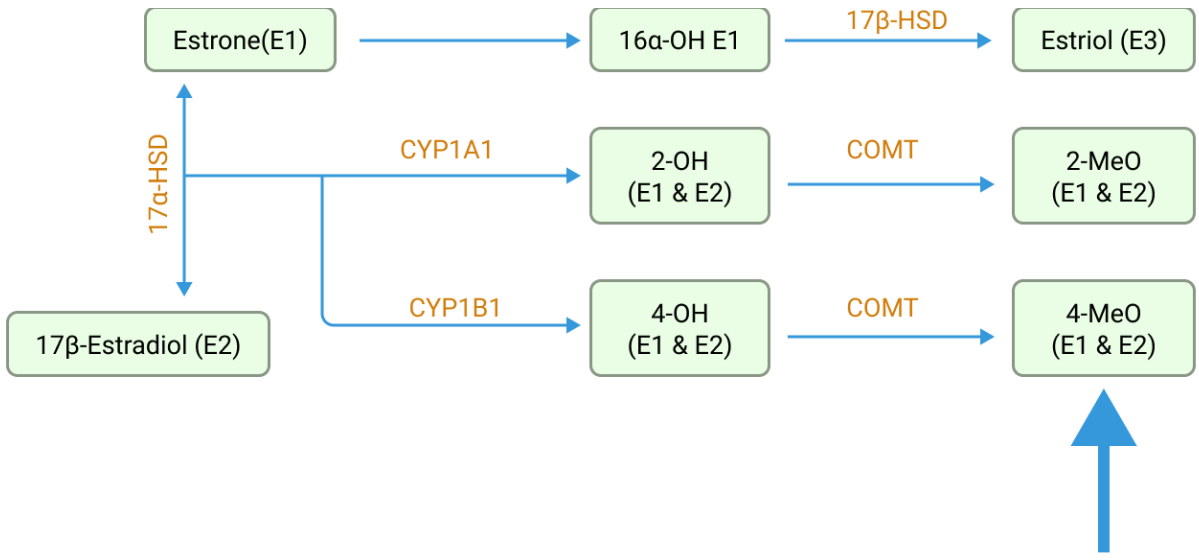
COMT Support

Catechol-O-methyltransferase (COMT) is an important phase II enzyme that inactivates the harmful potential of catechol estrogens through the methylation and formation of methoxy estrogens. Support for COMT function can be gained through optimization of nutrient cofactors and methyl donors, such as increasing intake of foods high in methionine, methyl vitamin B12 (methylcobalamin), pyridoxal-5-phosphate (P5P), betaine, choline, folate (5-methyltetrahydrofolate), and magnesium.[109] Also notable is that pre-clinical research suggests that a high sucrose diet may inhibit COMT activity.[110]

Table 5. Food Sources of Nutrients to Aid Methylation and COMT Enzyme Activity

Nutrients	Food Sources
Methionine	Turkey, pork, chicken, beef, fish, egg, Brazil nuts, soybeans, sesame and pumpkin seeds, teff, oats, spirulina[111]
Vitamin B12	Meats and meat products (especially liver and kidney), poultry, fish, shellfish, eggs[111]
Vitamin B6	Poultry, pork, beef, lentils, sunflower seeds, amaranth, garlic, prunes[111]
Betaine	Quinoa, beets, spinach, whole grains (especially rye, kamut, bulgur, amaranth, barley, and oats) sweet potato, meats, poultry[111]
Choline	Poultry, soy, egg, salmon, quinoa, wheat germ, milk, cauliflower, peas, tofu, broccoli[112]
Folate	Spinach, legumes, liver, sunflower seeds, quinoa, asparagus, avocados, mustard greens, artichokes[111]
Magnesium	Nuts, seeds, legumes, whole grains[111]

The 4 Methoxyestrogens - 4-MeO Estradiol and 4-MeO Estrone



As indicated previously, 4-OH metabolites from CYP1B1 enzyme activity are considered an unfavorable estrogen intermediate due to potential for quinone conjugates which can cause DNA damage and promote carcinogenicity. The solution for inactivation of 4-OH metabolites is rapid methylation of these products in Phase 2 with the formation of the 4-methoxyestrogens, 4 Me-O estradiol and 4-Me-O estrone. The methylation of 4-OH intermediates is governed by COMT and requires substrates such as Vitamins B6, B12, folate and betaine. COMT activity can be affected by genetic polymorphisms and nutrient status and thus can vary greatly among individuals. 4-Methoxyestrogens have been shown to have neutralizing activity on the harmful effects of 4-OH metabolites via prevention of oxidative damage.[113] There have also been other benefits shown by 4-MeO estrogens. In a pre-clinical study, 4-MEO's showed a strong neuroprotective effect against neuronal oxidative damage, stronger than parent estrogens.[114]

↑ Potential Causes of Increased 4-MeO Metabolites

- Generally considered beneficial
- Increased 4-OH intermediates resulting in efficient methylation
- Increased COMT activity from genetic polymorphisms or increased demand

↓ Potential Causes of Decreased 4-MeO Metabolites

- Decreased 4-OH intermediates
- Decreased or inefficient COMT enzyme activity from genetic polymorphism or decreased demand
    - o Consider addition of methyl donors to increase 4-MeO metabolites (See above COMT Support section)
- Use ratio of 4-MeO/4-OH estrogens to aid in assessment of COMT methylation activity

Ratio of 4-MeO E1/4-OH E1 and 4-MeO E2/4-OH E2

↑ Causes of Increased 4-MeO/4-OH Ratios

Methylation of the 4-hydroxyestrogens is beneficial as this renders them inert and prevents estrogen quinone formation. A higher ratio is related to a lower risk of breast cancer.

- Elevations of the ratio of methylated estrogens to catechol estrogens generally indicate increased COMT activity and methylation.
    - o Thus, the conversion of catchol estrogen into methylated versions is considered optimal for reduction of carcinogenic potential.
- Causes are similar to causes of increased 4-MeO metabolites, refer to 4-MeO section.

↓ Causes of Decreased 4-MeO/4-OH Ratios

- Low ratios of methyoxylated estrogens compared to catechol estrogens indicate reduced or inadequate methylation. This may confer risks of increased DNA damage from oxidative stress of catechol estrogens and quinone conjugates leading to increased breast, and potentially additional, cancer risks.[115]
- Causes of a reduced ratio are similar to causes of decreased 4-MeO metabolites, refer to MeO section

Additional Estrogen Related Markers

Bisphenol A

Bisphenol A (BPA) is an industrial chemical which is produced in large volumes primarily for polycarbonate plastics and epoxy containing products. Humans have become ubiquitously exposed to BPA through food packaging, paper receipts, sports equipment, medical and dental supplies, and subsequent contamination in dust, food, and water. BPA is a known 'endocrine disruptor'. BPA has a low binding affinity with estrogen receptors, however, is thought to be able to stimulate estrogenic effects at low doses. BPA monitoring and avoidance is advised for conditions where estrogen excess related illness is a concern.[116] In addition to estrogenic concerns, studies show BPA exposure promotes the development of obesity and may play a role in the development of diabetes, hypertension, male and female infertility, hormone dependent tumors, PCOS, liver inflammation, COPD, cardiovascular disease as well as increased all-cause mortality.[117,118]

↑ Potential Causes of Increased BPA levels

Increased levels of BPA can indicate recent and/or ongoing exposure to plastic or epoxy resins. Items that frequently contain BPA include the following:

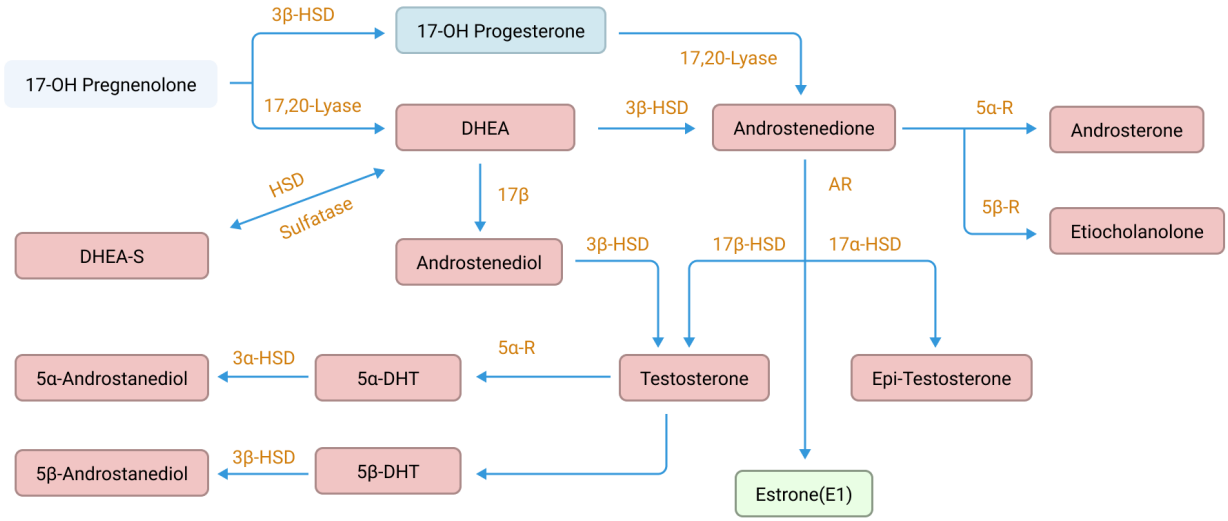
- Water bottles (#3, 6, or 7), children's toys, food can linings, food packaging, food storage containers, eyeglass lenses, baby bottles and nipples, water supply pipes, medical equipment, sports safety equipment, electronic devices, CD/DVD discs, thermal paper receipts, and dental sealants[119]
- Lifestyle considerations for elevated BPA
    - o BPA avoidance is advised wherever possible.
    - o Antioxidants
        - Curcumin, NAC, melatonin, selenium, alpha lipoic acid, vitamin A, quercetin, lycopene, and vitamin E have shown mitigation of BPA harms in pre-clinical evidence.[118,120]
    - o Increased elimination
        - Sauna has shown the ability to increase excretion of BPA through the skin[121]
        - Wheat sprout juice (Triticum aestivum) (100ml/day) consistently reduced urine BPA levels in a cohort of young women, suggesting potential detoxification of BPA-toxicity via antioxidative mechanisms and interference of absorption and distribution.[122]

Total Estrogen

The total estrogens are the sum of all estrogens and estrogen metabolites. High or low levels of these values can be used in conjunction with individual estrogen values as part of an overall estrogen dominance or deficiency evaluation.

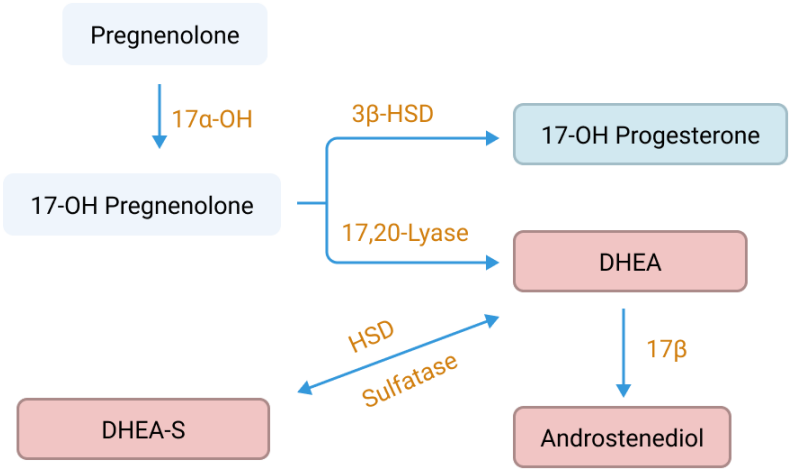
Androgens

Androgens	DHEA	Etiocholanolone
	Testosterone	5α-DHT
	Epi-Testosterone	5α,3α-Androstanediol
	Androstenedione	5α-Androstanediol
	Androsterone	DHEA-S



Total Estrogen

DHEA and DHEA-S are quantitatively the most abundant steroid hormones in circulation in humans. DHEA is considered slightly androgenic while DHEA-S is relatively inactive at the steroid receptor. However, DHEA, and inactive DHEA-S converted to active DHEA, serve as a precursor to androgens and estrogen in the periphery.[123] Generally, the level of DHEA and androgen metabolites should trend in similar directions. If a single androgen metabolite is markedly elevated or decreased, then consideration can be given to possible inhibition or upregulation of the enzymatic pathway regulating the metabolite.



DHEA

DHEA begins with the conversion of cholesterol into pregnenolone by the mitochondrial enzyme p450scc. Pregnenolone is then converted into 17-OH pregnenolone by a 17α-hydroxylase reaction. The 17,20-lyase reaction follows which converts 17-OH pregnenolone to DHEA.

DHEA can be either converted to DHEA-S or go through reactions to produce other androgens, such as androstenedione, etiocholanolone and androsterone, as well as estrone. DHEA also plays a role in the physiology of adipose and studies indicated it can reduce adipose tissue mass, inhibit the proliferation of adipocytes and may protect against obesity.[124]

DHEA-S

DHEA-S occurs by the sulfation of DHEA and this process is catalyzed by the enzyme hydroxysteroid sulfotransferase (HST, SULT2A1), commonly known as DHEA sulfotransferase. DHEA-S can also be converted back into DHEA by steroid sulfatase (STS). DHEA-S is the sulphated, most abundant, version of DHEA. Compared to DHEA, DHEA-S it has a longer half-life, does not have diurnal variation, and provides a stable circulating pool from which to measure adrenal androgen activity. Thus, DHEA-S it is often used to measure effects of DHEA supplementation. As mentioned, DHEA and DHEA-S can interconvert through enzymatic actions.

While DHEA-S has little androgenic capacity, it is known to be active as a neurosteroid and a buffer from the effects of oxidation and glucocorticoids.[125]

Typically, females are referred for DHEA/DHEA-S testing for virilization and/or PCOS evaluation. Males are referred for DHEA/DHEA-S testing for congenital adrenal hyperplasia, primary or secondary adrenal insufficiency, adrenal tumors hypertension and alopecia.[126]

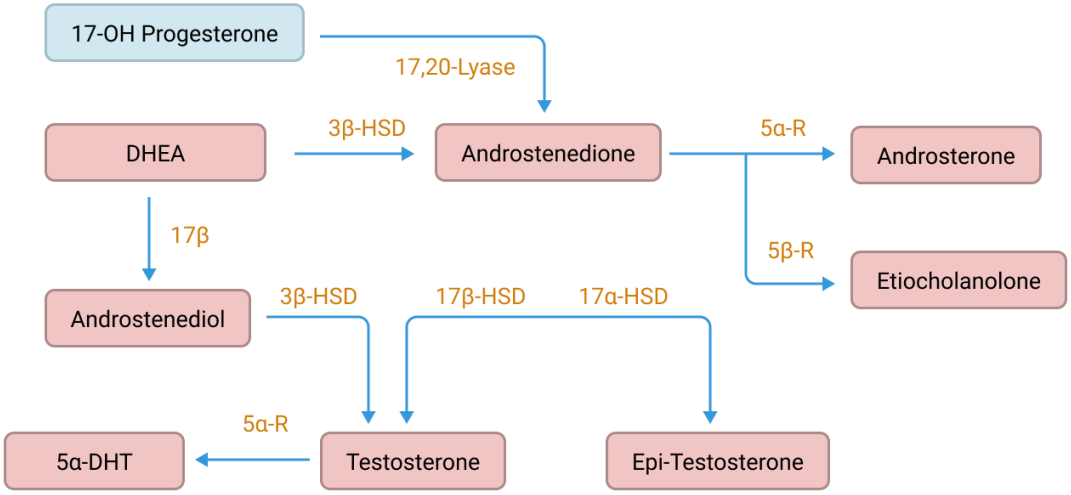
↑ Potential Causes of High DHEA/DHEA-S

- DHEA and DHEA-S can be mildly elevated for idiopathic reasons
- Exogenous supplementation
- Androgen secreting adrenal tumor
- Elevated cortisol
- PCOS
- Steroid sulfatase (STS) deficiency (in cases of DHEA-S elevations)[127]
- Precocious puberty
- Congenital adrenal hyperplasia[128]
- If DHEA is high but DHEA-S is low – consider polymorphisms or inflammation as a cause of reduced DHEA sulfotransferase enzyme activity
- Lifestyle considerations for androgen excess can be seen in Table 6.0 in the Testosterone Section.

↓ Potential Causes of Low DHEA/DHEA-S

- Biological aging in males and females
- Chronic stress
- Chronic inflammation[129]
- Primary and secondary adrenal insufficiency
- Hypothyroidism
- Anencephaly in a developing fetus
- Drugs include carbamazepine, dexamethasone, opioids, phenytoin.[128]
- Lifestyle considerations for androgen deficiency, if clinically relevant can be seen in Table 7.0 in the Testosterone Section.

Androstenedione (A4)



Androstenedione is a key intermediate and hormone precursor of estrone and testosterone. Androstenedione is synthesized from DHEA and then either converted into testosterone through the action of 17β- hydroxysteroid dehydrogenase (17β -HSD), or to estrone via the aromatase enzyme (AR) complex. It is primarily secreted by the adrenal gland and governed by ACTH. It can be secondarily secreted in the testes or ovaries from DHEA-S. Males rapidly convert androstenedione to testosterone in the testes. Females use androstenedione as a precursor for all estrone production and about half of testosterone production.[130] Androstenedione is often measured as a diagnostic aid for hyperandrogenism, premature adrenarche, and diagnosis and monitoring of congenital adrenal hyperplasia (CAH). It was once sold as a popular athletic enhancer, however, since 2004, has been classified as a Schedule III-controlled substance and is not available for over the counter or prescription use. In peripheral tissues, testosterone can be converted back to the inactive androstenedione by 17β -HSD-2, as a means to regulate intracellular androgen levels.[131]

↑ Causes of Increased Androstenedione

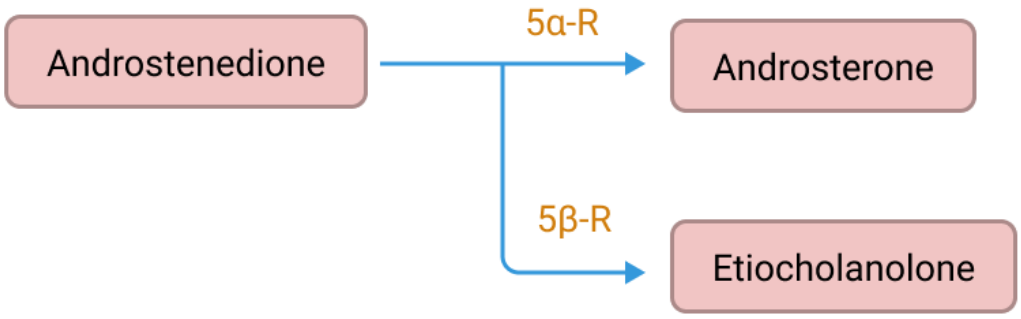
- Mild elevations are usually idiopathic in men
  - o Peripheral conversion to estrogens may produce gynecomastia in men
- May relate to insulin resistance and PCOS in women
- Marked elevations can be caused by adrenal gland disorders
  - o Adrenal tumors, congenital adrenal hyperplasia,[132] Cushing's syndrome
- Androstenedione producing gonadal tumors (testes, ovaries)
- Genetic polymorphisms: CYP19, CYP17A1[133]
- Exogenous hormone or dietary supplement use
- Elevated 17-beta dehydrogenase-2 which increases peripheral testosterone inactivation to androstenedione
- Lifestyle considerations for androgen excess, if clinically relevant, can be seen in Table 6.0 in the Testosterone Section.

↓ Causes of Decreased Androstenedione

- Aging
- Adrenal insufficiency - low cortisol production
- Osteoporosis

- Medications: such as glucocorticoids and testosterone replacement therapy
- Poor nutrition: A diet that is low in essential vitamins and minerals can also lead to reduced androstenedione
- Rare causes include 17Beta HSD-3 deficiency, STAR (steroidogenic acute regulatory protein) deficiency and 17-α -hydroxylase deficiency
- Lifestyle considerations for androgen deficiency, if clinically relevant can be seen in Table 7.0 in the Testosterone Section

The Metabolites of Androstenedione: Androsterone and Etiocholanolone



Assessment of androstenedione metabolites can provide insight around androsterone and etiocholanolone levels as well as insight into 5α and 5β-reductase activities. 5α-reductase, as a precursor to androgenic metabolites, is often a therapeutic target for androgen excess conditions, while 5β-reductase yields less androgenic effects.

Androsterone

Androsterone is a metabolite of androstenedione, governed by enzymatic effects of 5α-reductase. It is one indicator of 5α-reductase activity. Androsterone is thought to be devoid of androgenic activity. Androsterone has been found to have neurosteroid effects and anticonvulsant properties through modulation of the GABA receptor.[134] Androstenedione, the parent compound of androsterone and etiocholanolone, along with both androsterone and etiocholanolone, have been found to be increased in androgenic alopecia.[135]

↑ Causes of Increased Androsterone

- Increased levels of DHEA
- Increased levels of androstenedione
- Increased activity of 5α-reductase

↓ Causes of Decreased Androsterone

- Decreased levels of DHEA
- Decreased levels of androstenedione
- Decreased activity of 5α-reductase
    - o Finasteride is a 5α -reductase inhibiting drug, used for alopecia and benign prostatic hypertrophy (BPH), which can cause decreased androsterone[136]

Etiocholanolone

Etiocholanolone is a metabolite of androstenedione, governed by the enzymatic actions of 5-beta reductase. It is thought to be one indicator of 5-beta reductase activity. It does not have androgenic action and is classified as a ketosteroid. It has a role in fever production through stimulation of interleukin-1 and other pro-inflammatory cytokines from leukocytes.[137]

Both etiocholanolone and androsterone have been found to have neurosteroid effects and anticonvulsant properties through modulation of the GABA receptor.[134] Also, both androsterone and etiocholanolone, along with androstenedione, have been found to be increased in androgenic alopecia.[138]

Causes of Increased Etiocholanolone

- Increased levels of DHEA
- Increased levels of androstenedione
- Increased activity of 5-beta reductase

Causes of Decreased Etiocholanolone

- Decreased levels of DHEA
- Decreased levels of androstenedione
- Decreased activity of 5-beta reductase

If either metabolite of androstenedione is high while the other is low, then the enzymatic pathway leading to that metabolite can reflect an upregulation in that preferred metabolic pathway. For example: high androsterone level with low etiocholanolone level may indicate that 5-alpha-reductase pathway is more upregulated or active. The 5-alpha- reductase pathway also governs DHT metabolite production, which is androgenic, so looking at these two hormones, alongside glucocorticoid metabolites, may provide helpful information (see insert on 5α-reductase enzymes below).

Spotlight on 5α-Reductase Enzymes

The 5α-reductase family of NADPH dependent enzymes reduces several steroids to more potent forms. Much is known about the 5α-reductase pathway due to its role in converting testosterone to the more potent dihydrotestosterone (DHT), primarily in target tissues such as skin, liver, and prostate. Pharmaceutical targeting of this pathway is common for treatment of BPH and lower urinary tract symptoms in men and androgen stimulated skin/hair disorders in both genders.

Less is commonly known about the role of 5α-reductase reduction activities in progesterone, glucocorticoid, and mineral corticosteroid pathways. Research on finasteride, a prescription 5α-reductase inhibitor, has indicated several systemic side effects resulting from actions across multiple hormone targets during and after 5α-reductase inhibition therapy. In addition to expected androgen related sexual health side effects, effects also include cognitive complaints, gynecomastia, depression and suicidality, anxiety, and headaches, among other complaints.[139,140]

Natural compounds with 5α-reductase inhibitory activity have this action through diverse plant constituents and mechanisms. Consequently, many natural compounds with 5α-reductase activity do not appear to have the same severity of side effects when used in moderate dosages.[141]

How Do You Evaluate 5α-Reductase Levels?

Evaluation of the ratio of metabolites produced from 5β-reductase and 5α-reductase pathways can highlight which may be more metabolically active. Vibrant's Urinary Hormones Panel measures the following metabolites for comparison.

- Androgen Metabolites: Androsterone vs. Etiocholanolone, Testosterone vs. 5α-DHT
- Glucocorticoid Metabolites: Ratio of α -Tetrahydrocortisol to β -Tetrahydrocortisol[142]
- Progesterone Metabolites: 5-α Pregnanediol vs. β-Pregnanediol

What is Associated with High Levels of 5α-Reductase?

- Obesity[143]
- Insulin resistance
- Elevated DHEA
- PCOS
- Hirsutism

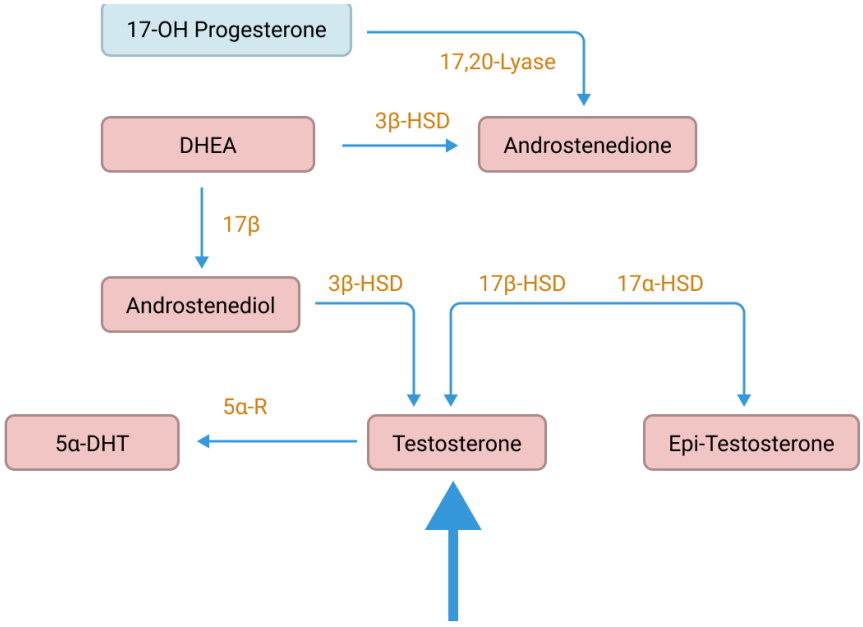
Natural Compounds with 5α-Reductase Inhibition Activity

- Flax Lignan[144] (Typical dose 10—30g/day)
- Saw Palmetto [145,146] (Typical dose 160mg/2x per day)
- Stinging Nettle[145]*
- Free Fatty Acids[145]* (i.e., GLA, DHA)
- Green Tea, Quercetin, and other Polyphenols[147]*
- Reishi Mushroom[148]*
- Zinc[149]*
- Pumpkin Seed Oil[150]*

*Dose which increases 5α-reductase inhibition activity is not well established

Testosterone (T)

Testosterone is produced primarily by the gonads, i.e., testes and ovaries, in men and women. Secondarily, it is produced by the adrenal glands, as well as in peripheral tissues from metabolites originating in gonads and adrenal glands. The main precursor is DHEA, which is converted to androstenedione or androstenediol by the enzyme 3ß-HSD2, and then to testosterone via 17ß-HSD or 3ß-HSD2 respectively.



Testosterone is the predominant androgen produced in males and females. In both males and females, testosterone supports reproductive function and libido, maintains muscle mass and bone structure, supports cardiac health and promotes optimal brain function.151 In males, at puberty, testosterone promotes the development of the male sexual organs and secondary sex characteristics such as deep voice, body hair and libido. It also contributes to the anabolic status of tissues such as red blood cells, muscle mass, linear growth, and bone density.[152]

In females, ovarian production of testosterone increases during the follicular phase of the menstrual cycle and reaches the highest levels at ovulation and the luteal phase. In advancing age, ovarian production of testosterone decreases gradually throughout age, rather than suddenly in a menopausal transition.[153] Nonetheless, by menopause total testosterone levels in women aged 65–74 years is approximately one-third that observed in women who are aged 20 years.[151]

The benefit of urinary testosterone testing is that it is more closely correlated with serum free testosterone rather than total testosterone levels. Levels of serum total testosterone are often influenced by sex hormone binding globulin levels which are affected by numerous conditions such as obesity and metabolic syndrome, thyroid disorders, steroid use, PCOS, pregnancy, etc.[154]

↑ Potential Causes of High Testosterone

- Endogenously high testosterone in males is uncommon, however can be a result of precocious puberty, adrenal hyperplasia or tumor, testicular tumor, and CNS lesion.
- Exogenous supplementation from prescriptions or adulterated over the counter “libido boosting” or “male enhancing” supplements
- In females, high testosterone can be a result of idiopathic hirsutism, polycystic ovary syndrome, abnormal menstrual cycles, congenital adrenal hyperplasia, ovarian tumors, and intersex physical characteristics.
- Cigarette smoking due to a nicotine metabolite which inhibits androgen breakdown
- Pollutant exposure (polychlorobiphenyls, hexachlorobenzene, etc.)
- Eating disorders
- Drugs - anticonvulsants, atrial natriuretic hormone, barbiturates, cimetidine, clomiphene, estrogens, gonadotropin (males), kaliuretic hormone, oral contraceptives, and vessel dilator hormone[155]

↓ Potential Causes of Low Testosterone

- There can be many common causes of low testosterone such as: Increased body mass, heavy alcohol use, hypopituitarism, hyperprolactinemia, hypothyroidism, and late-onset hypogonadism (andropause).156 In women, oophorectomy and menopause are also common contributing causes.[155]
- Other causes can be the following: cirrhosis, COPD (moderate to severe), Klinefelter syndrome, Down syndrome, obstructive sleep apnea, end-stage renal disease, adrenal insufficiency, epilepsy, trauma to gonads or head, hemochromatosis, human immunodeficiency virus, and male hypogonadism.
- Drugs which contribute to low testosterone include anabolic steroids, cyproterone, dexamethasone, diethylstilbestrol, digitalis, digoxin (males), estrogen therapy (increases SHBG), ethyl alcohol, glucose, glucosteroids, gonadotropin-releasing hormone analogs, finasteride, halothane, ketoconazole, metoprolol, metyrapone, opioids, phenothiazines, spironolactone, and tetracycline.[155]
- It is important to note that individuals with a polymorphism of the UGT2B17 gene have low or undetectable urinary testosterone levels, thus in these individuals’ serum or saliva testing of testosterone will provide a more accurate result in free testosterone levels.

Treatment Considerations for High Testosterone and Low Testosterone

Testosterone/Androgen Excess

Treatment Objectives: Consider which of the following single or combination of therapeutic approaches is the best fit for the patient with symptoms related to androgen excess.

- Reduce circulating free androgens
- Increase insulin sensitivity
- Increase sex hormone binding globulin (SHBG) to decrease circulating free androgens
- Weight loss if obesity is present

Table 6. Holistic Treatment Considerations for Androgen Excess

Lifestyle Considerations	Reduce Free Androgens/Improve Insulin Sensitivity	Interventions to Increase SHBG (Sex Hormone Binding Globulin)
Specific Supplements	**NAC** in PCOS patients 600mg/3x per day[157] **Vitamin D** 4000 IU/day158 Additionally, repletion in deficient women (to blood levels above 32ng/mL) ↑SHBG and ↓bioavailable E2 and testosterone[11] **Myo-inositol** 4g/day or **D-chiro inositol** 1g/day[159-161] **Soy isoflavones** 50g/day[161] **Berberine** 400mg/TID – improves insulin sensitivity[162] **Chromium*** Improve insulin resistance, may lower DHEA levels in adipose tissue in PCOS patients[163] **Zinc, Magnesium and Selenium** to counter deficiencies in diet[164]	**DIM** 300mg/day[165] **Refer to Insulin Modulating interventions** increased insulin decreases SHBG[166]
Diet	**DASH Diet** reduce insulin resistance promotes weight loss in PCOS[167] **Mediterranean Diet** reduces insulin[167] **Calorie Restriction Diets** reduce insulin resistance promotes weight loss in PCOS[167] **Low Glycemic/Ketogenic diet**[164] **Almonds** 46 g/d[168] **Walnuts** 36 g/d[168] **Prebiotics/ Probiotics*** -Increased microbiome diversity[169] **Soy isoflavones in diet** 50g/d[161] **Spearmint tea**[164] **Green tea**[164] **Licorice tea**[164] **Flax seed meal** 30g/day[170]	**Coffee, Tea and other caffeine containing beverages**[171]* **Walnuts** 36g/day[168] **Soy milk** 30g/day[171] **Olive Oil**[173]* **High Fiber diet, vegetarian diet**[173] **Insulin Modulating interventions** as increased insulin decreases SHBG[174]

Exercise/Sauna	**Vigorous aerobic exercise – minimum of 120 minutes per week alone or with resistance training** reduce insulin resistance in PCOS, aid body composition[164]	**Moderate intensity exercise** 225 min/week[175]
Misc. Factors	**Optimizing sleep** aids with regulation of dysglycemia[164]	**Drugs that increase SHBG include** Tamoxifen and other selective estrogen-receptor modulators (SERM's), oral estrogen, metformin, and anti-seizure medications

* Dosage information is variable or not well established for this specific benefit; refer to empiric or published standards of safe use

Additional treatment options for androgen excess:

- If hirsutism is present, and signs of elevated 5α-reductase upregulation are shown, consider modulation of this pathway, see 5α-reductase insert.
- Pharmacologic therapy such as spironolactone, or spironolactone in combination with licorice[132]

Further testing considerations for androgen excess:

- **Saliva Hormone** test to determine bioavailable and tissue levels of hormones
- **Environmental Toxins and Heavy Metals** tests to assess xenoestrogen compounds
- **Cardiac Health and Diabetes Panel** to assess cardiometabolic function
- **Thyroid Panel**
- **IGF-1**
- **17-OH Progesterone** – for non-classical congenital adrenal hyperplasia
- **Prolactin** (can be ordered alone)
- **Liver function tests** (included in Comprehensive Metabolic Panel, cannot be ordered alone)
- **SHBG** (included in Serum Hormones Panel, can be ordered alone)

Testosterone/Androgen Deficiency

Treatment Objectives: Consider which of the following single or combination of therapeutic approaches is the best fit for the patient symptoms of testosterone/androgen deficiency.

- Reduce aromatase activity
- Support symptoms of low androgens
- Reduce obesity and insulin resistance to reduce aromatase production
- Reduce exposure to phthalates and heavy metals

Table 7. Holistic Treatment Considerations for Testosterone Deficiency

Lifestyle Interventions	Symptomatic Support/Increase Testosterone (T)	Decrease Aromatase Activity
Supplements	**Withania somnifera (Ashwagandha)** studied to boost testosterone in men (i.e **KSM-66** 300mg/BID or TID), **Shoden** brand 21mg withanolide glycoside/d or **5g root powder**/day[176,177] **Trigonella foenum-graceum (Fenugreek)** 500-600mg/day seed extract boosts testosterone & libido in males[178] **Mucuna pruriens** 5g/day seed powder improves testosterone in infertile men and boosts semen quality[179] **Eurycoma longifolia (Long jack)** 200-400mg/day increased libido, testosterone, ED in men,[178] conflicting studies on efficacy **Korean Red Ginseng** 1500mg/BID improves serum testosterone in men with metabolic syndrome,[180] conflicting studies on efficacy[176] **DHEA** >50g/day[181]** may boost testosterone levels, especially in women **Nigella Sativa** improves semen parameters in infertile men[178] **Tribulus terrestris** 750mg/day improved sexual enhancement in women,[178]several studies show no increase in testosterone in men,[176] may enhance libido **Maca** 1500mg root/BID improvement in testosterone/libido/sexual function in women on SSRI,[29] several studies in men show no increase in testosterone[178] **Saw palmetto** Increased T, with decreased DHT and Estrogen in men at 800mg-2000mg/day[14]	Numerous **flavonoids** show preclinical evidence of decreased aromatase activity: * **Resveratrol**[13] **Grape Seed extract**[14] **Citrus Peels/citrus flavonoids**[182] **Apigenin**[13] **Chrysin** (Honey/propolis)[16]
Diet	**Diets that aid weight loss** - In obese men and/or men with metabolic syndrome, weight loss through a variety of diet and exercise or bariatric surgery programs consistently increase testosterone[183]	**White button mushrooms[14]*** **Foods with apigenin and/or resveratrol:** grapes, berries, celery, parsley, onions, oranges, chamomile, thyme, basil, oregano, red wine[184] Kale, collards[14]
Exercise/Sauna	**Exercise programs that aid weight loss** - In obese men and/or men with metabolic syndrome, multiple, varied diet/exercise programs that resulted in weight loss significantly improve testosterone levels.[183]	**150 minutes moderate activity** per week[185]

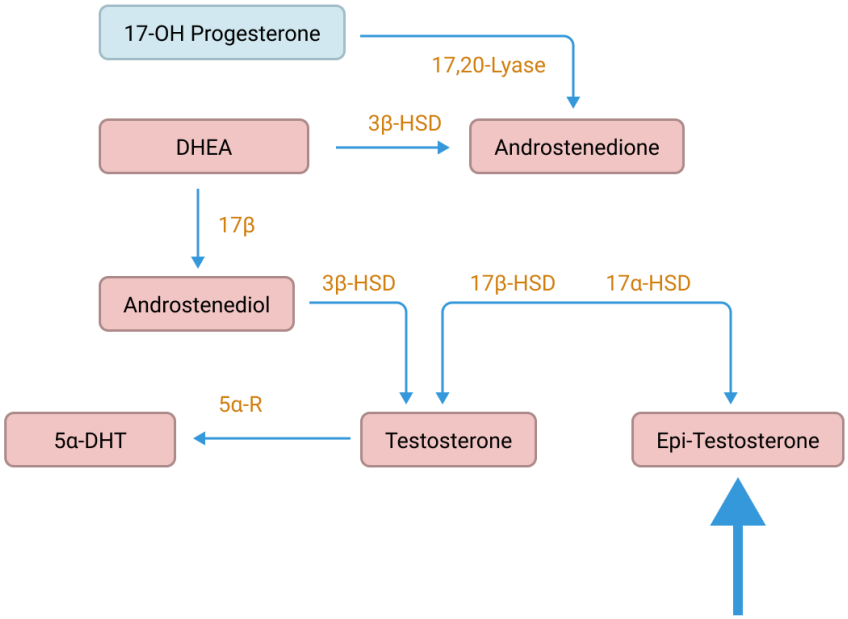
Stress Management	**Optimize sleep,** sleep deprivation (<5hours/night) linked to decreased testosterone[183] High occupational stress is linked to lower testosterone levels.[183] Stress management interventions may be helpful.	
Miscellaneous Support	Aromatase inhibitors, SERM's and hCG have been shown to raise testosterone[186] Varicocele repair significantly improves testosterone levels[183] Testosterone replacement therapy (TRT) is an option for hypogonadal men. It has shown to improve sexual function, muscle strength, bone density, and mood and cognition***	Metformin may aid increases in testosterone in men with metabolic syndrome[187]

* Dosage information for this indication is not well established, refer to empiric guidelines of safe and effective use.

** May increase estrogen and other hormone levels, use under physician supervision and hormonal lab monitoring.

*** Fully research the individual patient's risk/benefit analysis regarding hormone related cancer and other side effects of testosterone replacement therapy (TRT) prior to recommending. Side effects of TRT include erythrocytosis, male infertility, testicular atrophy, and gynecomastia.[186] There are also concerns but inconclusive data regarding the role of exogenous testosterone with cardiovascular risks, prostate cancer risks and venous thromboembolism.[186]

Epi-Testosterone (Epi-T)



Epi-testosterone is an isomer of testosterone that is endogenously produced by the gonads in response to LH. It is a competitive antagonist of the androgen receptor and as such, demonstrates a weak anti-androgen effect. It is also a strong inhibitor of 5-alpha-reductase activity in studies.[188] The ratio of testosterone to epi-testosterone is used to monitor anabolic drug use in athletic competitions.

↑ Causes of Increased Epitestosterone

- Highest levels are seen in adolescent males
- Levels can be elevated in exogenous epi-testosterone use

↓ Causes of Decreased Epitestosterone

- Exogenous use of testosterone or other androgens
- Oral contraceptive use
- Levels can vary with menstrual cycle fluctuations

T/Epi-T Ratio (Testosterone/Epi-Testosterone Ratio)

This ratio is a well-known biomarker of androgen abuse or doping. A typical T/Epi-T ratio is about 1:1. Use of exogenous synthetic testosterone or testosterone precursors results in elevations in urinary testosterone with a corresponding decrease in urinary epitestosterone. While some factors can alter the ratio aside from doping, a urinary T/Epi-T ratio value which is ≥ 4 is suspicious for exogenous use of androgens. In evaluation of androgen abuse, serial measurement of individual T/Epi-T ratios are preferred, rather than single point in time testing.

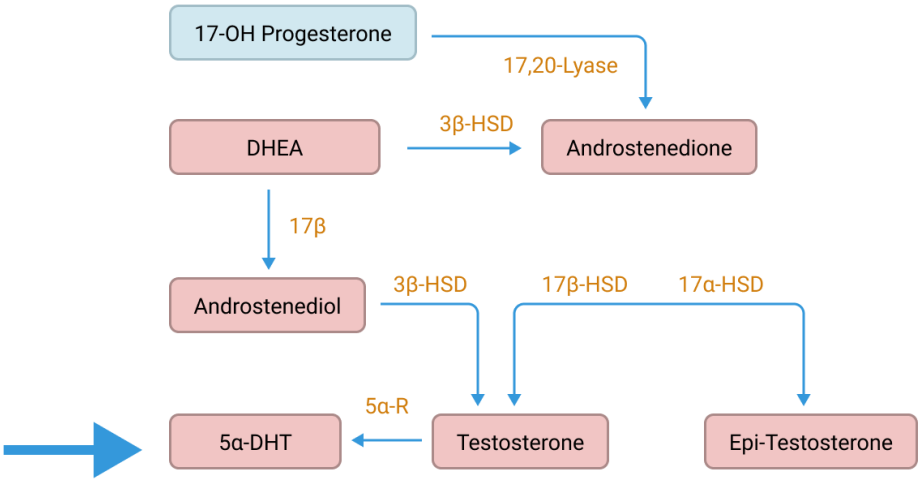
↑ Causes of Increased T/Epi-T ratio

- Exogenous androgen use
- Increased alcohol consumption, particularly in women

↓ Causes of Decreased T/Epi-T Ratio

- Ketoconazole use[189]
- Genetic polymorphism of UGT2B17 (common in Asian populations)
    - o Lowers urinary levels of testosterone, 5α-DHT and 5β-Androstanediol

T/Epi-T Ratio (Testosterone/Epi-Testosterone Ratio)



5α-dihydrotestosterone (DHT) is an androgenic hormone that is formed from testosterone through the action of the enzyme 5α-reductase in the gonads, as well as other tissues such as prostate, skin, and liver. DHT can also be indirectly formed through "backdoor pathways" and the action of 5α-reductase on other androgen intermediates.[190]
DHT is a more potent androgen than testosterone due to increased affinity for the androgen receptor. Biologically, DHT plays important roles in the maturity of male genitalia and secondary sex characteristics during male development stages, as well as a primary role in prostate growth and function. In females, DHT is known to contribute to puberty and the development of secondary sex characteristics, however other roles are somewhat unclear. Metabolites of DHT have beneficial neurosteroid and cognitive effects. Unlike testosterone, DHT cannot be aromatized into estradiol.[191] DHT levels are currently used to monitor 5α-reductase deficiency, male pattern baldness, and monitoring of 5α-reductase therapy.

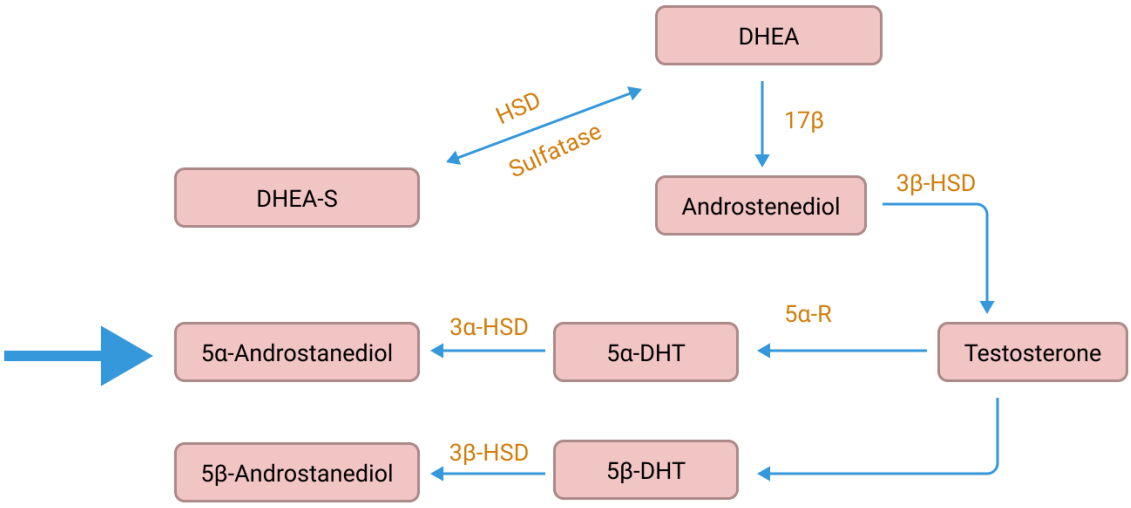
↑ Potential Causes of Increased 5α-DHT

- PCOS and hirsutism in females
- Androgenic alopecia
- BPH
- Increased 5α-reductase activity
    - o Refer to Spotlight on 5α-Reductase Enzymes section
    - o Obesity, insulin resistance, DHEA supplementation
- Lifestyle considerations for androgen excess, if clinically relevant, can be seen in Table 6.0 in the Testosterone Section.

↓ Potential Causes of Decreased 5α-DHT

- Reduced testosterone
- Prostatectomy
- 5α-reductase inhibition or deficiency
    - o Refer to Spotlight on 5α-Reductase Enzymes section
- Hypopituitarism
- Lifestyle considerations for androgen deficiency, if clinically relevant can be seen in Table 7.0 in the Testosterone Section

DHT Metabolites: 5a,3a-Androstanediol and 5b-Androstanediol



5a,3a-Androstanediol

5α-DHT is reduced to 5α, 3α-androstanediol via the activity of the enzyme 3α-HSD. 5α, 3α-androstanediol (often referred to as 3α-diol) can be a useful marker for peripheral androgen activity in conditions such as PCOS, hirsutism and acne. 5α, 3α-androstanediol may be a more sensitive marker than DHT for peripheral androgen production because DHT is often bound to sex hormone binding globulin and it is metabolized rapidly.[192,193] Also considered a neurosteroid, 5α, 3α-androstanediol has shown to have anxiolytic effects through actions on the GABA receptor as well as estrogenic effects from binding to estrogen receptor beta (ER-β).[194]

↑ Causes of Increased 5α, 3α-androstanediol (known as 3α-diol)

- Increases in 5α -DHT
    - o PCOS and hirsutism in females
    - o Androgenic alopecia
    - o Benign prostatic hypertrophy
    - o Increased 5α-reductase activity
        - - Obesity, insulin resistance, high DHEA
- Increased 3α-HSD activity

↓ Causes of Decreased 5α, 3α-androstanediol (known as 3α-diol)

- Decreases in 5α-DHT
    - o Reduced testosterone
    - o Prostatectomy
    - o 5α-reductase inhibition or deficiency
    - o Hypopituitarism

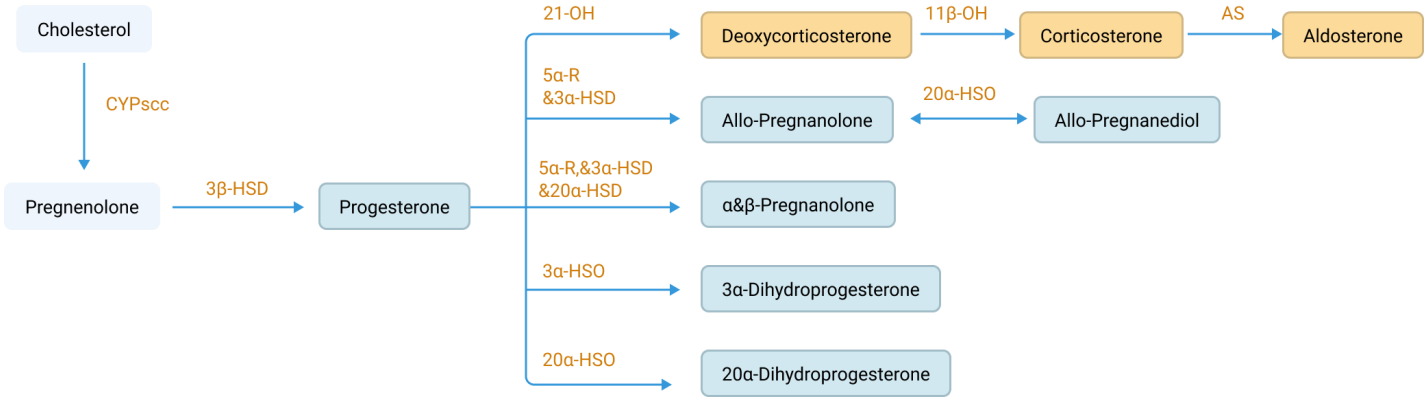
5β-Androstanediol

Testosterone can also be metabolized by 5β-reductase into 5β-DHT, a metabolite with insignificant androgenic action. 5β-DHT is reduced to 5β-androstanediol (also known as etiocholanediol or 5β-androstane-3α,17β-diol) through the action of 3β-HSD. Clinical implications of high and low levels of 5β-androstanediol have not been well studied. High and low levels of 5β-androstanediol are affected by upstream hormone and metabolite levels such as DHEA, testosterone, and 5β-DHT values, as well as 5β-reductase and 3β-HSD enzyme activity.

Progestogens

Progestogens	Progesterone	20α-Dihydroprogesterone
	Allopregnanolone	β-Pregnanediol
	Allopregnanediol	α-Pregnanediol
	3α-Dihydroprogesterone	

The Progesterone Metabolites



Progesterone and its metabolites are vital to normal health and reproduction and have roles across many systems in both men and women. While the role of progesterone in normal menstruation and pregnancy is well known, there are other systemic roles that progesterone fulfills. For example, progesterone is a precursor to critical steroid hormones such as aldosterone, cortisol, testosterone, and estradiol. Also, progesterone and its metabolites have important roles in the cardiovascular, renal, and musculoskeletal systems. Further, progesterone is a neuroprotectant, neuromodulator and aids with relaxation and sleep. Importantly, progesterone plays a role in immune support and cancer protection against endometrial, colorectal cancers and potentially others. In addition to these benefits, in men, progesterone also influences spermiogenesis and testosterone biosynthesis.

Progesterone metabolizes into isomers of dihydroprogesterone, pregnanolone and pregnanediol. All of these metabolites then undergo phase I and phase II reactions and result in significantly more progesterone metabolites.[195]

Beta(β)-Pregnanediol

Progesterone itself cannot be measured directly in urine, therefore, beta (β) and alpha (α) pregnanediol, the main progesterone metabolites in urine, are used in urine as surrogate progesterone markers. β-pregnanediol is considered the primary metabolite resulting from progesterone metabolism and is inactive. β-pregnanediol is produced through 5β-reductase enzyme activity. Factors which elevate or decrease progesterone will correspondingly increase or decrease β -pregnanediol. In addition, upregulation or down regulation of 5β-reductase enzyme activity can influence overall levels of β-pregnanediol.

↑ Potential Causes of Increased Progesterone/β-Pregnanediol

- Pregnancy
- Exogenous progesterone supplementation (non-topical forms)
- Ovarian cysts
- Ovarian tumors and testicular tumors
- Adrenal hyperplasia
- Stress and caffeine have also been linked to slightly elevated levels.[196,197]

↑ Potential Causes of Decreased Progesterone/β-Pregnanediol

- Irregular or anovulatory cycles, including during breastfeeding
- PCOS
- Aging and menopause
- Thyroid disorders
- Obesity
- Over exercise
- Hyperprolactinemia
- Anorexia
- Long term use of NSAIDs[198]
- Oral contraceptives[199]
- Endometriosis
- Environmental toxicants such as phthalates, pesticides, herbicides, etc.,[200]
- Low progesterone in pregnancy can be caused by ectopic pregnancy or complications or failure with the fetus or placenta
    - o Later in pregnancy, low progesterone can be caused by toxemia or pre-eclampsia of pregnancy
- In men, while sudden dramatic reductions in progesterone are uncommon, waning progesterone can occur as a sequalae of reduced androgens that occur gradually over time after the 4th decade in men[201]

Table 8. Lifestyle Considerations to Support Reduced Progesterone or PG/E2 Ratio

1. Vitex agnus castus 40mg per day[202]
2. Vitamin C 750mg/day[203]
3. B6 pyridoxine 200mg per day[204]
4. White peony root daily tea or 3-5 g of root/day[205]
5. Evening Primrose Oil[206]
6. Increase foods high in zinc, B6, Vitamin C, Magnesium[203]
7. Consider Progesterone replacement therapy with oral micronized, transdermal, or compounded prescription.
8. Assess risk/benefit analysis for the specific individual prior to giving hormone replacement therapy

Alpha-Pregnanediol (5α-Pregnanediol)

5α-pregnanediol has been studied to stimulate GABA receptor action and promote relaxation and sleep.[207] 5α-pregnanediol can be a substrate of allopregnanolone and can convert into allopregnanolone through the action of 20-hydroxysteroid dehydrogenase.[208]

Potential Causes of Increased and Decreased 5α-Pregnanediol

- Elevations and decreases in 5α-pregnanediol correspond to those listed for general progesterone and β-pregnanediol, (see above) however may independently vary due to increases or decreases in 5α-reductase activity.
- Medications which block 5α-reductase activity, such as those used for enlarged prostate and male pattern baldness, can correspondingly cause decreases in 5α-pregnanediol.

Allopregnanolone

Allopregnanolone (3α -THP) is formed when progesterone is reduced by 5α-reductase to 5α-dihydroprogesterone and this is further reduced by 3α -hydroxysteroid dehydrogenase to create allopregnanolone. Allopregnanolone is a regulator of female reproductive function and lactation, but also has been greatly studied for its neurosteroid effects. Allopregnanolone is a modulator of the $GABA^A$ receptor.

Potential Causes of Increased Allopregnanolone

- Typically increases or decreases in allopregnanolone relate to increased or decreased progesterone levels.
- Refer to the Beta (β)-Pregnanediol section for a complete listing of factors affecting progesterone levels.
- Common scenarios causing increased allopregnanolone include the luteal phase of the menstrual cycle, oral progesterone supplementation and pregnancy.
- It has been found that premenopausal women have higher allopregnanolone than postmenopausal women.[209]

Potential Causes of Decreased Allopregnanolone

- Common scenarios causing decreased allopregnanolone include situations of low progesterone such as perimenopause and menopause, luteal phase defect or anovulation, and pharmaceutical interventions such as finasteride or oral contraceptives.
- Variations in enzyme activity of 5α-reductase, such as those seen with pharmaceuticals, as well as 3α -hydroxysteroid dehydrogenase levels, can also affect allopregnanolone levels.
- Decreased production of allopregnanolone or fluctuations in levels have been associated with increased depression and anxiety, premenstrual dysphoric disorder, increased seizure activity in epileptics, Parkinson's disease and Alzheimer's disease.[210,211,212]

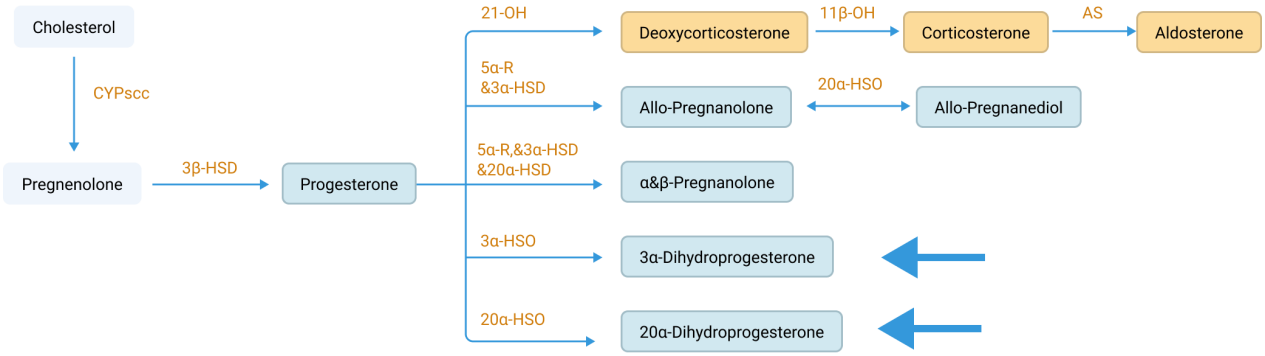
A Note About 4-Pregnenes and 5α-Pregnanes

Progesterone metabolites that are formed from 5α reductase activity have been termed 5α-pregnanes. As indicated, these metabolites have been well studied for calming, anti-seizure, and sleep benefits due to stimulation of $GABA^A$ receptors. Metabolites that have been formed through other enzymatic reactions have been termed 4-pregnenes, such as 3α-dihydroprogesterone and 20α-dihydroprogesterone.

Contrary to the calming benefits of 5α-pregnanes, some pre-clinical studies have demonstrated a potential role 5α-pregnanes may have in proliferation and cancer promotion effects in breast cancer cells as well as colon, ovarian and glioblastoma tumor tissue.[213,214] In opposition to this, the 4-pregnenes, such as 3α-dihydroprogesterone and 20α-dihydroprogesterone have demonstrated cancer inhibiting effects. Early studies show that in the microenvironment of a tumor cell, the ratio of 5α-pregnanes to 4-pregnenes is

much higher than a normal cell, which is supportive of tumorigenesis.[215,216] While more research is needed, it may be pragmatic to evaluate overall levels of 5α-pregnanes in comparison to 4-pregnenes regarding questions surrounding potential oral progesterone supplementation. This would be most relevant in clinical situations where recurrence of breast cancer and/or other cancer types is a concern.

3α-Dihydroprogesterone and 20α-Dihydroprogesterone



3α-Dihydroprogesterone

3α-dihydroprogesterone is created from progesterone through the enzymatic actions of 3α-hydroxysteroid oxidoreductase (3α-HSO) in tissues. It has been identified as a metabolite which has anti-proliferative effects in tumor cells.[217] Variations in activity of 3α-HSO and levels of precursor hormones can affect levels of 3α-dihydroprogesterone.

20-Dihydroprogesterone

20α-dihydroprogesterone is created from progesterone through the enzymatic action of 20α-hydroxysteroid oxidoreductase (20α-HSO) enzymes. 20α-dihydroprogesterone has a lower affinity for the progesterone receptor and is significantly less potent than progesterone.[218] 20α -has been studied to have drug metabolizing, anti-aromatase, and anti-proliferative effects.[219]

Variations in 20α-HSO enzymes and precursor hormones can contribute to elevations and reductions in 20α-dihydroprogesterone.

β-Pregnanediol/E2 (β-pregnanediol/estradiol ratio)

The β-pregnanediol/estradiol (β-pregnanediol/E2) ratio is used similarly to the progesterone/E2 ratio. This was commonly used empirically as a marker of "Estrogen Dominance," developed, and popularized by John Lee, MD.[220] Conventionally, the Pg/E2 ratio is also used in IVF research for pregnancy rates and has been studied for assorted characteristics of menstrual cycles and fibrocystic breast disease.[221,222]

↑ Causes of Increased β-Pregnanediol/E2 – High Progesterone Relative to Estradiol

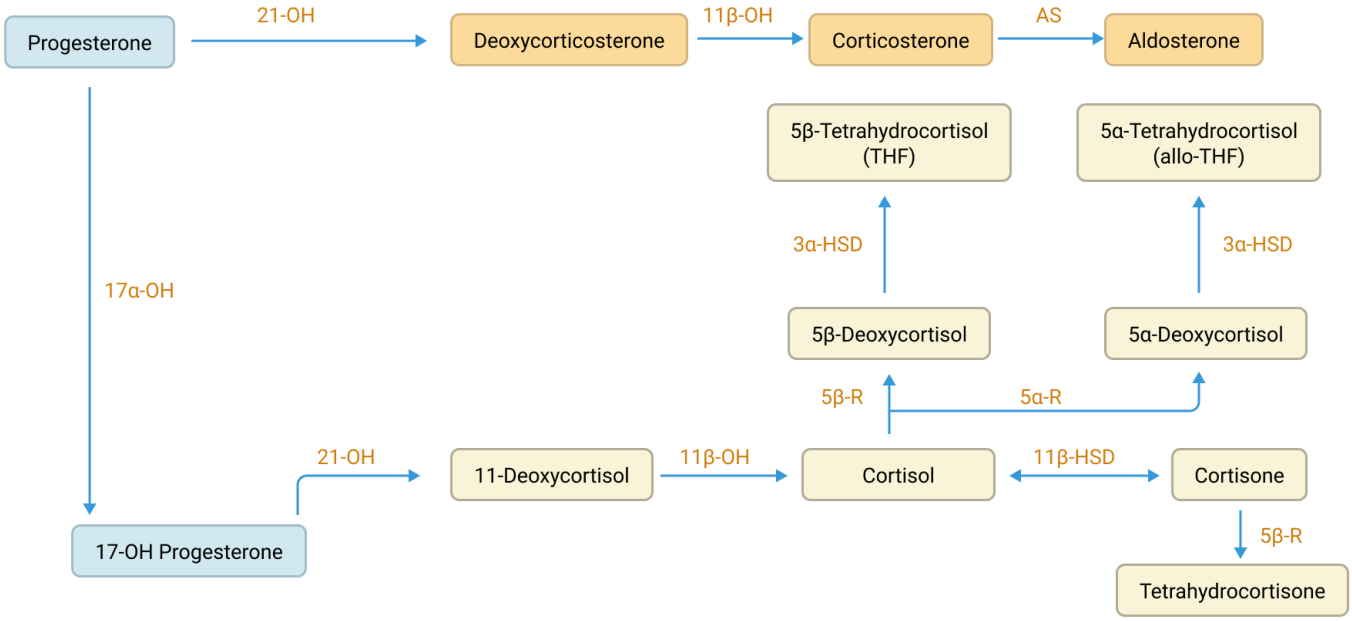
- Exogenous oral progesterone use (most common)
- Increased endogenous progesterone production (i.e., common in pregnancy)
- Decreased estradiol production

↓ Causes of Decreased β-Pregnanediol/E2 – Low Progesterone Relative to Estradiol

- Natural aging in men and women – common in 4th and 5th decades of life and later
- Sub-optimal clearance of estradiol and/or estradiol metabolites
    - o Detoxification pathways
    - o Environmental xenoestrogen
    - o Exposure

Glucocorticoids and Glucocorticoid Metabolites

Glucocorticoids	Cortisol	α-Tetrahydrocortisol (α-THF)
	Cortisone	β-Tetrahydrocortisone (β-THE)
	β-Tetrahydrocortisol (β-THF)	Corticosterone



Cortisol is widely known as "the stress hormone," and nearly all tissues of the body have glucocorticoid receptors to respond to the actions of cortisol. Cortisol plays a significant role in maintaining glucose and protein homeostasis, mediation of the stress and immune response, and suppression of inflammation.[223]

The hypothalamus-pituitary-adrenal axis (HPA axis) regulates production and secretion of cortisol. It does this through release of CRH, corticotropic releasing hormone, from the hypothalamus which signals ACTH, adrenocorticotropic hormone, release from the pituitary gland to the adrenal cortex, which then releases cortisol. After cortisol is released in response to these signals, cortisol sends a negative feedback loop to suppress further production of ACTH and CRF. See Figure A.

The HPA axis function follows a diurnal pattern of release, therefore cortisol levels are highest in the morning after waking and lowest at night around bedtime.[223] Aside from diurnal secretion of hormones, and measured pulsatile releases of hormones, HPA axis function and release of cortisol are also triggered by stressors, both acute and chronic.

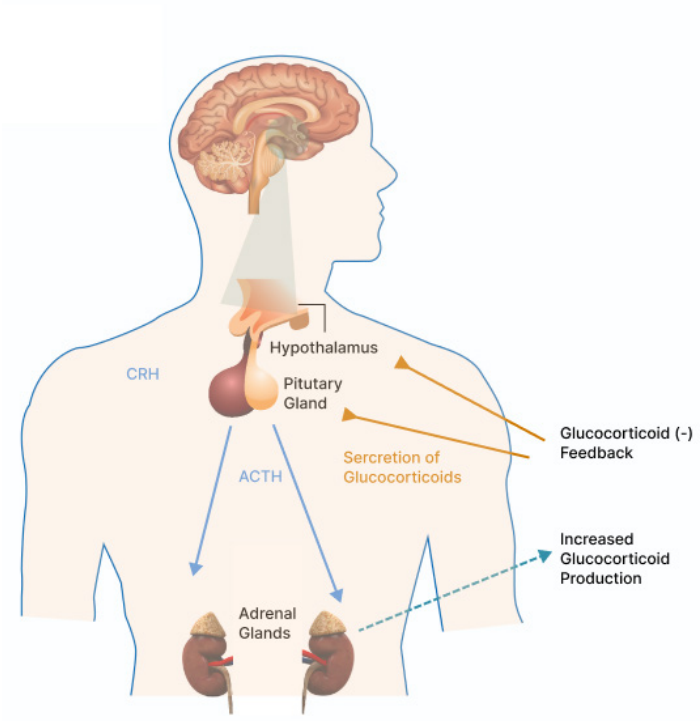
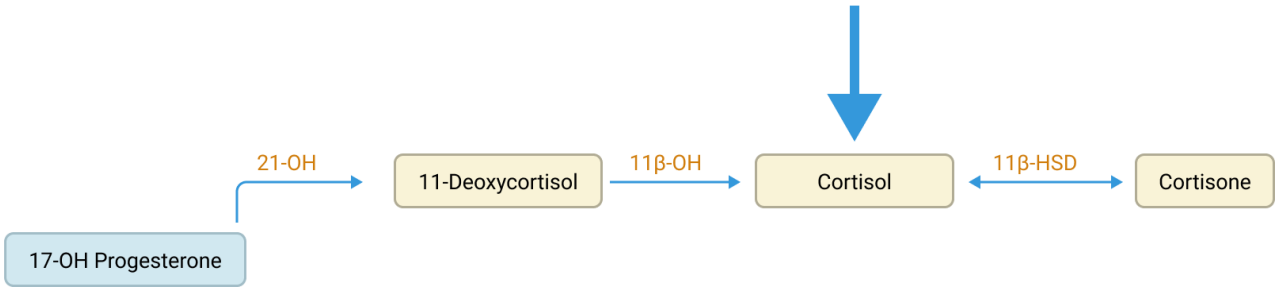


Figure A.

Total Cortisol



Total cortisol is a measure of the sum of free cortisol and conjugated cortisol excreted in the urine. Cortisol undergoes steroidogenesis similarly to other steroid hormones, originating from cholesterol and progressing to progesterone. From 17-OH progesterone, cortisol goes through two hydroxylation steps to arrive at 11-deoxycortisol which is then further hydroxylated by 11β-hydroxylase to arrive at cortisol. In the tissues, the glucocorticoids (cortisol, cortisone, and corticosterone) are regulated by 11β-hydroxysteroid dehydrogenases, type 1 and type 2.

↑ Table 9. Potential Causes of Increased Cortisol

Cushing's Disease/Syndrome	Infectious Disease	Psychological Stress
Adrenal Neoplastic Disease	Crohn's Disease	Obesity
Hyperpituitarism or Hyperthyroidism	Stress: Heat/Cold/Trauma/Pain	Night Shift Work/Circadian Disruption
Diabetes Mellitus (uncontrolled)	Burns	Alcoholism and Alcohol Withdrawal
Shock	Surgery/Post Op Recovery	Anorexia/Hypoglycemia
Cigarette Smoking	Chronic Renal Disease	HIV
Pregnancy	Virilism	Smoking
Hypertension	Exercise or Chronic Overexercise	Glucocorticoid Resistance
Eclampsia	Severe Hepatic Disease	
Opioid Withdrawal	Depression/Neuropsychiatric disease	

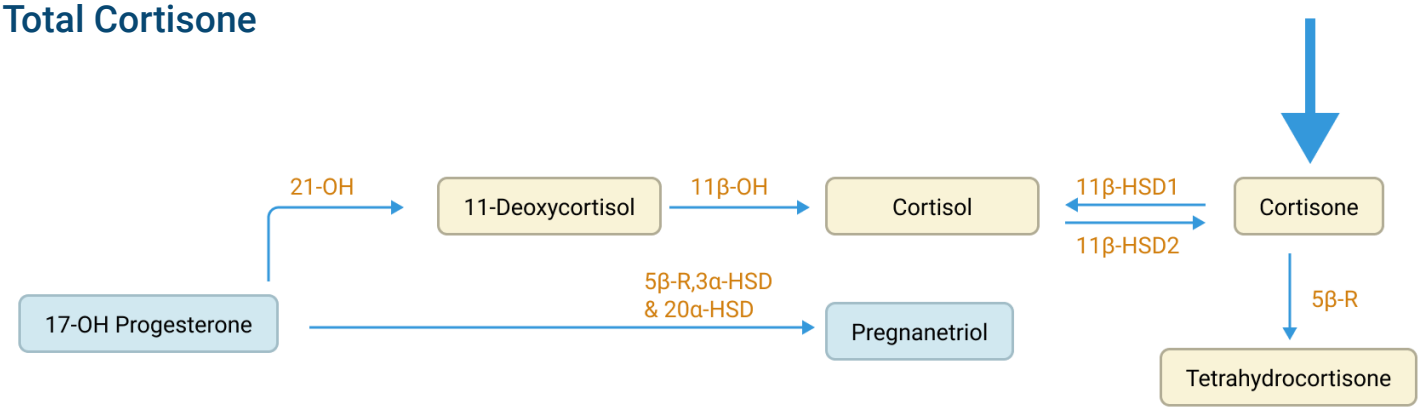
Drugs which can increase cortisol levels include glucocorticoids, caffeine, nicotine, corticotropin, estrogens, oral contraceptives, yohimbine, and vasopressin.[224] Initial or short-term use of marijuana can increase cortisol, while opposite effects may result from chronic or heavy use.[225]

Table 10. Potential Causes of Decreased Cortisol

Primary adrenal insufficiency- due to Addison's disease	Primary adrenal insufficiency- due to adrenal destruction from other causes (malignancy, surgery, etc.)
Primary adrenal insufficiency due to genetic conditions (i.e., CAH - congenital adrenal hyperplasia)	Secondary adrenal insufficiency (i.e., withdrawal from glucocorticoid therapy)
Liver disease	Long term use or recent withdrawal from corticosteroid therapy
Pituitary adenoma	Craniopharyngioma/hypophysectomy
	Hypopituitarism
Postpartum pituitary necrosis	Rheumatoid arthritis
Early traumatic experiences, chronic stress, PTSD	Hypothyroidism
Recurrent infectious processes	Waterhouse-Friderichsen syndrome

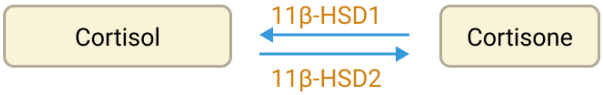
Drugs which can decrease cortisol levels include opioids,[226] ketoconazole, rifampin, phenytoin, dexamethasone, dexamethasone acetate, and dexamethasone sodium phosphate. There is some evidence that marijuana affects cortisol response, namely that heavy/chronic use can blunt cortisol response.[227]

Total Cortisone



Total cortisone is a measure of the sum of free cortisone and conjugated cortisone excreted in the urine. Cortisol is converted to cortisone, an inactive metabolite by 11-β-hydroxysteroid dehydrogenase type 2 (11β-HSD2). 11β-HSD2 is highly expressed in aldosterone-selective target tissues such as the distal nephron, colon, skin, and the salivary glands.[228]

The function of 11β-HSD2 to convert cortisol to cortisone is critical in tissues to protect from overexposure to active cortisol and corticosterone.[229] Otherwise, these active hormones would occupy mineralocorticoid receptors and produce a cascade effect of mineralocorticoid excess symptoms such as sodium retention, hypertension, and hypokalemia.[230] In contrast, in metabolically active tissues such as the liver (20-40% of daily production) adipose tissue, and skeletal muscle, 11β-Hydroxysteroid dehydrogenase, type 1 (11β-HSD1), reversibly catalyzes the 11β-reduction of cortisone to cortisol, and regenerates cortisol within these tissues.[230]



Which is More Accurate: Cortisol or Cortisone?

There are specific clinical situations in which there would be expected differing results between cortisol and cortisone. In these clinical situations, cortisone may reflect a greater accuracy than cortisol.

- Systemic glucocorticoid deficiency or excess
- Use of oral glucocorticoid therapy
- Directly following acute stress

While cortisone itself is an inactive metabolite, it has been shown, like cortisol, to directly compare to serum free cortisol levels.[231,232] In general, cortisone values tend to parallel cortisol values. Thus, similar situational or condition triggers cause elevations or depressions in cortisone values as cortisol values. Diurnal cortisone values have not yet been studied for normative value comparisons.[231]

11β-HSD Enzyme Activity

11β-HSD1 and 11β-HSD2 enzyme activity affect cortisol and cortisone levels. These enzymes affect local target tissues in an intracrine manner, as in 11β-HSD2 upregulating in the distal nephron and 11β-HSD1 upregulating in adipose tissue and the brain. In these scenarios, the effect is intracrine, i.e., local, and does not affect systemic cortisol production.[233]

However, these enzymes also work in an endocrine manner, and may affect cortisone and cortisol levels systemically as well. For example, 11β-HSD1 in the splanchnic bed generates 30–40% of the total daily production of cortisol in humans, while 11β-HSD2 in the kidney deactivates a similar percentage.[233] Thus, up or downregulation of these enzymes can play a role in cortisone and cortisol dynamic systemic levels.

Careful evaluation of cortisol/cortisone ratio (below) and levels of terminal cortisol and cortisone metabolites, 5β THF, 5α -THF, and 5β-THE, can yield insights about systemic 11β-HSD enzyme activity. For example, the trend of increased cortisol terminal metabolites (β -THF + α -THF) compared to tetrahydrocortisone from cortisone, can indicate increased 11β-HSD1 activity. Similarly, an examination of the cortisol/cortisone ratio can provide insight on 11β-HSD2 activity.

Cortisol/Cortisone Ratio

This value measures the ratio of total cortisol to total cortisone.

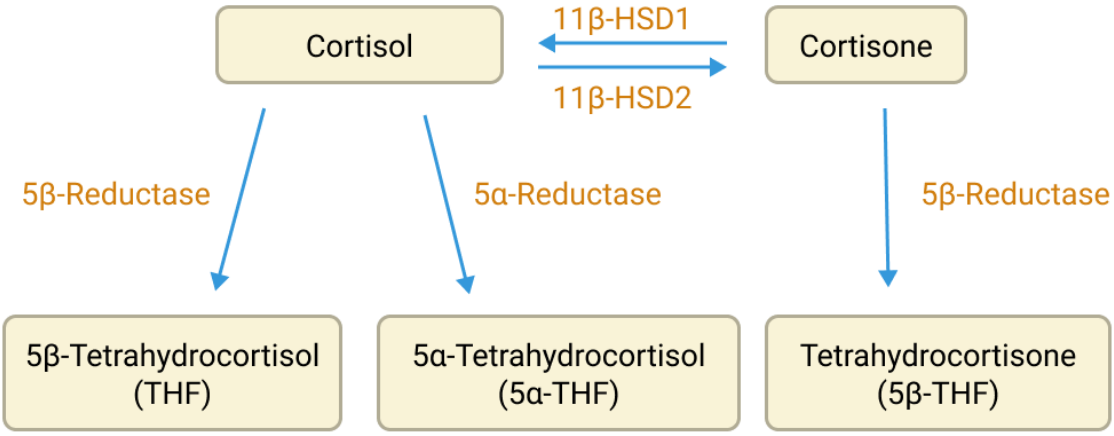
Potential Causes of Increased Cortisol/Cortisone Ratio

- Upregulated 11β-HSD1 activity which is linked to the following:
  - o Inflammatory diseases such as joint disorders, neurodegenerative disorders, diabetes,[234] metabolic syndrome, cognitive decline, and obesity[235]
  - o Drugs, foods, and herbs such as grapefruit juice, licorice, glucocorticoids, progesterone[236]
  - o Synthetic endocrine disruptors such as phthalates, organotins (tin compounds widely used as pesticide, biocides and in PVC materials), alkylphenols[233]

Potential Causes of Decreased Cortisol/Cortisone Ratio

- Upregulated 11β-HSD2 activity which is linked to the following:
  - o Hormones such as estrogen, progesterone, 7-keto-DHEA, thyroid[233]
    - Pregnancy upregulates cortisone and 11β-HSD2[2387]
    - PCOS upregulates cortisone and 11β -HSD2[238]
- Supplements, drugs, and foods that upregulate 11β-HSD2 activity
  - o Green tea, coffee, holy basil, curcumin, vitamin A, pravastatin, bile acids.[239,240.241]

Cortisol and Cortisone Metabolites



Evaluation of cortisol metabolites can provide insight into trends of enzyme activity and show patterns in chronic diseases such as PCOS, obesity, apparent mineralocorticoid excess, hyper or hypothyroidism, anorexia, and others.[242, 243] In the metabolism process, cortisol is reduced by either 5β-reductase or 5α-reductase, often in the liver, to form dihydrocortisol intermediates. These are further reduced by 3α-hydroxysteroid dehydrogenases to form 5β-tetrahydrocortisol (5β-THF) and 5a- tetrahydrocortisol (5α -THF), the terminal metabolites of cortisol. Cortisone is reduced by 5β-reductase to produce tetrahydrocortisone (5β-THE), the final product of cortisone metabolism.

Cortisol Metabolites: β -Tetrahydrocortisol (β -THF or THF) and α -Tetrahydrocortisol (α -THF or Allo-THF)

These metabolites can be used alongside cortisol measures to aid assessment of glandular cortisol production as well as shine a spotlight on overall cortisol metabolism in the body. β -THF is the metabolite from active cortisol which has been reduced by 5β-reductase. α -THF is the metabolite from active cortisol which has been reduced by 5α-reductase.

These markers also offer insight into respective 5α–reductase or 5β-reductase enzyme activity, along with other metabolites produced from the same enzymatic pathway. See the Spotlight on 5α-Reductase Enzymes section for further information regarding specific enzymatic influences.

↑ Potential Causes of Increased β -THF and α -THF

- If cortisol metabolites, β -THF and α -THF, are elevated it can reflect excess cortisol production or increased cortisol metabolism. Conditions such as stress, insulin resistance, obesity and hyperthyroidism can cause increased cortisol production and/or metabolism.
- Refer to Table 9., Potential Causes of Increased Cortisol, for further possible causes.

↓ Potential Causes of Decreased β -THF and α -THF

- If cortisol metabolites are decreased it can potentially illustrate reduced glandular output, but also depending on other markers, reflect decreased metabolism and clearance as seen in adrenal insufficiency, suboptimal liver function and hypothyroid.
- Refer to Table 10., Potential Causes of Decreased Cortisol, for further causes of decreased cortisol metabolites.

Cortisone Metabolite: β -Tetrahydrocortisone (β -THE)

β -THE is the metabolite from cortisone which has been reduced by 5β-reductase. This metabolite, in conjunction with β-THF and α -THF can aid in evaluation of overall cortisol production and metabolism in the body. If this marker is elevated or decreased, it can reflect situations of cortisol excess or deficiency such as those listed in Tables 9. and 10.

This marker can also offer insight into 5β – reductase enzyme activity, along with other metabolites produced from the same pathway. See the Spotlight on 5α-Reductase Enzymes section for further information regarding specific enzymatic influences.

Metabolized Cortisol (THF+THE)

Metabolized cortisol reflects the sum of α -THF, β -THF, and β -THE. Metabolized cortisol is the best measure of overall cortisol production and clearance through the liver. It does not include the fraction of cortisol that is free and bioavailable for use, generally about 5-10% of total production.[244]

The Mineralocorticoids: Deoxycorticosterone (11-Deoxycorticosterone) and Corticosterone

Deoxycorticosterone (11-Deoxycorticosterone)

Deoxycorticosterone (DOC) is an intermediate steroid hormone synthesized from progesterone through the action of 21α-hydroxylase and is a precursor for the synthesis of cortisol and aldosterone. It has negligible mineralocorticoid activity. Typically, this measure is helpful for diagnosis of disorders of steroid synthesis such as congenital adrenal hyperplasia.[245]

↑ Potential Causes of Increased Deoxycorticosterone

- Congenital adrenal hyperplasia due to 11 beta-hydroxylase 2 (CYP11B2) deficiency
- Pregnancy and newborns
- DOC producing adrenal tumor
- Cushing's disease
- Primary cortisol resistance syndrome.

↓ Potential Causes of Decreased Deoxycorticosterone

- Congenital adrenal hyperplasia due to 21-hydroxylase deficiency, 3-beta-steroid dehydrogenase deficiency, or steroidogenic acute regulatory protein (StAR) deficiency.
- Primary or secondary adrenal insufficiency

Corticosterone

Corticosterone is an intermediate metabolite produced from deoxycorticosterone through 11β-hydroxylase activity in the pathway of aldosterone formation. It circulates at 10–20-fold lower concentrations than cortisol.[246] In other species, such as rodents and birds, corticosterone acts as the main glucocorticoid, however, in humans, the role of corticosterone, other than as an intermediate, is somewhat undefined. There is ongoing research into the role of corticosterone in insulin sensitivity, HPA axis function and mineralocorticoid effects.[247]

Testing levels of corticosterone plays a role, in conjunction with deoxycorticosterone, for diagnosis of disorders of steroid synthesis, especially relating to congenital adrenal hyperplasia and 11-beta-hydroxylase activity.

Oxidative Stress

Other	Melatonin 8-OHdG

8-OHdG

8-hydroxy-2'-deoxyguanosine (8-OHdG, or 8-oxodG) is a marker of oxidative damage to DNA from reactive oxygen species (ROS). The guanine molecule, one of 4 nucleobases in DNA, has a high susceptibility to ROS oxidative damage. When oxidative damage to guanine occurs, 8-OHdG is formed. When this damage is repaired by the cell, the 8-OHdG is released into the urine.

8-OHdG formation is considered to have a close relationship with tumors, cell aging, exposure to xenobiotics, cardiovascular disease, and other degenerative diseases. A meta-analysis of 8-OHdG in solid tumor cancers found that in most solid tumors, except breast cancer, elevated 8-OHdG was associated with poorer survival outcomes.[248] Some researched associations to elevated 8-OHdG are as follows:

Causes of Increased 8-OHdG

- Increased cortisol[249]
- Smoking[250]
- Pollutants such as benzene, styrene, toluene, PAH, asbestos, BPA, heavy metals[251]
- Cancer (numerous solid tumor types and leukemia)[252]
- Occupational radiation exposure[253]
- Atherosclerosis[254]
- Hypertension[254]
- Diabetes[255]
- COPD
- Recent, acute exercise[256] (moderate exercise over time shows reductions in 8-OHdG[257])

The Good News about Diet and 8-OHdG

The good news is that a variety of food-based interventions with antioxidant effects have been shown to reduce 8-OHdG levels.

- Diets rich in colorful fruits, vegetables and polyphenols have been shown to reduce 8-OHdG. The inclusion of foods such as onions, almonds, green tea, lycopene, curcumin, and red wine have independently shown the ability to reduce 8-OHdG.[118]
- Simply increasing the number of fruits and vegetables per day in one study, i.e., up to 12 servings, has shown decreased 8-OHdG markers in study participants.[258]
- The Mediterranean diet is a cohesive diet that brings these elements together and has shown reductions in 8-OHdG in metabolic syndrome patients.[118]

Diurnal Values of Free Cortisol and Cortisone

Urinary Free Cortisol

Urinary Free Diurnal Cortisol	Free Cortisol (1st morning) Free Cortisol (2nd morning) Free Cortisol (Evening) Free Cortisol (Night) Free Cortisol (Pooled)

Free cortisol is a measure of unbound cortisol. Serum testing typically measures the carrier or protein-bound version of hormone that is travelling through the blood. While free and albumin-bound hormones can enter tissues, only unbound hormones can exert actions on target cells. Typically, about 5-10% of cortisol is thought to be free and 90-95% bound to binding proteins.[259,260]

Urine free cortisol has been shown to strongly correlate with mean serum-free cortisol in cortisol excess situations.[261] Measurement of free cortisol is helpful for determination of HPA axis function. It is also relevant for patients who are on drugs which affect binding proteins of cortisol, such as contraceptives, low dose prednisone and others.[262]

↑ Potential Causes of Elevated Timed Cortisol Samples

Much of the research on diurnal patterns of cortisol has been conducted on salivary cortisol, rather than urine, however, summaries of those findings are listed here.

↑ **Free Cortisol (1st morning) –** Elevated waking cortisol values can specifically reflect depression, early stages of burnout, pain, glycemic dysregulation, or job-related stress.[263] Severe obstructive sleep apnea has been shown to produce irregular AM cortisol values in studies (both high and low).[264, 265]

↑ **Free Cortisol (2nd morning) –** As a solo elevation, this generally reflects sporadic or situational stress triggers. One recent study showed that elevations specifically of noon or night cortisol accurately predicted job stress and/or burnout better than frequently studied cortisol awakening response (CAR) values (not measured with this test) which show conflicting results regarding burnout.[266]

↑ **Free Cortisol (Evening) –** In addition to situational stress triggers, high evening cortisol can be related to glycemic dysregulation due to late-day, prior to dinner collection time. In the literature it has also been associated with autism spectrum disorder, adolescence, and home related stressors such as divorce and financial strain.[267, 268]

↑ **Free Cortisol (Night) –** This value reflects the expected nadir of cortisol output and therefore, baseline cortisol levels. Increased levels are related to insomnia, situational stressors, inflammation, and disease conditions such as the ones in the above table. Markedly increased bedtime cortisol values should prompt consideration of Cushing's syndrome or disease.[269]

↑ **Free Cortisol (Pooled) -** Pooled cortisol reflects overall diurnal cortisol output, collected from all samples given. Thus, it can be a measure of general HPA axis functionality. Some evidence shows that elevations in pooled cortisol reflect chronic stress.[268]

↓ Potential Causes of Decreased Timed Cortisol Samples

↓ **Free Cortisol (1st morning)** – Severe exhaustion can result in a decreased AM cortisol, in addition to severe obstructive sleep apnea and conditions of adrenal insufficiency such as Addison's Disease.[270, 265]

↓ **Free Cortisol (2nd morning)** – Generally, decreased values represent incomplete recovery from acute stress exposures, exhaustion from chronic stress and/or conditions related to low cortisol output.

↓ **Free Cortisol (Evening)** – Any of the conditions listed in the Causes of Decreased Cortisol Table listed earlier in this section can produce a decreased pooled cortisol value. Chronic stress has been linked to a flattened or attenuated diurnal output of cortisol, however the opposite has also been shown. This discrepancy has been theorized to be related to changes in HPA axis output related to long-term exposure to chronic stress.[271]

Urinary Free Cortisol

Urinary Free Diurnal Cortisol	Free Cortisol (1st morning) Free Cortisol (2nd morning) Free Cortisol (Evening) Free Cortisol (Night) Free Cortisol (Pooled)

Diurnal cortisone values have not been well studied for normative value comparisons to diurnal cortisol. Nonetheless, while urinary cortisone itself is an inactive metabolite, it has been shown, like urinary cortisol, to directly compare to serum free cortisol levels. Thus, elevations or depressions in urinary cortisone values tend to parallel cortisol values. However, differences between urinary free cortisone and cortisol can result from activity in 11-βHSD enzyme function. Evaluation of the ratio of cortisol/cortisone values and cortisol and cortisone metabolites can shed light on these enzymatic actions. Refer to the 11β-HSD Activity section under Total Cortisone for further information.

Interpretation of Cortisol Circadian Rhythm – HPA Axis Dysregulation

Our collective understanding of the stress response and the HPA axis largely comes from the pioneering work of Hans Selye. His theory of General Adaptation Syndrome described a limited, yet functional, model of stress response and adaptation to persistent stress. It is based on a stressor producing 3 stages of response.

Stage 1 – The first stage that one goes through in response to a stressor is termed "alarm" and indicates a release in catecholamines along with a transient increase in corticosteroids.

Stage 2 – The second stage of stress response is termed "resistance" and describes a heightened stress response with persistently elevated cortisol and other physiological adaptations necessary to resist the stressor.

Stage 3 – The third stage is termed "exhaustion" and results in persistent hypocortisolism and depletion of other biological resources needed to maintain the stress response.[272]

As research evolves it can be said that numerous factors influence the stress response and subsequent HPA axis result, and the issue is more complex than originally modeled. Selye and others have noted,[273] timing is a critical element. Hormonal activity is elevated at stressor onset but reduces as time passes. However, how much time passes, and how much the cortisol response is affected, is widely variable among persons. This heterogeneity may relate to HPA axis linked genetic variation among other factors.[271] Second, ongoing stressors that threaten physical integrity, involve trauma, and are uncontrollable can elicit a high, flat diurnal profile of cortisol secretion.[273] However, it is demonstrated that this is not the case in every situation. For example, it is lower in people with posttraumatic stress, and it may be lower after many years of persistent stress. Third, regarding hypocortisolemia, situations that provoke repeated or sustained cortisol elevations may lead to a breakdown in the negative feedback system of cortisol secretion, ultimately resulting in low flattened slopes.[273]

HPA Axis Function

A normal cortisol circadian rhythm is that cortisol is elevated in the morning, peaks within 30-45 minutes following waking, known as the cortisol awakening response or CAR, then begins a steady downward trend until it reaches its nadir at bedtime.[271]

Questions to Ask in an HPA Axis Evaluation

1. Does the Diurnal rhythm peak in the morning and then trend downward from AM to PM?
2. Is there a single elevation only, that can be explained by a situational trigger or chronic condition?
3. Is there a combination of either high or low elevations with loss of diurnal rhythm?

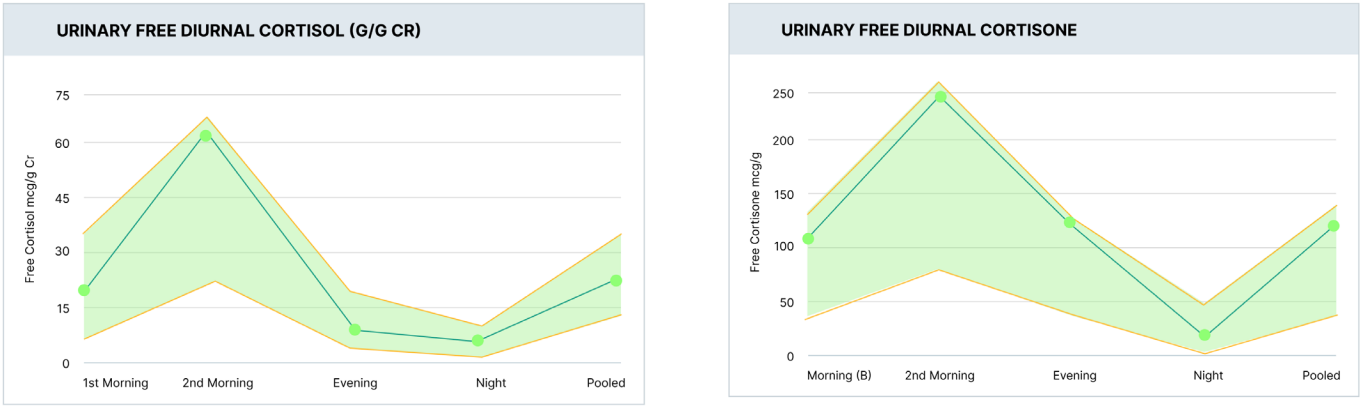


Figure 1. Normal Variation of HPA Axis Curve

The graph above has values within an expected HPA axis configuration. Although a typical HPA axis curve shows all values trending downward from the morning cortisol value, in this test, the second morning cortisol, taken 2 hours after waking, is higher than the first morning collection. In urine testing this can be a normal variant. As urine captures hormone levels accumulated in the urine since the previous urine sample, it is possible that the increased hormone produced from the cortisol awakening response (CAR) has been captured in this 2nd morning sample. Clinical correlation would be helpful to rule in or out situational triggers as well. The evening and night values proceed in a typical downward trend with the expected nadir at the night sample.

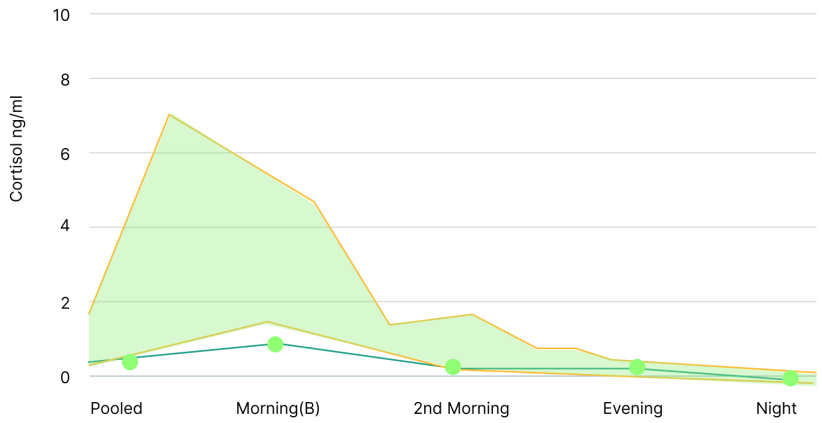


Figure 2. Flattened Diurnal Slope

A flattened diurnal slope is found when there is a pattern of blunted response to stress throughout the day, typically with hypocortisolemia. This flattened diurnal slope pattern represents a more advanced state of HPA axis dysregulation. This pattern has been studied in relationship to chronic stress, early childhood adversity, burnout, etc. and has associations with many clinical conditions as seen on the right.[274]

Clinical Tip

A Flattened HPA Axis Curve is an Important Finding!

The most studied variation in diurnal cortisol slope pattern is a flattened or blunted slope. The following conditions have been associated with a flatter diurnal cortisol slope[274]

- Immune dysregulation
- Inflammatory dysregulation
- Chronic fatigue syndrome
- Chronic stress
- Breast cancer mortality
- Obesity/BMI/adiposity
- Depression and negative affect
- Cardiovascular disease
- Recent withdrawal from corticosteroid therapy

Lifestyle Treatment Considerations for Glucocorticoids

Hypercortisolism – Treatment Objectives

Consider which of the following single or combination of therapeutic approaches is the best fit for the patient with hypercortisolism. Transient elevated cortisol values may be corrected by remediation of situational triggers. Further modifiable HPA axis triggers for persistent hypercortisolism include dysglycemic and inflammatory conditions as listed below.

- Identify stressful situational triggers and offer lifestyle remediation long commutes, caffeine, jet lag, stressful life event, hypoglycemia, insomnia
- Identify and treat glycemic dysregulation diabetes, hypoglycemia, stress/comfort eating, eating disorder
- Identify and treat chronic inflammatory conditions which may promote hyper signaling of HPA axis such as:autoimmune disease, arthritis or chronic musculoskeletal pain, chronic sinusitis, CVD, GI inflammation

Table 11. Holistic Treatment Considerations for High Cortisol Effects

Lifestyle Considerations	To Aid Cortisol Reduction/Mitigate Effects of High Cortisol
Supplements	**Ashwagandha** 300m/ BID KSM-66 brand (Chandrasekhar), also + studies for Sensoril and Shoden brands[275] **Phosphatidyl serine/phosphatidic acid** 400mg[276] **L-theanine** 200mg[277] anti-stress and cortisol lowering effect **Curcumin** 500mg BID lowered AM cortisol[278] Curserin brand (Phosphatidyl serine/curcumin blend -800mg/BID) lowers AM cortisol[279] **Panax Ginseng** 1.5g/ Korean ginseng BID reduced cortisol in metabolic syndrome[180] **Rhodiola** 300mg/BID[275] **Relora** Magnolia/phellodendron 250mg BID[280] **DHEA*** 10-450mg/d in meta-analysis of multiple studies reduces cortisol, considered safe under doctor monitoring (avg. dose 50 or 100mg), moderate's effects of high cortisol in target tissues[281]
Diet	**Low sucrose diet/carbohydrate restriction** May modulates 11B-HSD enzyme activity to reduce truncal obesity[282] **Prebiotics, high fiber diet** may reduce cortisol levels, anxiety, and related cognitive function[283] **Cherry juice** 125 ml/BID[284]enhances mood, reduces cortisol In Cushing's syndrome and prolonged hypercortisolism consider the following dietary measures: **Low sodium diet**, (excess cortisol can lead to hypertension), **higher calcium diet** (to offset osteoporosis), **higher protein diet** (offset muscle wasting)

Exercise	**Yoga** heterogenous mix of styles, is associated with decreased evening and/or waking cortisol, improved regulation of HPA axis system[285] **Habitual exercise** (low or moderate intensity) regulates HPA axis function, buffers anxiety response and rumination time[286,287] however extreme exercise is associated with prolonged cortisol elevations and decreased immunity **Weight bearing exercise** to offset bone loss from chronic hypercortisolism
Stress Management	**Meditation** Numerous styles of meditation are associated with decreases in salivary cortisol and anxiety, rumination[288,289] **Forest Bathing** Decreases cortisol after forest walking, anticipation of stress relief is also helpful[290] **Biofeedback** 5 minutes once a day decreases stress and cortisol levels[291] **QiGong** Reduces cortisol, raises serotonin and brain derived neurotropic factor (BDNF)[292] **Sleep** Maximize sleep – at least 7 hours each night for prevention of high cortisol

***Not advised to use DHEA without physician supervision, monitoring cortisol values, and monitoring for excess androgenic side effects/symptoms**

Further Testing Considerations for Elevated Cortisol

- **Cardiac Health Panel** and **Diabetes Panel**
- **Methylation Panel**
- **Tickborne** or Infections tests to explore occult infections
- **Gut Zoomer** for inflammation if cause of persistent inflammation is unknown or clinical symptoms correlate
- **hs-CRP** for inflammation
- **Thyroid Panel**
- **DEXA scan** for osteoporosis
- **Blood pressure** monitoring
- Extreme elevations of cortisol require a more **extensive endocrine workup** including workup for Cushing's syndrome, adrenal tumor/hyperplasia and/or pituitary tumor etc. Endocrinologist referral is advised.

Hypocortisolism – Treatment Objectives

- Differentiate low to moderate hypocortisolism, which is more common, from adrenal insufficiency which is more uncommon.
- Hypocortisolism, sub-optimal adrenal output, and/or HPA axis dysregulation are all terms which i ndicate maladaptation to chronic stress and are associated with conditions such as burnout, PTSD, impacts of early life trauma, chronic fatigue syndrome, etc.
- Adrenal insufficiency (Primary, secondary, or tertiary) is extreme hypocortisolism; it can lead to life threatening events and necessitates glucocorticoid and possibly mineralocorticoid replacement therapy. Similarly, a blunted or flattened circadian release of cortisol should prompt a workup for adrenal insufficiency.
- Maximize sleep – at least 7 hours each night for restoration and repair
- Address chronic life/job stressors/major depression and provide relevant support
- Re-establish circadian rhythm through morning light, early, daytime exercise and sleeping in total darkness
- Consider nutrient and herbal support for adrenal nourishment and stress buffering
- Regular macronutrient balanced meals to stabilize blood glucose levels

Table 12. Holistic Treatment Considerations for Low Cortisol Effects

Lifestyle Considerations	Improve Low Cortisol/Re-establish Diurnal Rhythm
Supplements	**Phosphatidyl serine/omega 3 fatty acid** blend 300mg normalization of blunted cortisol response[293] **St John's Wort** 600mg/d increased cortisol at 600mg/d in 2 studies, null cortisol effect in others at varying doses[275] **Licorice** Inhibits 11β-HSD2 – increases cortisol: cortisone ratio in target tissues. dosage varies based on glycyrrhizin content of product, genetics, gut flora, blood pressure. Licorice may promote pseudoaldosteronism effects if dose is too high – monitor BP[294] **Adaptogens** such as **Rhodiola rosea, Eleutherococcus senticosus** and **Schisandra chinensis** show benefits for fatigue, endurance and cognitive performance in several studies, however, no evidence found for increased cortisol with use[295] Hydrocortisone (Cortef) for adrenal insufficiency*
Diet	**Grapefruit Juice -** inhibits 11β-HSD2 – can increase cortisol: cortisone ratio
Exercise	Conflicting studies - **Low intensity exercise** only, to patient tolerance
Stress Management	**Mindfulness meditation** 12-minute meditation scan reduces blunted cortisol in cancer patients[296]

***Hydrocortisone/Cortef is a prescription glucocorticoid replacement used for adrenal insufficiency. Lower doses (2.5-10mg daily) are sometimes used by integrative physicians for low or moderate hypocortisolism or HPA axis dysregulation with blunted cortisol levels. Risk reward considerations, frequent cortisol/DHEAS monitoring, evaluation for side effects and established tapering programs are advised with any glucocorticoid replacement plan.**

Further Testing Considerations for Low Cortisol

- **Cardiac Health Panel**
- **Neurotransmitter test**
- **Total Tox Burden** (Heavy Metals, Environmental Toxins, and Mycotoxins tests)
- **Thyroid Panel**
- Extreme hypocortisolism requires a more extensive endocrine workup including ACTH stimulation test, etc., for adrenal insufficiency. Endocrinologist referral is advised.

Urinary Free Melatonin - 6-Hydroxymelatonin Sulfate (MT6s)

Urinary Diurnal Melatonin Mt6s	Melatonin (1st morning) Melatonin (2nd morning) Melatonin (Evening) Melatonin (Night) Melatonin (pooled)

Melatonin is a hormone synthesized from tryptophan and secreted by the pineal gland. Its principal role is in maintaining circadian rhythm and sleep induction; however, it also has antioxidant, anti-inflammatory,immunomodulatory, and anticancer effects.[297] Disruption in melatonin secretion is positively correlated with certain clinical conditions such as major depressive disorder, metabolic syndrome, Alzheimer's disease, Parkinson's disease, fibromyalgia, hypertension, and cancer.[298-301]

Melatonin is metabolized to 6-hydroxymelatonin, primarily in the liver, and then conjugated with sulfuric acid to form 6-sulfatoxymelatonin sulfate (MT6s). This is the primary melatonin metabolite found in the urine.[302] Urinary MT6s strongly correlates with plasma melatonin rhythm and is an established marker for evaluation of plasma melatonin.[303]

Melatonin is primarily secreted in response to environmental light cues. Melatonin production begins to increase about 2 hours prior to an established bedtime and increases throughout the sleep cycle. During peak darkness, the highest amount of melatonin is produced, typically around 3-4am in a typical sleep cycle. As light enters the room in the early daytime, melatonin levels drop off. Melatonin production is at its lowest at midday.

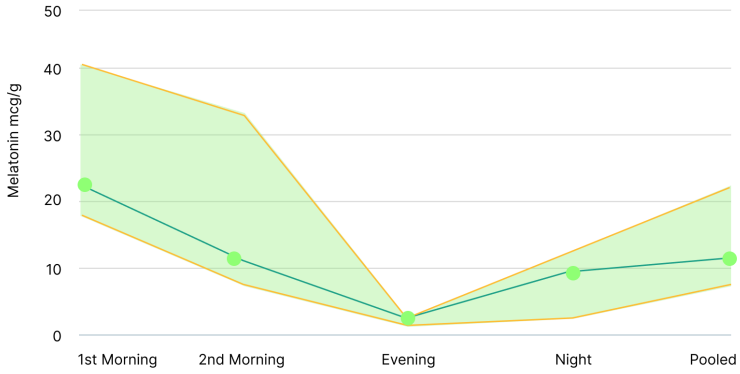


Figure 3. Diurnal Melatonin

This graph is an average result of melatonin urine results from 4x a day testing. The peak morning result is the collection of excreted overnight urine, which typically has the highest amount of melatonin. Epidemiological studies have shown a significant relationship between nocturnal plasma melatonin and morning urinary MT6 levels.[302]

Melatonin levels typically drop off at midday and results of that urine collection containing metabolized melatonin are seen in the evening sample. Melatonin metabolism studies have shown that there is a time lag seen between plasma melatonin values and urine values that is expected to be about 2 hours due to melatonin metabolism in the liver and subsequent renal clearance.[304] This timing is affected by individual renal variability. Towards the end of day, melatonin levels rise again, and this initial rise is seen in the night urine sample.

↑ Potential Causes of Increased Melatonin

- Use of supplemental melatonin
- Use of SSRI antidepressants and MAO inhibitors or supplements such as 5-HTP or tryptophan
- Caffeine)[305]
- Oral contraceptives (mixed studies)[305]

↓ Potential Causes of Decreased Melatonin

- Age – In the 40's melatonin production begins to decline, and declines increase with age related calcification of the pineal gland
- Exposure to blue light from computer screens in the evening and electromagnetic frequencies
- Exercise at night can delay or reduce melatonin secretion
- Comorbid heath conditions such as: renal disease, hepatic disease, ophthalmic disease, spinal cord disease
- Medications: β1-adrenergic blockers (i.e., propranolol), α2 adrenergic agonists (i.e., clonidine), NSAIDS, benzodiazepines[302,305]
- Consider melatonin supplementation if clinically relevant

Urinary Creatinine

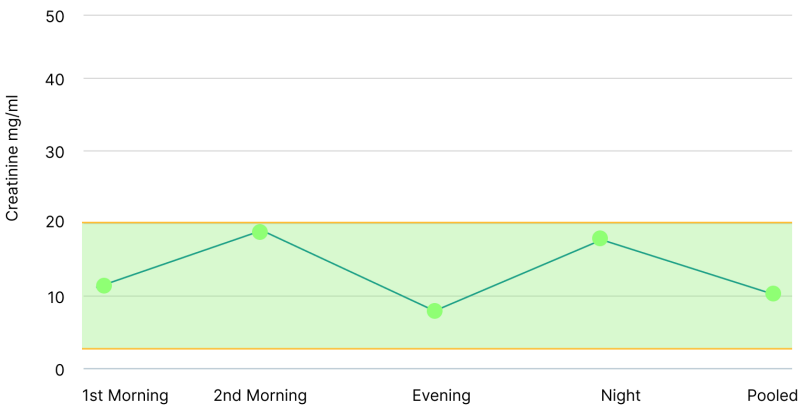


Figure 4. Diurnal Creatinine

Urine creatinine, a metabolite of creatine from muscle and protein metabolism, is often used as a biomarker of renal function and/or can reflect a number of systemic conditions. Creatinine elimination is stable, largely independent of diet and hydration fluctuations. Thus, creatinine values in the urine, in addition to use as a disease biomarker, can also be used to adjust for hydration status in urine lab testing.[306] All the hormones tested on Vibrant Wellness Urine Hormones test are divided by urinary creatinine concentration values to improve accuracy of results which may be otherwise skewed by hydration variability. Individual creatinine concentrations are charted here for additional reference.

Disclaimer

The Urine Hormone test is a general wellness test. This interpretive guide is intended to offer information for improvement of functions associated with a general state of health while making references to diseases or conditions. The content in this guide is not meant to diagnose, treat, or cure any disease or condition. The clients who receive Vibrant Wellness Urine Hormone test results are advised to consult their physician, and/or health care provider team for diagnosis and further follow up care, including but not limited to additional testing, prescription medication, and any treatment interventions including diet, exercise, or lifestyle management.

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