VibrantWellness

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Saliva Hormones Interpretive Guide

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Table of Contents

Disclaimer and Regulatory Statement

This Saliva Hormones Interpretive Guide is intended to be used in tandem with Vibrant Wellness's Salivary 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. 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 general wellness test and interpretive guide intended use relates to sustaining or offering general improvement to functions associated with a general state of health while making reference 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 Salivary 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.

The Vibrant Wellness platform provides tools to track and analyze general wellness profiles and encourage a general state of health and well-being.

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. So, these tests are for wellness and informational purpose only.

Vibrant is actively doing clinical research on these samples, de-identified from patients under an IRB and will make research publications towards the same as and when the clinical utility is well established. These tests have been laboratory developed and their performance characteristics determined by Vibrant America LLC, a CLIA-certified laboratory performing the test CLIA#:05D2078809. The test has not been cleared or approved by the U.S. Food and Drug Administration (FDA). Although FDA does not currently clear or approve laboratory-developed tests in the U.S., certification of the laboratory is required under CLIA to ensure the quality and validity of the tests.

Advantages of Salivary Testing of Steroid Hormones

Saliva, as a diagnostic biofluid, has been labelled the 'mirror of the body' as it can reflect the health and disease processes within.[1] In the endocrine work up specifically, saliva offers several distinct advantages over serum and other testing methodologies.

The primary benefit of salivary testing is that steroid hormones collected from saliva are unconjugated and/or bioavailable rather than the total, or protein bound, hormones measured in serum.[2,3] Due to this unique feature, salivary hormone testing has been well established to confer reliability and accuracy in many clinical scenarios.[1,3,4] These include adrenal glucocorticoid measurements and diagnosis of Cushing's disease and adrenal insufficiency[5] and more direct assessment of target tissue hormone levels.[6] Salivary testing also assesses baseline hormone levels of menstruating females (i.e., luteal phase testing).

Regarding use of transdermal creams, such as progesterone, it has been shown that serum and whole blood levels consistently underestimate tissue levels of hormones following transdermal progesterone use.[7] Due to the sensitivity of saliva in reflecting transdermal progesterone levels, and likelihood of underestimation of these values in serum, salivary progesterone testing is currently one of the more reliable testing methods available to prevent overdosages of progesterone from topical creams and gels.[7,8]

In addition to these clinical advantages, collection of salivary hormones conveys more convenience than serum testing. Patients collect samples themselves, in their home. There is no incidence of "needle stick" injuries to medical personnel with salivary collection. There is no increased glucocorticoid release from the stress of venipuncture for the patient. Lastly, because of this ease of collection, salivary samples are convenient to collect throughout several points throughout the day and several points in a menstrual cycle, if desired.

SALIVARY TESTING STRENGTHS

Convenient & painless collection	Can be used to measure baseline levels of sex hormones	Can be used to measure bioavailable hormone	Can be used to measure cortisol & HPA axis dysregulation	Can be used to monitor various methods of HRT
✓	✓	✓	✓	✓

Advantages of Salivary Testing of Steroid Hormones

Saliva originates from pairs of the major salivary glands, the parotid, submandibular, and sublingual glands. Saliva also includes fluid from numerous small buccal glands which line the mouth.[9]

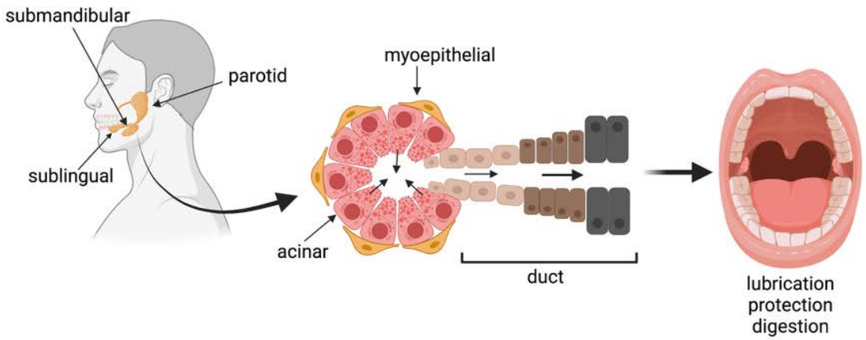


Figure 1. Piraino LR, Benoit DSW, DeLouise LA. Salivary Gland Tissue Engineering Approaches: State of the Art and Future Directions. Cells. 2021; 10(7):1723. https://doi.org/10.3390/cells10071723 Open Access. CC by 4.0.

The salivary glands are lined with secretory units called acini, made up of acinar cells. (See Fig. 1) The acinar cells are surrounded by contractile cells, called myoepithelial cells. The myoepithelial cells contract the cell to produce the flow of secretions. Once the secretions are pulsed, salivary ducts which are connected to the acinar cells then collect the saliva and distribute it into the oral cavity.[10]

Hormones can enter saliva by a variety of mechanisms. For steroids such as cortisol and testosterone, which are neutrally charged, hormones enter the gland from rapid diffusion from the capillaries through the acinar cells of the glands.[4] For positively charged steroids, like DHEAS, the mode of hormone entry is by diffusion between the tight junctions of the acinar cells. Steroids can also enter saliva from blood or plasma via oral abrasions or directly from oral intake. [4]

While small, lipophilic steroid hormones enter the salivary gland by passive diffusion, the gland membrane acts as a barrier to conjugated or tightly bound hormones. This results in free, unbound hormone in the saliva. [6]

What are Biologically Available Hormones and Why Should We Test Them?

Serum testing typically measures the carrier or protein-bound version of hormone that is travelling through the blood. Conversely, saliva measures biologically available hormone.

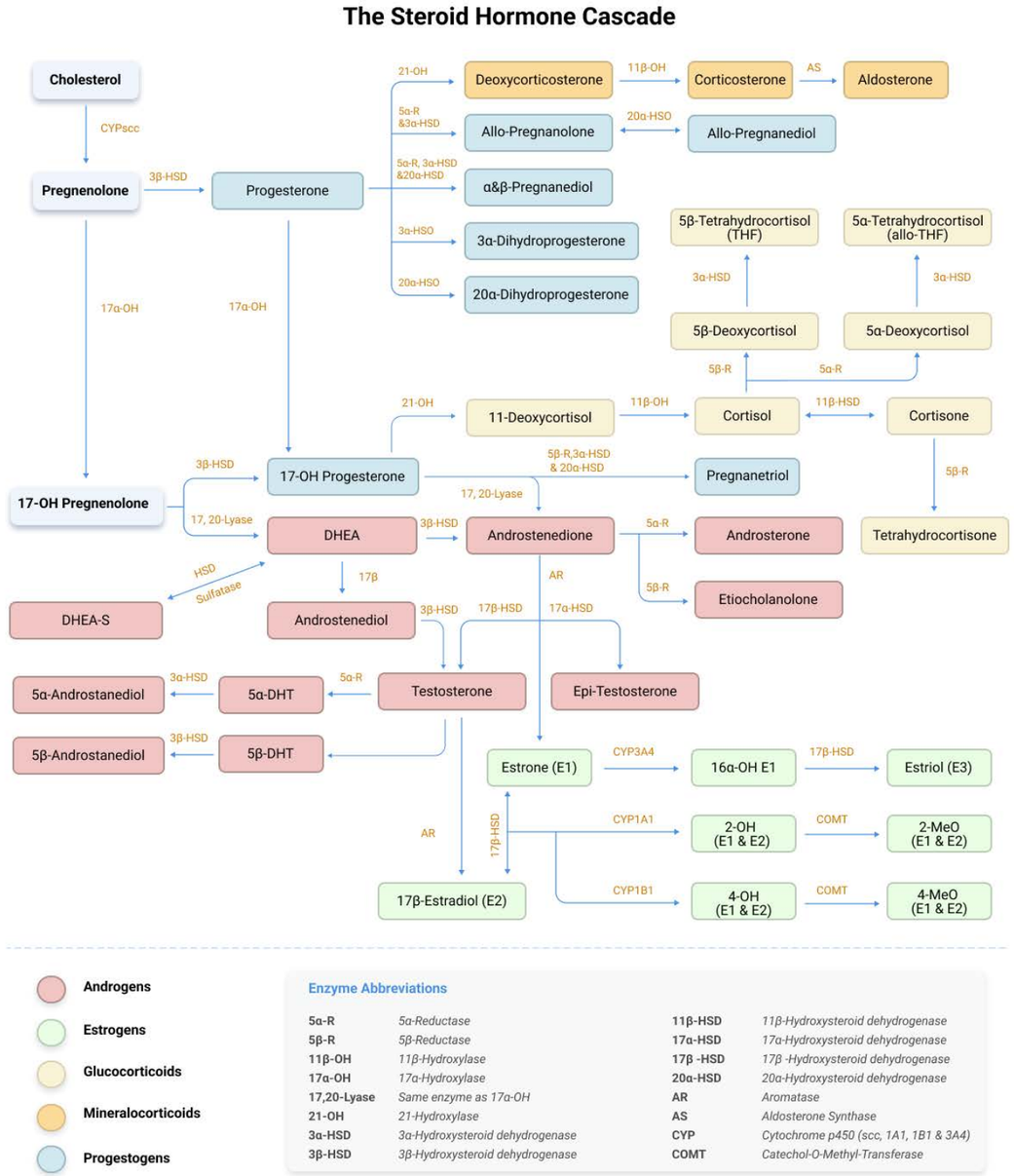
While free and albumin-bound hormone can enter tissues, only unbound hormone can exert actions on target cells. It is the combination of free hormones as well as loosely bound hormones (which can rapidly become unbound or free in tissue capillaries) that are collectively termed “bioavailable hormones” [2]

As changing physiological conditions affect levels of binding proteins, measurement of bioavailable hormones is thought to reflect hormone activity more accurately in many clinical situations.

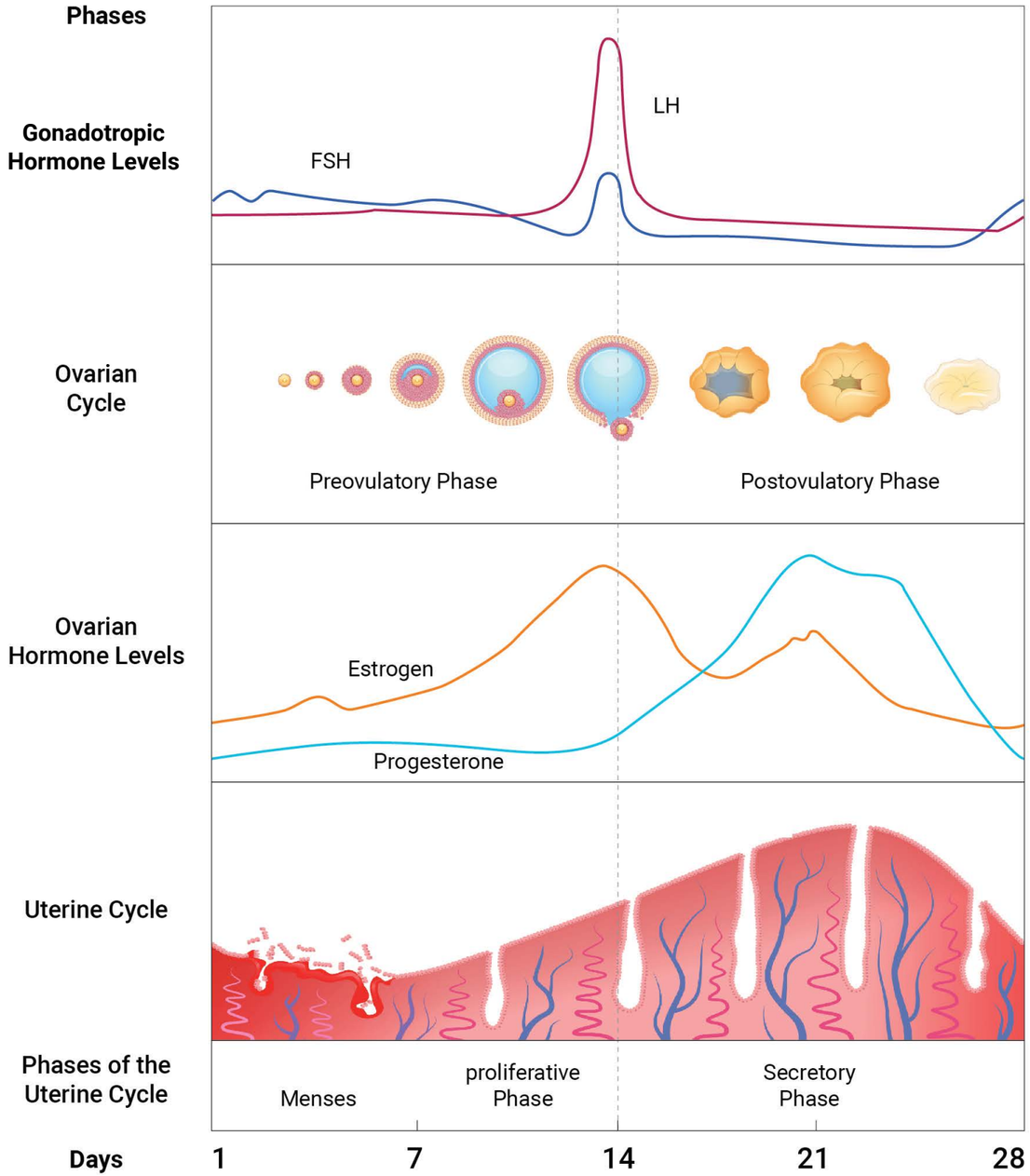
Lab Methodology for Salivary Hormone Testing at Vibrant America

Vibrant America utilizes liquid chromatography-tandem mass spectrometry (LC-MS/MS) exclusively for salivary hormone testing. This has the advantage of high specificity compared to immunoassays. LC-MS/MS also allows simultaneous measurements of multiple analytes and has a low limit of quantification which means precision and accuracy despite low levels of hormone in the sample.[5]

Hormone Cascade



The Menstrual Cycle

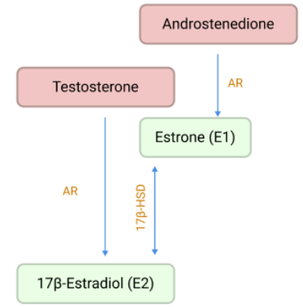


What is Estradiol (E2)?

The major estrogens produced are estradiol (17β-estradiol, E2), estrone (E1), and estriol (E3). Estradiol, commonly known as E2 or 17B-Estradiol, is the predominant and most biologically active estrogen in circulation in males and females.[11,12] Estradiol plays a key role in the development of the female reproductive system 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.[13]

How is Estradiol (E2) Made?

E2 originates from cholesterol, which is converted through a cascade of progesterone and androgen intermediates, finally resulting in estradiol through aromatization from testosterone. [14] 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-estradiol.[12,13] In postmenopausal women, the estradiol acts locally in a paracrine or endocrine manner and circulating estradiol spills over from these local areas.[15]



In males, E2 is predominantly produced by the testes.[13] Males and postmenopausal women have significantly lower levels of estradiol than premenopausal women.

Causes of High Estradiol (E3)

High estradiol can be the results of excess aromatase activity, environmental exposures to xenoestrogens, chronic liver disease, hyperthyroidism, dysregulated gut microbiome, or ectopic production of estradiol from cancerous tissues.[13,16,17,18] Inflammation can also contribute to excess estrogen, as acidic diet and other inflammatory triggers cause lowered ph., which activates aromatase enzyme.[14] Other causes of increased aromatase activity include factors such as age, obesity, insulin, gonadotropins, and alcohol.[19]

Conditions associated with High Estradiol (E3)[13,14]

- PCOS
- Uterine Cancer
- Gastric Cancer
- Multiple Sclerosis
- Obesity
- Endometriosis
- Prostate Cancer
- Thyroid Cancer/goiter
- Schizophrenia
- Gynecomastia
- Breast Cancer
- Pituitary Cancer
- SLE
- Oligospermia
- Male hypogonadism
- Breast Cysts
- Gallbladder Dz
- Fibroids
- Ovarian Cancer

Symptoms of High Estradiol (E2) [14,20,21,22]

- Heavy menstruation
- Nervousness/irritability
- Mood swings
- Headaches
- Weight gain
- Sleep disturbances
- Fibrocystic breasts
- Bloating
- Worsened PMS
- Fatigue
- In males, excess estrogen is linked to depression
- infertility, and enlarged breasts

Causes of Low Estradiol (E2)

Most commonly, menopause, ovariectomy, and aging result in low estradiol. However, there can be many other causes. Compounds which cause aromatase inhibition or increased prolactin (luteotropic hormone or luteotropin) will reduce aromatase activity resulting in lower estrogen.

Drugs which increase prolactin include the following: antidepressants, antipsychotics, anticonvulsants, opiates, estrogens, anti-androgens, anti-hypertensive drugs, and H2-receptor antagonists.

Drugs or toxicants which increase aromatase inhibition include the following: aromatase inhibitors (anastrazole, exemestane, herbicides (glyphosates, Roundup etc.) agricultural antifungals, immunosuppressive drugs (glucocorticosteroids, methotrexate), antimalarials and cigarette smoke.[14]

Anti-mullerian hormone and smoking are also miscellaneous factors that reduce aromatase activity. *Please note that Vibrant America Clinical Lab does not measure anti-mullerian hormone at this time.*

Lastly, hypogonadism and genetic disorders such as 17α-hydroxylase/17,20-lyase deficiency or estrogen resistance syndrome can result in low estradiol levels.[13]

Conditions Associated with or Worsened by Low Estradiol (E3)[14,23]

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

Low Estrogen Symptoms[12–14, 21,22,24]

- Hot flashes
- Night sweats
- Urinary infections
- Urinary incontinence
- Irregular bleeding
- Low libido
- Painful intercourse
- Osteoporosis
- Episodes of rapid heartbeat
- Depression
- Emotional instability
- Deficits in multi-tasking, short term memory and executive function

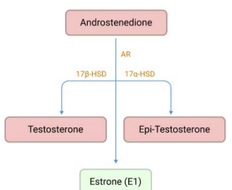
Estrone (E1)

What is Estrone (E1)?

Estrone (E1) is a weaker estrogen than estradiol (E2); by some estimates it has less than 10% the strength of estradiol.[25] While levels of E1 do not differ significantly in pre- vs. post-menopausal women,[15] E1 is the predominant estrogen in post-menopausal women[26] by a factor of 100-fold compared to E2.[25] In post-menopausal women, E1 concentrations have been positively correlated with bone mineral density and breast cancer risk, and inversely correlated with colon cancer risk.[15] Serum estrone levels are an important indicator of serum estradiol levels in post-menopausal women.[15]

How is Estrone (E1) Made?

Like all estrogens, estrone originates from cholesterol and subsequent progesterone and androgen intermediates. Specifically, estrone (E1) is derived from the conversion of androstenedione by an aromatase enzyme, 17- βHSD, found in peripheral and adipose tissues. Estrone is converted to small amounts of estradiol in peripheral tissues.



Causes of High Estrone (E1)

High levels of estrone are commonly found in women who use hormone replacement therapy (HRT), even if the estrogen is estradiol rather than estrone, as they can interconvert.[27] Of note is that advanced age and obesity will further elevate estrone values in users of HRT. [28] Liver disease,[29] hyperthyroidism, [30] and hormonally producing tumors of ovary, adrenal gland, etc also can raise estrone. [31,32]

Conditions associated with High Estrone (E1)

Refer to section on elevated estradiol for a full list of estrogen associated conditions.

Causes of Low Estrone (E1)

Estrone levels appear to be similar in pre and postmenopausal women, [15] 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. [15] Low estrone levels, below reference ranges, primarily result from decreased aromatase activity. Refer to the Low Estradiol section of this guide for a comprehensive list of medications that can decrease aromatase activity.

Conditions Associated with Low Estrone (E1)

Refer to section on low estradiol for a full list of low estrogen associated conditions.

Estriol (E3)

What is Estriol (E3)?

Estriol (E3) is the weakest of the three estrogens: it dissociates rapidly from estrogen receptors.[33] 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.[33] E3, as a hormone, 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.[34]

How is Estriol (E3) Made?

All estrogens originate from cholesterol and androgen intermediates, notably aromatization from testosterone and androstenedione. E2 is reversibly oxidized to estrone and most estriol is formed from estrone via CYP3A4 metabolism and 17-Beta HSD, as seen to the right.[34] Both E2 and E1 can be irreversibly converted to estriol in the liver.

Causes of High Estriol (E3)

Elevated estriol is most common in pregnancy or use of bioidentical hormone replacement prescriptions.[35]

Conditions that create elevated estradiol may also increase estriol, refer to Estradiol section for further reference.

Causes of Low Estriol (E3)

Most of the data on low estriol results from studies involving maternal fetal screening results. Low estriol during pregnancy is a marker of fetal compromise and can indicate genetic disorders, fetal growth restriction, placental sulfatase deficiency.[36] It can also be associated with congenital adrenal hyperplasia, aromatase deficiency, fetal adrenal insufficiency and/or fetal loss.[37]

Low estradiol conditions may contribute to a low estriol status in non-pregnant women, for further reference refer to section on Low Estradiol.

Ratio of E3/E1+E2

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.[38]

Vibrant America's salivary 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 salivary 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.

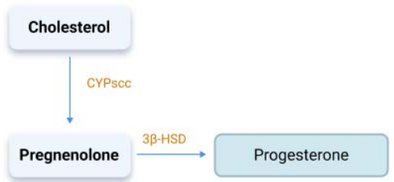
Progesterone

What is Progesterone?

Some researchers have postulated[39] that "Life is not possible without progesterone" due to its critical role across multiple systems. In a menstruating woman, after monthly ovulation the corpus luteum in the ovary produces progesterone, which halts endometrial growth and induces secretory changes in the uterine lining to promote successful implantation. Withdrawal of progesterone is associated with the onset of menses.[40] Progesterone also has vital roles on breast development and during pregnancy and lactation. In men, progesterone influences spermiogenesis and testosterone biosynthesis in the Leydig cells.[41] In men and women progesterone plays a vital role as a precursor to critical steroid hormones such as aldosterone, cortisol, testosterone, and estradiol. It also has important roles in the cardiovascular, renal, and musculoskeletal systems.[42] Further, progesterone is a neuroprotectant and neuromodulator and aids with sleep. Lastly, progesterone plays a role in immune support and cancer protection against endometrial, colorectal cancers and potentially others.[39]

How is Progesterone Formed?

Progesterone can be formed from tissues in ovaries and placenta, testes, adrenal gland, and brain. Mechanistically, free cholesterol converts to pregnenolone via CYP450scc enzyme activity and then converts to progesterone via 3 –β-HSD (hydroxysteroid dehydrogenase) activity.[42]Progesterone produced from the gonads travels through the blood and exerts hormonal effects, while progesterone of adrenal origin is converted into glucocorticoids and androgens.[42]



Causes of High Progesterone

Progesterone is commonly elevated beyond luteal phase values in pregnancy and with exogenous progesterone supplementation or exposure. Other situations whereby progesterone can be increased are ovarian cysts, ovarian tumors and testicular tumors which increase progesterone secretion. Also, adrenal hyperplasia from congenital or oncologic origin can increase progesterone through overproduction of progesterone precursors. Stress and caffeine have also been linked to slightly elevated levels.[43,44]

Conditions of High Progesterone

It is unclear if endogenously produced progesterone elevations result in any specific medical condition, aside from elevated progesterone symptoms, listed below.

Synthetic progestins have been positively associated with breast cancer risk.[45,46] Observational studies suggest, in menopausal women, estrogen and (bioidentical) progesterone use may be associated with lower breast cancer risk compared to estrogen and synthetic progestin combinations.[47] One recent study, in postmenopausal women not on hormone replacement, suggests that endogenously elevated progesterone is associated with elevated breast cancer risk in menopausal women, however this predominates in women with moderate to high estradiol levels. In this study, women with low estradiol levels and elevated progesterone had a reduced breast cancer risk.[48] More studies in all aspects of bioidentical progesterone and breast cancer risk would help clarify ongoing questions.

Symptoms Associated with High Progesterone [49]

- Drowsiness
- Dizziness
- Headache
- Weight gain
- Acne
- Water retention/bloating

Causes of Low Progesterone

In a non-pregnant woman, low progesterone can be caused by irregular or anovulatory cycles (including during breastfeeding), PCOS, aging and menopause, thyroid disorders, obesity, over exercise, hyperprolactinemia, anorexia, long term use of NSAIDs,[50] oral contraceptives,[51] and endometriosis. Environmental toxicants such as phthalates, pesticides, herbicides, etc., show pre-clinical evidence of reduced steroidogenesis, including progesterone.[52]

Low progesterone in pregnancy can be caused by ectopic pregnancy or complications or failure with the fetus or placenta. 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.[53]

Conditions Associated or Worsened by Low Progesterone [54]

- Amenorrhea
- Luteal deficiency
- Ectopic pregnancy
- PCOS
- Endometrial hyperplasia
- Decreased ovarian function
- Turner's syndrome
- Eclampsia
- Abnormal uterine bleeding
- Anovulatory menstrual cycles
- Hypogonadism
- Miscarriage
- Menopause and aging

Symptoms Associated with Low Progesterone[55]

- Headaches
- Bloating
- Breast tenderness
- PMS Symptoms
- Insomnia
- Anxiety/depression
- Mood swings
- Infertility
- Dysmenorrhea
- Irregular menstrual cycles

The Progesterone to Estradiol Ratio – PG/E2 Ratio

What is the Progesterone to Estradiol Ratio – PG/E2 Ratio?

The Pg/E2 ratio is commonly used empirically as a marker of "Estrogen Dominance," developed, and popularized by the late John Lee, MD.[56] 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.[57–59]

Elevated Pg/E2 Ratio Causes

An elevated Pg/E2 ratio can be caused by exogenous progesterone use (most common), increased endogenous progesterone production (i.e., common in pregnancy), and/or decreased estradiol production. Empirically this is termed "Progesterone Dominance."

Conditions Associated with Increased PG/E2 ratio

Clinically, progesterone dominance can be associated with symptoms of increased progesterone (refer to progesterone section) as well as increased symptoms of estrogen deficiency due to hormone receptor down-regulation.

Progesterone is hyperthermic, thus, an increase in the Pg/E2 ratio across the menstrual cycle is associated with increased average body temperature.[57]

In IVF treatments, increased Pg/E2 ratio during the follicular phase is associated with decreased pregnancy as compared to later luteal rise in Pg/E2.[58]

Decreased Pg/E2 Ratio Causes

A decreased Pg/E2 ratio infers low progesterone relative to estradiol concentrations. A low ratio can be caused by natural perimenopausal reductions in progesterone which precede reductions in estradiol. It can also occur later in menopause and andropause with natural aging and reduced progesterone levels.

A decreased Pg/E2 ratio can additionally reflect sub-optimal clearance of estradiol and/or estradiol metabolites related to metabolic health conditions, impaired detoxification pathways and/or environmental xenoestrogen exposure.

Conditions Associated with Decreased Pg/E2 Ratio

A decreased Pg/E2 ratio is empirically known as "Estrogen Dominance" as coined by John Lee, MD.[56] Clinically this produces estrogen excess symptoms and progesterone deficiency symptoms. Refer to respective progesterone and estradiol sections for a thorough listing of these symptoms.

There is some research to suggest decreased Pg/E2 ratio is associated with fibrocystic breast disease and luteal mastodynia.[59]

In IVF treatments, a significantly decreased Pg/E2 ratio during the late follicular phase is associated with decreased pregnancy rates.[60]

Lifestyle Considerations for Estrogen and Progesterone Hormone Imbalances

ESTROGEN DOMINANCE/PROGESTERONE DEFICIENCY

Treatment objectives: Consider which of the following single or combination of therapeutic approaches is the best fit for the patient with estrogen dominance and/or progesterone deficiency

- Reduce circulating estradiol, estrone and xenoestrogens
- Target microbiome support for enhanced estrogen clearance
- Decrease aromatization from androgens to estrogens, especially with excess adiposity
- Increase progesterone and Pg/E2 ratio when indicated

HOLISTIC TREATMENT CONSIDERATIONS FOR ESTROGEN DOMINANCE/PROGESTERONE DEFICIENCY

Lifestyle Considerations	Reduction of Estrogens and Xenoestrogens	Support Microbiome for Estrogen Clearance	Decrease Aromatase Activity	Increase Progesterone and Pg/E2 Ratio
Specific Supplements Additional Pathways to consider for support:	**DIM** up to 300mg per day [61] **Sulforaphane*** [62] **Vitamin D** Repletion in deficient women (to blood levels above 32ng/mL) ↑SHBG and ↓ bioavailable E2 and testosterone[63] **Phase II Liver Support** **Methylation** Support **COMT Support** (if genetic variants are present)	**Lactobacillus spp**[64]* **Calcium D - glucarate** 1500mg or higher/day*	Numerous **flavonoids** show preclinical evidence of decreased aromatase activity*: **Resveratrol** [65] **Grape Seed extract** [66] **Citrus Peels/citrus flavonoids** [67] **Apigenin** [65] **Chrysin**[68,69]	**Vitex agnus castus** 40mg per day[70] **Vitamin C** 750mg/day[71] **B6 pyridoxine** 200mg per day[72] **White peony** root–daily tea or 3-5 g of root/day[73] **Evening Primrose Oil** [74]*
Diet	**Brassica family vegetables – 1.5 servings/day**[63] **Wheat sprout juice -100ml/day**[75] **Broccoli sprouts**[62]*	**Fiber from fruit/veggies - 30 g/day**[76] **Flaxseed meal – 10 -30 g/day**[77,78]**, positive benefits with 50mg lignans per day**[77] **Kefir** [64]*	White button mushrooms[66]* Foods with apigenin and/or resveratrol: grapes, berries, celery, parsley, onions, oranges, chamomile, thyme, basil, oregano, red wine[79] Kale, collards[66]	**Increase foods high in zinc, B6, Vitamin C, Magnesium**[71,72]

Exercise/ Sauna	**Dry Sauna- 15 minutes 3x per week*** **Exercise – 100-300minutes/week treadmill/aerobic reduces estrogen[80]**	**Moderate exercise benefits microbiome diversity[81]**	150 minutes moderate activity per week if weight loss is needed	
Misc. Lifestyle Factors	Reduce or avoid exogenous OCP, HRT Avoid endocrine disruptors such as phthalates, BPA, growth hormones in meat, pesticides[82]		Weight loss if obesity or PCOS is present[83] Lifestyle support to reduce inflammation secondary to obesity[84]	**Consider Progesterone** replacement therapy with oral micronized, transdermal, or compounded prescription. **

* 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 dominance:

1. **Nutripro or Methylation panel** by Vibrant Wellness testing for genetic snp's which may lead to estrogen metabolism issues (MTHFR, COMT)
2. **Gut Zoomer** testing for microbiome and beta glucuronidase influences
3. **Urinary Hormones** testing for estrogen and progesterone metabolites
4. **Environmental Toxins** Test- for plasticizers and other environmental toxicant burden
5. **Cardiometabolic** Testing with Vibrant America Cardiac Health and Diabetes Panel.
6. **Neural Zoomer Plus or Neurotransmitter** testing – progesterone is a neuroprotectant and neuromodulator, progesterone deficiency may affect these pathways

ESTROGEN (E2) DEFICIENCY SYMPTOMS

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

HOLISTIC TREATMENT CONSIDERATIONS FOR ESTROGEN (E2) DEFICIENCY

Lifestyle Considerations	Vasomotor symptom/mood support
Supplements *	**Black Cohosh** for mood and hot flashes 40-127mg/day[85] **Soy Isoflavones** for vasomotor symptoms, bone loss, hypertension – trial doses ranged from 40-120mg isoflavones per day [86] Use organic, non-GMO isoflavones **Maca** 3.0 g/d for libido, depression in menopausal women; improvement in testosterone, but not estradiol, in women[87] **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[88,89,90] **Red Clover** for hot flashes ≥ 80mg/d[91] **Red Ginseng** 0.9-3mg/day for fatigue, memory, hot flashes[92] **Ashwagandha** 300mg 2x/day for vasomotor symptoms, increased estradiol in perimenopausal women in one study[93] **Vitamin E** 800 IU/day for hot flashes[94] **Fenugreek seed** 600mg/day de-husked seed extract for vasomotor symptoms[95]
Diet	**Mediterranean diet** to reduce menopausal symptoms[96]& cardiometabolic issues[97] **Diet with increased fruit/veg, fiber & less sugar and fat** improves vasomotor symptoms[96] **High fiber and soy isoflavones** decrease vasomotor symptoms[96]
Exercise/Sauna	**150 minutes moderate aerobic + strength training** [98] - support for bone health, general estrogen deficiency symptoms **Yoga** – variable practices aid vasomotor and psychological health[99]
Stress Management	**Cognitive Behavioral Therapy** for vasomotor symptoms, insomnia[100] **Hypnosis** for reduction in hot flash severity[101] **Aromatherapy/massage**[101] **Mindfulness Meditation** for distress from vasomotor symptoms[102] **Acupuncture** for hot flashes[103]
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

- **Cardiovascular Health** and CardiaX tests from Vibrant America
- **DEXA** testing for osteoporosis
- **APOE** testing for Alzheimer's Disease
- **Colorectal cancer screening**

LIFESTYLE CONSIDERATIONS FOR LOW ESTRIOL RATIO - E3/E2+E1

Treatment objectives for estriol ratio lower than 1.0:

- Modify excess E2 and E2 through lifestyle interventions as suggested in estrogen dominance section
- Assess and correct for any pre-existing iodine deficiency

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 clinical studies regarding estriol risk and fibrocystic breast disease and breast cancer,[38,104] as well as pre-clinical evidence that iodine/iodide can modulate estrogen metabolic pathways to favor inactivation of E2.[105–108] Further clinical trials needed.

Iodine interventions, beyond diet suggestions for deficiency, require physician oversight including measures such as initial thyroid and iodine testing, ongoing monitoring, and careful consideration of dose, form, and length of iodine usage. Iodine excess may precipitate hyperthyroidism, hypothyroidism, goiter, and/or thyroid autoimmunity.[109]

LIFESTYLE CONSIDERATIONS FOR LOW PROGESTERONE/ESTRADIOL RATIO

Refer to table for Holistic Treatment Considerations for Estrogen Dominance/Progesterone Deficiency.

Testosterone

What is Testosterone?

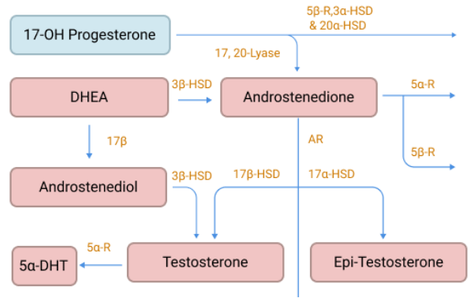
Testosterone is the predominant androgen produced in males and females. In both males and females, testosterone supports reproductive function and libido, maintains muscle mass and bone structure, supports cardiac health and promotes optimal brain function.[110] In males, at puberty, testosterone promotes 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.[111]

In females, testosterone is produced by ovarian production as well as adrenal secretion. 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.[112] 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.[110]

The benefit of salivary testosterone testing is that it is consistent 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.[113]

How is Testosterone made ?

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.



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. Commonly it can also reflect exogenous supplementation whether 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.

It can also be higher in smokers, those exposed to pollutants (polychlorobiphenyls, hexachlorobenzene), and with eating disorders. It can also be high with use of the following drugs: anticonvulsants, atrial natriuretic hormone, barbiturates, cimetidine, clomiphene, estrogens, gonadotropin (males), kaliuretic hormone, oral contraceptives, and vessel dilator hormone.[114]

Conditions Associated with High Testosterone[115]

- Androgenetic alopecia (males)
- Infertility
- Breast Cancer (inconclusive studies with exogenous testosterone use)
- Polycythemia/erythrocytosis (with exogenous testosterone use)
- Obstructive sleep apnea with polycythemia (with exogenous testosterone use
- Prostate enlargement/prostate carcinoma (exogenous testosterone use, conflicting studies)
- Hepatotoxicity (with methyl testosterone use)
- Hypertension and cardiovascular events (inconclusive studies with exogenous testosterone use)
- PCOS (female)
- Virilization in pre-pubertal males and females

Symptoms of High Testosterone

In adult women, increased adrenal androgen production causes hirsutism, acne, male-pattern baldness, menstrual irregularities, oligomenorrhea or amenorrhea, infertility. In more extreme clinical situations, frank virilization can occur at varying ages, which involves a more severe hirsutism along with broad range of signs suggestive of androgen excess such as ambiguous external genitalia, deepening of the voice as well as the above symptoms.

In males, symptoms of acne, hirsutism, oligospermia, hypo-fertility, and elevated anxiety and aggression can be caused from excess endogenous androgen production.[11]

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).[117] In women, oophorectomy and menopause are also common contributing causes.[114]

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.[114]

Conditions Associated with Low Testosterone[118]

- Diabetes Mellitus Type ² (Total but not free testosterone)
- Frailty, Sarcopenia
- All-cause mortality, cardiovascular mortality
- Alzheimer's Disease (Free but not total testosterone)
- Adiposity
- Anemia
- Dysthymia
- Osteoporosis and Fractures
- Androgenetic alopecia
- Gynecomastia (males)
- Infertility
- Erectile dysfunction

Symptoms Associated with Low Testosterone[117]

Symptoms of androgen deficiency are numerous and include the following:

- Low libido
- Reduced bone strength
- Poor concentration
- Depression
- Breast discomfort in males
- Fatigue
- Decreased motivation
- Loss of muscle mass
- Memory issues
- Disturbance of normal sleep pattern
- Reduced physical performance ability
- Delayed development of secondary sex characteristics in prepubertal males
- Decreased body hair
- Weight gain

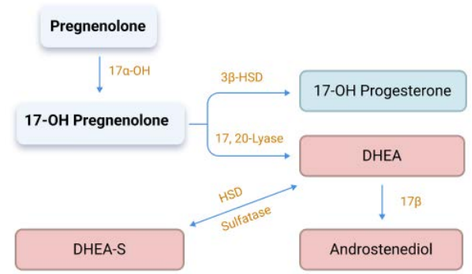
DHEA-S

What is DHEA-S?

DHEA-S is quantitatively the most abundant circulating steroid hormone produced by the adrenal glands. DHEA-S is the sulphated, most abundant, version of DHEA; compared to DHEA it has a longer half-life, does not have diurnal variation, and provides a stable circulating pool from which to measure adrenal androgen activity. By itself, DHEA and DHEA-S are just slightly androgenic but serve as a precursor to androgens and estrogen in the periphery.[119] DHEA-S is known to be active as a neurosteroid and a buffer from the effects of oxidation and glucocorticoids.[120,121] Typically, females are referred for DHEA-S testing for virilization and/or PCOS evaluation. Males are referred for DHEA-S testing for congenital adrenal hyperplasia, primary or secondary adrenal insufficiency, adrenal tumors hypertension and alopecia.

How is DHEA-S Made?

DHEA-S, like all steroid hormones, 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. The sulfation of DHEA into DHEA-S 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).[121]



Causes of High DHEA-S

DHEAS can be mildly elevated for idiopathic reasons; other considerations include exogenous supplementation, androgen secreting adrenal tumor, elevated cortisol, PCOS, steroid sulfatase (STS) deficiency, precocious puberty, and congenital adrenal hyperplasia.[122,123]

Symptoms of High DHEA-S

In males, often there are not noticeable symptoms of elevated DHEA. Men can, however, experience symptoms of estrogen excess through peripheral conversion of androgens to estrogen. Women experience androgenic symptoms from elevated DHEA.

Causes of Low DHEA-S

Biological aging in males and females produces lower DHEA-S levels. Also, chronic stress, chronic inflammation,[124] primary and secondary adrenal insufficiency, and hypothyroidism can result in lower DHEA-S levels. Low levels in amniotic fluid indicate anencephaly in the fetus. Drugs include carbamazepine, dexamethasone, opioids, phenytoin.[122]

Conditions Associated with Low DHEA-S:

There are numerous conditions in which there are associated low DHEA-S values as seen below.[120,121]

- SLE
- Progressive systemic sclerosis
- Inflammatory Bowel Disease
- Rheumatoid arthritis
- Inflammatory Bowel Disease
- Increased coronary heart disease
- Increased All-Cause Mortality
- Septic Shock
- Depression/Anxiety
- Biopolar Disorder
- Alzheimer's Disease
- Sarcopenia
- Androgen Deficiency symptoms

Lifestyle Treatment Considerations for Androgens

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

HOLISTIC TREATMENT CONSIDERATIONS FOR ANDROGEN EXCESS

Lifestyle Considerations	Reduce free androgens in women/improve insulin sensitivity	Interventions that may increase SHBG (Sex Hormone Binding Globulin)
Supplements	**NAC** in PCOS patients 600mg/3x per day[125] **Vitamin D** – 4000iu/day[126] Additionally, repletion in deficient women (to blood levels above 32ng/mL) ↑SHBG and ↓bioavailable E2 and testosterone[63] **Myo**-inositol 4g/day or D-chiro inositol 1g/day[127,128] **Soy isoflavones** 50g/day[129] **Berberine** – 400mg/TID – improves insulin sensitivity[130] **Chromium*** – Improve insulin resistance, may lower DHEA levels in adipose tissue in PCOS patients[131] **Zinc, Magnesium and Selenium** to counter deficiencies in diet[132]	**DIM** 300mg/day[133] **Refer to Insulin Modulating interventions -** increased insulin decreases SHBG[134]
Diet	**DASH Diet** -reduce insulin resistance promotes weight loss in PCOS[135] **Mediterranean Diet** – reduces insulin[135] **Calorie Restriction Diets** -reduce insulin resistance promotes weight loss in PCOS [135] **Low Glycemic/Ketogenic diet** [132] **Almonds** 46 g/d[136] **Walnuts** 36 g/d [136] **Prebiotics/ Probiotics*** -Increased microbiome diversity [137] **Soy isoflavones in diet** – 50g/d [129] **Spearmint tea**[132]	**Coffee, Tea and other caffeine containing beverages ***[139] **Walnuts** 36g/day [136] **Soy milk** 30g/day [140] **Olive Oil*** [141] **High Fiber diet, vegetarian diet**[141] **Insulin Modulating interventions** as increased insulin decreases SHBG [134]
	Green tea [132] **Licorice tea** [132] **Flax seed meal** 30g/day [138]	
Exercise/ Sauna	**Vigorous aerobic exercise – minimum of 120 minutes per week alone or with resistance training** – reduce insulin resistance in PCOS, aid body composition[132]	**Moderate intensity exercise** 225 min/week[142]
Misc Factors	**Optimizing sleep aids** with regulation of dysglycemia[132]	**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:

- If hirsutism is present consider including use of 5-alpha-reductase enzyme blocking herbal supplements such as: Serenoa repens, Camellia sinensis, Rosmarinus officinalis, Glycyrrhiza glabra, etc. [132]
- Pharmacologic therapy such as spironolactone, or spironolactone in combination with licorice[132]

Further testing considerations for androgen excess:

- **Fasting Glucose, Insulin, HgbA¹c**
- **Thyroid Panel**
- **IGF-¹**
- **Prolactin**
- **Liver function tests**
- **SHBG**
- **Urine Hormones Test** from Vibrant America for testosterone and estrogen metabolites
- **Environmental Toxins Test** from Vibrant America to assess xenoestrogen compounds

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

HOLISTIC TREATMENT CONSIDERATIONS FOR TESTOSTERONE DEFICIENCY

Lifestyle Interventions	Symptomatic Support/Increase Testosterone	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**[143–146] **Trigonella foenum-graceum (Fenugreek)** 500-600mg/day seed extract boosts testosterone & libido in males[147] **Mucuna pruriens** - 5g/day seed powder improves T in infertile men and boosts semen quality[148] **Eurycoma longifolia (Long jack)** – 200-400mg/day increased libido, testosterone, ED in men, [147] conflicting studies on efficacy **Korean Red Ginseng** 1500mg/BID – Improves serum testosterone in men with metabolic syndrome,[149] conflicting studies on efficacy[143] **DHEA** >50g/day[150]** may boost T levels, esp. in women **Nigella Sativa** – improves semen parameters in infertile men[147] **Tribulus terrestris** – 750mg/day – improved sexual enhancement in women,[147] several studies show no increase in T in men,[143] may enhance libido **Maca** -1500mg root/BID – improvement in testosterone/libido/sexual function in women on SSRI,[87] several studies in men show no increase in T[143] **Saw palmetto** - Increased T, with decreased DHT and Estrogen in men at 800mg - 2000mg/day [66]	Numerous **flavonoids** show preclinical evidence of decreased aromatase activity*: **Resveratrol**[65] **Grape Seed extract**[66] **Citrus Peels/citrus flavonoids**[67] **Apigenin**[65] **Chrysin** (Honey/propolis) [68,69]
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 [151]	**White button mushrooms*** [66] **Foods with apigenin and/or resveratrol:** grapes, berries, celery, parsley, onions, oranges, chamomile, thyme, basil, oregano, red wine [79] Kale, collards [66]
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. [151]	**150 minutes moderate activity** per week[83]
Stress Management	**Optimize sleep,** sleep deprivation (<5hours/night) linked to decreased testosterone [151] High occupational stress is linked to lower testosterone levels.[151] Stress management interventions may be helpful.	
Miscellaneous Support	Aromatase inhibitors, SERM's and hCG have been shown to raise testosterone[151] Varicocele repair significantly improves testosterone levels[152] 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 T in men with metabolic syndrome[153]

* 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.152 There are also concerns but inconclusive data regarding the role of exogenous testosterone with cardiovascular risks, prostate cancer risks and venous thromboembolism.[152]

Further Support for Testosterone Deficiency includes:

- Avoidance of phthalates and heavy metals in foods and household products. These can reduce testosterone levels through various mechanisms. Further resources for avoidance and elimination of environmental toxicants can be found from the National Association of Environmental Medicine at https://envmedicine.com

<u>Further testing considerations for testosterone deficiency:</u>

- Serum LH and FSH to determine if hypogonadism is primary or secondary in males
- Semen analysis in men with infertility
- CBC to rule out anemia
- Environmental Toxins Test from Vibrant America for phthalates and heavy metals

Salivary Hormone Assessment, Monitoring, and Evaluation of Transgender Persons

Gender-affirming hormone therapy is a multidisciplinary treatment that may involve endocrinologists, primary care practitioners, and specialists. Clinicians should be knowledgeable about the diagnostic criteria for gender-affirming hormone therapy and should aim to maintain physiologic levels of gender-appropriate hormones and persistently monitor hormone levels and metabolic parameters for adverse effects, risks, and complications of treatment. A comprehensive resource to guide gender-affirming hormone therapy is the clinical practice guideline from the Endocrine Society entitled, Endocrine Treatment of Gender Dysphoric/ Gender Incongruent Persons: An Endocrine Society Clinical Practice Guideline.[154]

Adrenal Hormone Testing – A Window into HPA Axis Function

HYPOTHALAMIC PITUITARY AXIS INFOGRAPHIC

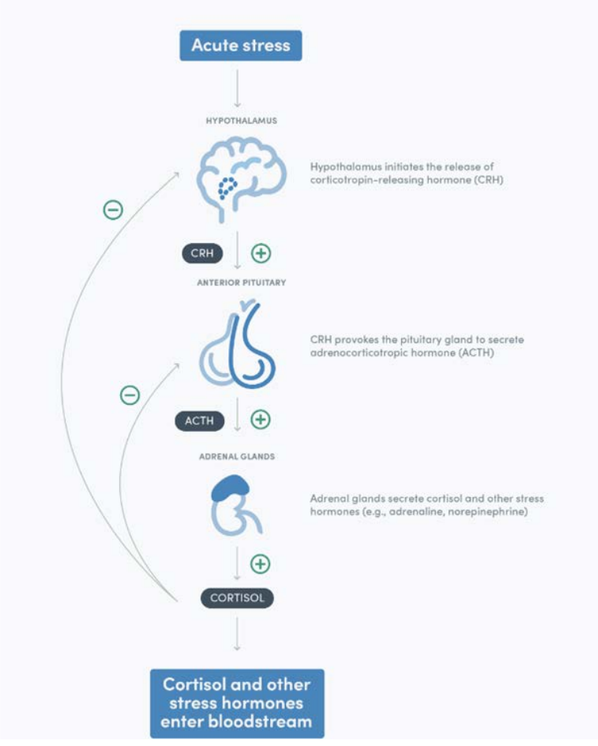


Figure 2. HPA Axis Infographic. Referenced from https://fullscript.com/practice-resources?g=all. Adapted and used with permission.

SALIVARY TESTING AND HPA AXIS DYSREGULATION

Testing of salivary cortisone and cortisol can offer a window into HPA (Hypothalamic pituitary axis) function and dysregulation. A normal physiological response of the HPA axis to a stressful event is characterized by a quick increase in cortisol level, followed by a decrease once the stressor is gone.[155] This process is controlled by the self-regulatory system of the HPA axis. Disturbances in this regulation can be observed in one-time or the diurnal pattern of cortisol release and have been linked to the development of stress-related body and mental disorders. These disorders include conditions such as: Type 2 diabetes, Cushing's disease, hypertension, CFS, fibromyalgia, chronic pain, depression, PTSD, and schizophrenia, among others.

As discussed previously, the primary advantage of salivary, versus plasma, cortisol and cortisone measurement in the endocrine workup is that it measures bioavailable, unbound hormones. Regarding glucocorticoids, approximately 90 -95% of serum total cortisol is bound to proteins such as corticosteroid-binding globulin (CBG) and albumin.[156,157] These proteins are affected by a wide variety of clinical conditions and medications, even common situations such as estrogen replacement therapy, which can increase CBG, and inflammatory states, which can decrease CBG. Thus, by measuring unbound hormones, the clinician obtains a more accurate picture of glucocorticoid activity in target tissues without fluctuations related to binding proteins.[156]

FOR MONITORING HPA AXIS PERFORMANCE, TEST, DON'T GUESS!

Uncovering an HPA axis dysregulation component of a clinical condition provides a new, actionable clinical target for improving outcomes. Furthermore, in monitoring glucocorticoid values over time, changes in patterns can be associated with aspects of clinical care such as treatment responsiveness, probability of disease relapse and recovery rate.[214]

RE-TEST, DON'T GUESS!

Glucocorticoids – Cortisol and Cortisone

Figure 3. Glucocorticoid Production.

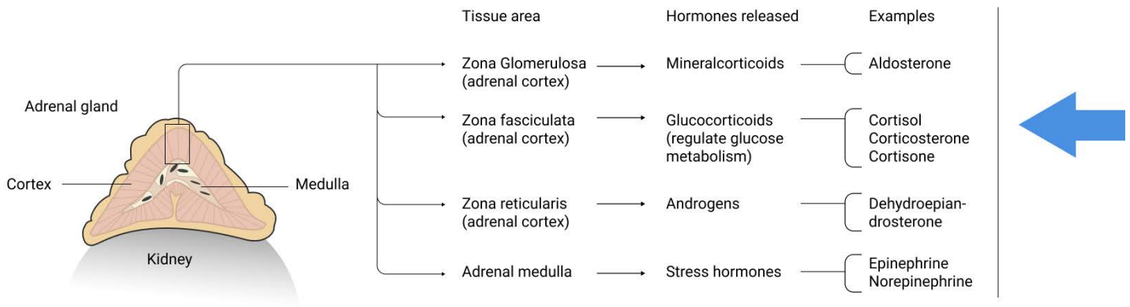


Illustration Source: Anatomy & Physiology, Connexions Web site. http://cnx.org/content/col11496/1.6/, Jun 19, 2013. OpenStax College, CC BY 3.0, via Wikimedia Commons. Adapted by Vibrant America.

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.[158]

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 on preceding page.)

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.[158] 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.

Cortisol and Cortisone

How is Cortisol Made?

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, as pictured to the right.

In the tissues, the glucocorticoids (cortisol, cortisone and corticosterone) are regulated by 11β hydroxysteroid dehydrogenases, type 1 and type 2.

Local Tissue Regulation of Cortisol Cortisone

Initially cortisol is unidirectionally converted to cortisone, an inactive metabolite, by 11β 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 in particular.[157] Due to this enzyme activity, concentration of cortisone in saliva is 2–6 times higher than that of cortisol.[159]

The function of 11β-HSD2 to convert cortisol to cortisone is critical in tissues to protect from overexposure to active cortisol and corticosterone.[160] Otherwise, these active hormones would occupy mineralocorticoid receptors and produce a cascade effect of mineralocorticoid excess symptoms such as sodium retention, hypertension, and hypokalemia.[161]

Local Tissue Regulation of Cortisone Cortisol

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. [161,162]

BENEFIT OF BOTH CORTISONE AND CORTISOL IN SALIVARY MEASUREMENTS

Salivary cortisol has been a mainstay of bioavailable glucocorticoid measurements for over 20 years. Salivary cortisone is a newer addition to salivary hormone testing due to advances in testing. Nonetheless, salivary cortisone, like salivary cortisol, has also been found to be strongly associated with serum cortisol levels.[155] In addition, salivary cortisone has been found to be a better marker of serum free cortisol than salivary cortisol in situations when serum cortisol levels are low, or during hydrocortisone therapy or excess.[159]

> *"Salivary cortisone has been found to be a better marker of serum free cortisol than salivary cortisol in situations where serum cortisol levels are low, or during hydrocortisone therapy or excess" [156]*

HIGH CORTISOL

CLINICAL TIP

How to Evaluate Diurnal Cortisol Values

Generally, environmental as well as biological stressors can influence cortisol output throughout the day. In addition to any underlying disease condition as noted in tables below, look for sporadic stressors such as **hunger or glycemic dysregulation, pain, caffeine, cigarette or drug intake, exercise, commuting, job stress, marital stress, depression, or even chronic loneliness** when evaluating out of range values.

Causes of Elevated Cortisol -Diseases or lifestyle factors which can cause elevated cortisol are as follows:[163,164,165]

- Cushing's Disease/Syndrome from ACTH secreting neoplasm
- Hyperpituitarism or Hyperthyroidism
- Severe hepatic disease
- Anorexia/Hypoglycemia
- Diabetes mellitus (uncontrolled)
- Adrenal neoplastic disease
- Eclampsia
- Shock
- Pregnancy
- Glucocorticoid resistance
- Infectious disease
- Burns
- Virilism
- HIV
- Opioid Withdrawal
- Crohn's disease
- Surgery/Post op recovery
- Chronic Renal Disease
- Hypertension
- Depression/Neuropsychiatric disease
- Stress: Heat/Cold/Trauma/Pain
- Psychological Stress
- Night Shift Work/Circadian Disruption
- Obesity
- Exercise or chronic overexercise
- Cigarette smoking
- Alcoholism and alcohol withdrawal

Drugs which can increase cortisol levels include glucocorticoids, caffeine, nicotine, corticotropin, estrogens, oral contraceptives, yohimbine, and vasopressin.[166] Initial or short-term use of marijuana can increase cortisol, while opposite effects may result from chronic or heavy use.[167]

Consequences of Persistent Hypercortisolism or Hyperactivation of HPA Axis[168]

- Bone Fragility
- Dyslipidemia
- Atherosclerosis
    - Coagulation
    - Cardiovascular Remodeling
- Increased infections
- Visceral adiposity
- Sarcopenia
- Diabetes
- Mood disorders
    - Depression
    - Anxiety
    - Bipolar Disorder
    - Memory Impairment

Elevated Timed Cortisol Samples (AM, NOON, EVENING, NIGHT)

Pooled Cortisol -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. [169]

AM Cortisol – In addition to the above variables, elevated waking AM cortisol values can specifically reflect depression, early stages of burnout, pain, glycemic dysregulation or job-related stress.[170] Severe obstructive sleep apnea has been shown to produce irregular AM cortisol values in studies (both high and low).[171,172]

Noon Cortisol – As a solo elevation, this generally reflects sporadic or situational stress triggers as listed above. 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.[173]

Evening Cortisol – In addition to the above variables, 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. [169,174]

Night Cortisol – 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, i.e., at or over 5.5 ng/ml, should prompt consideration of Cushing's syndrome or disease.[175]

Salivary cortisol measurements, both bedtime and midnight, have been shown to diagnose Cushing's disease as well as plasma and urine measurements.[175]

LOW CORTISOL

Causes of Decreased Cortisol - Diseases or lifestyle factors which can cause decreased cortisol are:[166,176,174]

- 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)
- Postpartum pituitary necrosis
- Liver Disease
- Pituitary adenoma
- Craniopharyngioma/ Hypophysectomy
- Long term use or recent withdrawal from corticosteroid therapy
- Hypopituitarism
- Rheumatoid arthritis
- Early traumatic experiences, Chronic stress, PTSD
- Hypothyroidism
- Waterhouse-Friderichsen syndrome
- Recurrent infectious processes

Drugs which can decrease cortisol levels include opioids,[178] 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.[167]

Symptoms of Persistent Hypocorticolism or Hypoactivation of HPA axis[179,180]

- Fatigue
- Muscle weakness
- Nausea/Vomiting
- Weight loss
- Myalgias
- Pale skin
- Hyponatremia
- Dizziness and Hypotension
- Depression
- Hyperpigmentation (in primary adrenal insufficiency)

Decreased Timed Cortisol Samples (AM, NOON, EVENING, NIGHT)

Decreased Pooled Cortisol -Any of the above conditions 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. [181]

Decreased AM Cortisol – Severe exhaustion can result in a decreased AM cortisol, in addition to severe obstructive sleep apnea[172] and pre-existing conditions listed above. Studies have shown morning salivary cortisol can be used to test for adrenal insufficiency (Addison's disease) and it is a "non-inferior" method as comparted to serum.[156] Researchers proposed a value lower than 1.16ng/ml (3.2nmol/L) for salivary AM cortisol should be followed up by further stimulation testing for adrenal insufficiency.[156]

Decreased Noon, Evening and Night Cortisol – Generally, decreased values represent incomplete recovery from acute stress exposures, exhaustion from chronic stress and/or conditions related to low cortisol output as seen above.

Salivary 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 salivary cortisone itself is an inactive metabolite, it has been shown, like cortisol, to directly compare to serum free cortisol levels. [155,157,159] In general, salivary cortisone values tend to parallel salivary cortisol values. Thus, similar situational or condition triggers cause elevations or depressions in salivary cortisone values as cortisol values. Diurnal cortisone values have not yet been studied for normative value comparisons.[155] The following considerations can be applied when reviewing cortisone values.

A NOTE ABOUT 11β-HSD ENZYMES

11β-HSD1 and 11β-HSD2 enzyme activity can affect cortisol and cortisone levels. These enzymes affect local target tissues in an intracrine manner, as in 11βHSD2 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.[162]

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-HSD1 in the kidney deactivates a similar percentage.[162] Thus, up or downregulation of these enzymes can play a role in cortisone and cortisol dynamic levels.

Increased Cortisone Relative to Cortisol

Factors which can promote actions of 11β-HSD1 Inhibition/11β-HSD1 Upregulation in select tissues

- Estrogen[162]
- Progesterone[162]
- Thyroid Hormone[151]
- 7-Keto DHEA[162]
- PCOS[182]
- Bile Acids[162]
- Pravastatin
- Coffee[160]
- Green Tea[183]
- Curcumin[184]
- Holy Basil
- Vitamin A185
- Pregnancy upregulates 11βHSD2186

Increased Cortisol Relative to Cortisone

Factors which may have actions of 11β-HSD2 Inhibition/11β-HSD1 Upregulation in select tissues

- Licorice (pastilles)[187]
- Grapefruit juice[187]
- Glucocorticoids[162]
- Progesterone[151]
- Proinflammatory cytokines (IL-6, TNF-α)[162]
- Synthetic endocrine disruptors (phthalates, organotins, alkylphenols)[162]
- Inflammatory Conditions such as osteoporosis, joint disorders, neurodegenerative diseases, diabetes, metabolic syndrome, obesity[188]

Clinical tip:

Clinical studies have shown increased 11β-HSD1 activity associated with type 2 diabetes, adipose tissue with obesity and metabolic syndrome.

11β-HSD1 activity is theorized to increase in target tissues, i.e abdominal adipose regions, while intact HPA axis feedback loops maintain glucocorticoid homeostasis in the plasma.

11β-HSD1 is also elevated in the aging brain, where it exacerbates glucocorticoid-related cognitive decline.

INTERPRETING CORTISOL CIRCADIAN RHYTHM GRAPH – G.A.S. AND 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.[189]

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,[190] 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.[181] Second, ongoing stressors that threaten physical integrity, involve trauma, and are uncontrollable can elicit a high, flat diurnal profile of cortisol secretion.[190] 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. Thirdly, 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.[190]

Clinical tip:

Circadian Rhythm Graph at a Glance

1. Does the Diurnal rhythm look normal from AM to PM?

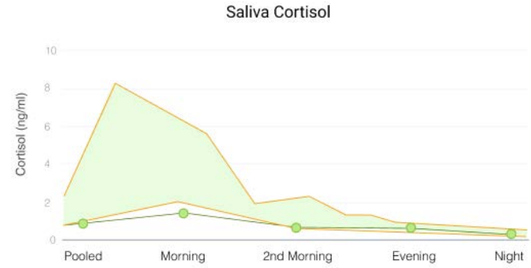
2. Is there a single elevation only, that can be explained by a situational trigger or chronic condition? (Reflects more need for stress trigger and condition support).

3. Is there a combination of either high or low elevations with loss of diurnal rhythm? (Reflects more need for condition, trigger and HPA axis support)

Normal HPA Axis Function

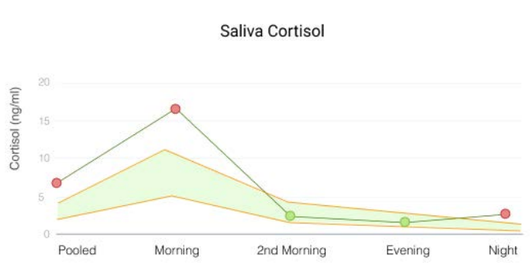
A normal cortisol circadian rhythm is that cortisol is elevated in the morning, peaks within 30-45 minutes following waking, known as the cortisol awakening response or CAR, then begins a steady downward trend until it reaches its nadir at bedtime.[181]

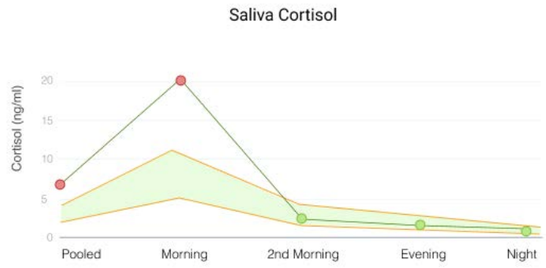
In the sample below, the circadian rhythm of this sample demonstrates intact HPA functionality, i.e., a smooth pattern from highest in the morning to lowest in the evening. One can observe the second morning cortisol is trending slightly high, but overall axis function and pooled cortisol value remains within the normal range.



HPA Axis Dysregulation

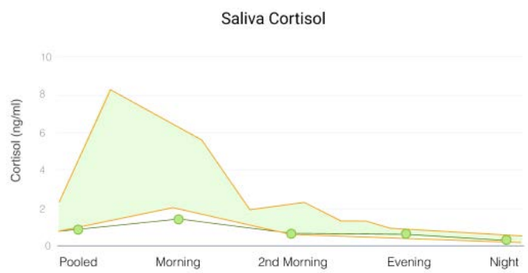
HPA dysregulation generally infers some loss of diurnal rhythm throughout the 12-hour waking period. This can be from a blunted diurnal rhythm or from more than one out of range elevation or depression in the diurnal sample. In the graphs below there is obvious HPA axis dysregulation. In both, there is an over-elevation of cortisol upon waking followed by a noticeable loss of HPA axis circadian rhythm after mid-morning. In the graph on the left, a nighttime spike in cortisol shows further dysregulation.





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.[191]



CLINICAL KEY

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:

- 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 – TRANSIENT OR PERSISTENT

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
  - ✓ Autoimmune disease, arthritis or chronic MSK pain, chronic sinusitis, CVD, GI inflammation

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 positive studies for Sensoril and Shoden brands [192] **Phosphatidyl serine/phosphatidic acid 400mg** [193] **L-theanine** 200mg[194]anti-stress and cortisol lowering effect **Curcumin** 500mg BID – lowered AM cortisol[195] Curserin brand - Phosphatidyl serine/curcumin blend -800mg/BID – lowers AM cortisol[196] **Panax Ginseng 1.5g**/ Korean ginseng BID reduced cortisol in metabolic syndrome[149] **Rhodiola** 300mg/BID[197] **Relora** – Magnolia/phellodendron 250mg BID[198] **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[199]
Diet	**Low sucrose diet/carbohydrate restriction**– May modulates 11β-HSD enzyme activity to reduce truncal obesity[200] **Prebiotics, high fiber diet** may reduce cortisol levels, anxiety, and related cognitive function[201] **Cherry juice (Jerte Valley Brand)** 125 ml/BID[202] 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[203] **Habitual exercise** (low or moderate intensity) regulates HPA axis function, buffers anxiety response and rumination time [204,205] 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[203,204] **Forest Bathing** – Decreases cortisol after forest walking, anticipation of stress relief is also helpful[207] **Biofeedback** – 5 minutes once a day decreases stress and cortisol levels[208] **QiGong** – Reduces cortisol, raises serotonin and brain derived neurotropic factor (BDNF)[209] **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

- **DEXA** scan for osteoporosis
- **Blood pressure** monitoring
- **Vibrant America Cardiovascular Panel and Diabetes Panel**
- **hs-CRP** for inflammation
- **Thyroid Panel**
- **Methylation Panel**
- 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.
- **Tickborne or Infections** tests to explore occult infections
- **Chronic Inflammation Panel, Gut Zoomer** for inflammation if cause of persistent inflammation is unknown or clinical symptoms correlate

LOW CORTISOL – TRANSIENT OR BLUNTED

Treatment approach to low cortisol

- 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 indicate 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. Researchers proposed a value lower than 1.16ng/ml (3.2nmol/L) for salivary AM cortisol should be followed up by further stimulation testing for adrenal insufficiency[156] 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

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[210] **St John's Wort** 600mg/d – increased cortisol at 600mg/d in 2 studies, null cortisol effect in others at varying doses[197] **Licorice** –Inhibits 11βHSD2 – 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. [211] **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.[212] Hydrocortisone (Cortef) for adrenal insufficiency*
Diet	**Grapefruit Juice** – inhibits 11BHSD2 – 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[213]

*Hydrocortisone/Cortef is a prescription glucocorticoid replacement used for adrenal insufficiency. Lower doses (2.5-10mg qd) 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 program is advised with any glucocorticoid replacement plan.

Further testing considerations for Low Cortisol

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

References

1. Kaczor-Urbanowicz KE, Martin Carreras-Presas C, Aro K, Tu M, Garcia-Godoy F, Wong DTW. Saliva diagnostics – Current views and directions. Exp Biol Med. 2017;242(5):459-472. doi:10.1177/1535370216681550

2. Stanczyk FZ. Treatment of postmenopausal women with topical progesterone creams and gels: Are they effective? Climacteric. 2014;17(Suppl 2):8-11. doi:10.3109/13697137.2014.944496

3. Kozloski MJ, Schumm LP, McClintock MK. The utility and dynamics of salivary sex hormone measurements in the national social life, health, and aging project, wave 2. Journals Gerontol - Ser B Psychol Sci Soc Sci. 2014;69:S215-S228. doi:10.1093/geronb/gbu123

4. Lewis JG. Steroid analysis in saliva: an overview. Clin Biochem Rev. 2006;27(3):139-146. http://www.ncbi.nlm.nih.gov/pubmed/17268582%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC1579286

5. Bäcklund N, Brattsand G, Israelsson M, et al. Reference intervals of salivary cortisol and cortisone and their diagnostic accuracy in Cushing's syndrome. Eur J Endocrinol. 2020;182(6):569-582. doi:10.1530/EJE-19-0872

6. Gröschl M. Saliva: A reliable sample matrix in bioanalytics. Bioanalysis. 2017;9(8):655-668. doi:10.4155/bio-2017-0010

7. Du JY, Sanchez P, Kim L, Azen CG, Zava DT, Stanczyk FZ. Percutaneous progesterone delivery via cream or gel application in postmenopausal women: a randomized cross-over study of progesterone levels in serum, whole blood, saliva, and capillary blood. Menopause. 2013;20(11):1169-1175. doi:10.1097/GME.0b013e31828d39a2

8. Stanczyk FZ, Matharu H, Winer SA. Bioidentical hormones. Climacteric. 2021;24(1):38-45. doi:10.1080/13697137.2020.1862079

9. Vining RF, Mcginley RA, Mcginley RA. Hormones in saliva. Crit Rev Clin Lab Sci. 1986;23(2):95-146. doi:10.3109/10408368609165797

10. Punj A. Secretions of the Human Salivary Gland. In: LewiNova, ed. Salivary Glands: New Approaches in Diagnostics and Treatment. Intechopen online; 2018. doi:10.5772/intechopen.75538

11. Files JA, Ko MG, Pruthi S. Bioidentical hormone therapy. Mayo Clin Proc. 2011;86(7):673-680. doi:10.4065/mcp.2010.0714

12. Wise PM, Suzuki S, Brown CM. Estradiol: A hormone with diverse and contradictory neuroprotective actions. Dialogues Clin Neurosci. 2009;11(3):297-303. doi:10.31887/dcns.2009.11.3/pmwise

13. Kumar A, Banerjee A, Singh D, et al. Estradiol: A Steroid with Multiple Facets. Horm Metab Res. 2018;50(5):359-374. doi:10.1055/s-0044-100920

14. Patel S, Homaei A, Raju AB, Meher BR. Estrogen: The necessary evil for human health, and ways to tame it. Biomed Pharmacother. 2018;102(March):403-411. doi:10.1016/j.biopha.2018.03.078

15. Davis SR, Martinez-Garcia A, Robinson PJ, et al. Estrone is a strong predictor of circulating estradiol in women age 70 years and older. J Clin Endocrinol Metab. 2020;105(9):E3348-E3354. doi:10.1210/clinem/dgaa429

16. X. Chai, S. Zeng WX. Estrogen-Metabolizing Enzymes in Systemic and Local Liver Injuries: A Case Study of Disease–Drug Interaction. In: Drug Metabolism in Diseases. ; 2017:241-255.

17. Parida S, Sharma D. The Microbiome-Estrogen Connection and Breast Cancer Risk. Cells. 2019;8(12). doi:10.3390/cells8121642

18. Shapira I, Sultan K, Lee A, Taioli E. Evolving Concepts: How Diet and the Intestinal Microbiome Act as Modulators of Breast Malignancy. ISRN Oncol. 2013;2013:1-10. doi:10.1155/2013/693920

19. Fukami M, Tsuchiya T, Vollbach H, et al. Genomic basis of aromatase excess syndrome: Recombination- and replication-Mediated rearrangements leading to CYP19A1 overexpression. J Clin Endocrinol Metab. 2013;98(12). doi:10.1210/jc.2013-2520

20. Stanikova D, Luck T, Bae YJ, Thiery J, Ceglarek U, Engel C, Enzenbach C, Wirkner K, Stanik J, Kratzsch J R-HS. Increased estrogen level can be associated with depression in males. Psychoneuroendocrinology. 2018;Jan(87):196-203.

21. He L, Guo W, Qiu J, An X, Lu W. Altered Spontaneous Brain Activity in Women During Menopause Transition and Its Association With Cognitive Function and Serum Estradiol Level. Front Endocrinol (Lausanne). 2021;12(May):1-8. doi:10.3389/fendo.2021.652512

22. Gholizadeh S, Sadatmahalleh SJ, Ziaei S. The association between estradiol levels and cognitive function in postmenopausal women. Int J Reprod Biomed. 2018;16(7):455-458. doi:10.29252/ijrm.16.7.455

23. Murphy N, Strickler HD, Stanczyk FZ, et al. A Prospective Evaluation of Endogenous Sex Hormone Levels and Colorectal Cancer Risk in Postmenopausal Women. J Natl Cancer Inst. 2015;107(10):1-10. doi:10.1093/jnci/djv210

24. Girard R, Météreau E, Thomas J, Pugeat M, Qu C, Dreher JC. Hormone therapy at early post-menopause increases cognitive control-related prefrontal activity. Sci Rep. 2017;7(December 2016):1-11. doi:10.1038/srep44917

25. Jeyakumar M, Carlson KE, Gunther JR KJ. Exploration of dimensions of estrogen potency: parsing ligand binding and coactivator binding affinities. J Biol Chem. 2011;286(15):12971-12982.

26. MA. JKA. “Estrogens.” In: Meyler’s Side Effects of Drugs. Elsevier Publishing; 2015:122-151.

27. Reed MJ, Beranek PA, Ghilchik MW JV. Conversion of estrone to estradiol and estradiol to estrone in postmenopausal women. Obs Gynecol. 1985;Sept(66):361-5.

28. Karim R, Mack WJ, Hodis HN, Roy S SF. Influence of age and obesity on serum estradiol, estrone, and sex hormone binding globulin concentrations following oral estrogen administration in post-menopausal women. J Clin Endocrinol Metab. 2009;Nov;94(11):4136-43. doi:10.1210/jc.2009-0643

29. Jasonni VM, Bulletti C, Bolelli GF, Franceschetti F, Bonavia M, Ciotti P FC. Estrone sulfate, es-trone and estradiol concentrations in normal and cirrhotic postmenopausal women. Steroids. 1983;May;41(5):569-73. doi:10.1016/0039-128x(83)90022-3.

30. Southren L. E al. The Conversion of Androgens to Estrogens in Hyperthyroidism. J Clin Endocrinol Metab. 1974;38(2):207–214.

31. Trabert B, Brinton LA E al. Circulating Estrogens and Postmenopausal Ovarian Cancer Risk in the Women’s Health Initiative Observational Study. Cancer Epidemiol Biomarkers Prev. 2016;Apr;25(4):648-656. doi:10.1158/1055-9965

32. Cavlan D, Bharwani N GA. Androgen- and estrogen-secreting adrenal cancers. Semin Oncol. 2010;Dec;37(6):638-648.

33. Falah N, Torday J, K. Quinney S, M. Haas D. Estriol review: Clinical applications and potential biomed-ical importance. Clin Res Trials. 2015;1(2):29-33. doi:10.15761/crt.1000109

34. Ali ES, Mangold C, Peiris AN. Estriol: Emerging clinical benefits. Menopause. 2017;24(9):1081-1085. doi:10.1097/GME.0000000000000855

35. Mucci LA, Lagiou P, Tamimi RM, Hsieh C, Adami H. Pregnancy estriol , estradiol , progesterone and prolactin in relation to birth weight and other birth size variables (United States). Published online 2003:311-318.

36. Settiyanan T, Wanapirak C, Sirichotiyakul S, et al. Association between isolated abnormal levels of maternal serum unconjugated estriol in the second trimester and adverse pregnancy outcomes unconjugated estriol in the second trimester and adverse pregnancy. 2016;7058(June). doi:10.3109/14767058.2015.1075503

37. Minsart A, Onderbergen A Van, Jacques F, Kurt C, Gillerot Y. Indication of prenatal diagnosis in pregnancies complicated by undetectable second-trimester maternal serum estriol levels. Published online 2008:27-30.

38. Lemon HM, Wotiz HH, Parsons L, Mozden PJ. Reduced Estriol Excretion in Patients with Breast Cancer Prior to Endocrine Therapy. JAMA J Am Med Assoc. 1966;196(13):1128-1136. doi:10.1001/jama.1966.03100260066020

39. Nagy B, Szekeres-Barthó J, Kovács GL, et al. Key to life: Physiological role and clinical implications of progesterone. Int J Mol Sci. 2021;22(20). doi:10.3390/ijms222011039

40. David H, Taylor H, Patrick R. Miscarriage of unclear etiology. Cochrane Database Syst Rev. 2018;11:16. doi:10.1002/14651858.CD003511.pub4.www.cochranelibrary.com

41. Oettel M, Mukhopadhyay AK. Progesterone: The forgotten hormone in men? Aging Male. 2004;7(3):236-257. doi:10.1080/13685530400004199

42. Taraborrelli S. Physiology, production and action of progesterone. Acta Obstet Gynecol Scand. 2015;94:8-16. doi:10.1111/aogs.12771

43. Herrera AY, Nielsen SE, Mather M. Stress-induced increases in progesterone and cortisol in naturally cycling women. Neurobiol Stress. 2016;3:96-104. doi:10.1016/j.ynstr.2016.02.006

44. Kotsopoulos J, Eliassen AH, Missmer SA, Susan E, Tworoger SS. NIH Public Access. 2010;115(12):2765-2774. doi:10.1002/cncr.24328.Relationship

45. Collins JA, Blake JM, Crosignani PG. Breast cancer risk with postmenopausal hormonal treatment. Hum Reprod Update. 2005;11(6):545-560. doi:10.1093/humupd/dmi028

46. Lieberman A, Curtis L. In defense of progesterone: A review of the literature. Altern Ther Health Med. 2017;23(6):24-32.

47. Asi N, Mohammed K, Haydour Q, et al. Progesterone vs. synthetic progestins and the risk of breast cancer: A systematic review and meta-analysis. Syst Rev. 2016;5(1):1-8. doi:10.1186/s13643-016-0294-5

48. Trabert B, Bauer DC, Buist DSM, et al. Association of Circulating Progesterone With Breast Cancer Risk Among Postmenopausal Women. JAMA Netw open. 2020;3(4):e203645. doi:10.1001/jamanetworkopen.2020.3645

49. Lexicomp. UpToDate. Progesterone Drug Information.

50. B.Q S, A.M DA-Z, S.S DS. Effects of Some Non Steroidal Anti-inflammatory Drugs on Ovulation in Women with Mild Musculoskeletal Pain (A Clinical Study). IOSR J Pharm Biol Sci. 2014;9(4):43-49. doi:10.9790/3008-09444349

51. Fleischman DS, Navarrete CD, Fessler DMT. Oral contraceptives suppress ovarian hormone production. Psychol Sci. 2010;21(5):750-752. doi:10.1177/0956797610368062

52. Piazza MJ, Urbanetz AA. Environmental toxins and the impact of other endocrine disrupting chemicals in women's reproductive health. J Bras Reprod Assist. 2019;23(2):154-164. doi:10.5935/1518-0557.20190016

53. Martelli M, Zingaretti L, Salvio G, Bracci M, Santarelli L. Influence of work on andropause and menopause: A systematic review. Int J Environ Res Public Health. 2021;18(19):1-20. doi:10.3390/ijerph181910074

54. Chernecky C. Progesterone. In: Chernecky, C., Berger B, ed. Laboratory Tests and Diagnostic Procedures. 6th ed. Saunders; 2013.

55. Ford O, Lethaby A, Roberts H, Mol BWJ. Progesterone for premenstrual syndrome. Cochrane Database Syst Rev. 2009;(2). doi:10.1002/14651858.CD003415.pub3

56. Lee, J., Hanley, J., Hopkins V. What Your Doctor May Not Tell You about Menopause: The Breakthrough Book on Natural Hormone Balance. Grand Central Publishing; 2004.

57. Grant LK, Gooley JJ, St Hilaire MA, et al. Menstrual phase-dependent differences in neurobehavioral performance: The role of temperature and the progesterone/estradiol ratio. Sleep. 2020;43(2):1-11. doi:10.1093/sleep/zsz227

58. Shalom-Paz E, Aslih N, Samara N, Michaeli M, Ellenbogen A. Late follicular progesterone to estradiol ratio is not influenced by protocols or gonadotropins used. Reprod Biol Endocrinol. 2015;13(1):1-7. doi:10.1186/s12958-015-0116-y

59. Brkić M, Vujović S, Ivović M, et al. The role of e2/p ratio in the etiology of fibrocystic breast disease, mastalgia and mastodynia. Acta Clin Croat. 2018;57(4):756-761. doi:10.20471/acc.2018.57.04.18

60. Li Y, Luo K, Tang Y, Lin G, Lu G, Gong F. Progesterone/estradiol ratio <0.25 on the day of human chorionic gonadotropin administration is associated with adverse pregnancy outcomes in prolonged protocols for in vitro fertilization/intracytoplasmic sperm injection. Taiwan J Obstet Gynecol. 2017;56(1):27-31. doi:10.1016/j.tjog.2016.05.013

61. Williams DE. Indoles Derived From Glucobrassicin: Cancer Chemoprevention by Indole-3-Carbinol and 3,3'-Diindolylmethane. Front Nutr. 2021;8(October):1-14. doi:10.3389/fnut.2021.734334

62. Yang L, Zahid M, Liao Y, et al. Reduced formation of depurinating estrogen-DNA adducts by sulforaphane or KEAP1 disruption in human mammary epithelial MCF-10A cells. Carcinogenesis. 2013;34(11):2587-2592. doi:10.1093/carcin/bgt246

63. Mason C, De Dieu Tapsoba J, Duggan C, Imayama I, Wang CY, Korde LA, Stanczyk F MA. Effects of vitamin D supplementation during weight loss on sex hormones in postmenopausal women. Menopause. 2016;Jun;23((6)):645-652.

64. Tao J, Li S, Gan RY, Zhao CN, Meng X, Li H Bin. Targeting gut microbiota with dietary components on cancer: Effects and potential mechanisms of action. Crit Rev Food Sci Nutr. 2020;60(6):1025-1037. doi:10.1080/10408398.2018.1555789

65. Balunas M, Su B, Brueggemeier R, Kinghorn A. Natural Products as Aromatase Inhibitors. Anticancer Agents Med Chem. 2013;8(6):646-682. doi:10.2174/187152008785133092

66. Zamir, A.; Ben-Zeev T., Hoffman J. Manipulation of Dietary Intake on Circulating Changes in CirculatingTestosterone Concentrations. Nutrients. 2021;13(3375). doi:10.3390/ nu13103375

67. El-Kersh DM, Ezzat SM, Salama MM, et al. Anti-estrogenic and anti-aromatase activities of citrus peels major compounds in breast cancer. Sci Rep. 2021;11(1):1-14. doi:10.1038/s41598-021-86599-z

68. Manso J, Sharifi-Rad J, Zam W, Tsouh Fokou PV, Martorell M, Pezzani R. Plant Natural Compounds in the Treatment of Adrenocortical Tumors. Int J Endocrinol. 2021;2021. doi:10.1155/2021/5516285

69. Balam FH, Ahmadi ZS, Ghorbani A. Inhibitory effect of chrysin on estrogen biosynthesis by suppression of enzyme aromatase (CYP19): A systematic review. Heliyon. 2020;6(3):e03557. doi:10.1016/j.heliyon.2020.e03557

70. Van Die MD, Burger H, Teede H, Bone K. Vitex agnus-castus extracts for female reproductive disorders: A systematic review of clinical trials. Planta Med. 2013;79(7):562-575. doi:10.1055/s-0032-1327831

71. Henmi H, Endo T, Kitajima Y, Manase K, Hata H, Kudo R. Effects of ascorbic acid supplementation on serum progesterone levels in patients with a luteal phase defect [2]. Fertil Steril. 2003;80(2):459-461. doi:10.1016/S0015-0282(03)00657-5

72. Abraham GE. Nutritional factors in the etiology of the premenstrual tension syndromes. J Reprod Med. 1983;Jul;28(7):446-464.

73. Kashani L, Akhondzadeh S. Herbal medicine in the treatment of polycystic ovary syndrome. J Med Plants. 2016;15(59):1-5.

74. Atteia HH, Alzahrani S, El-Sherbeeny NA, et al. Evening Primrose Oil Ameliorates Hyperleptinemia and Reproductive Hormone Disturbances in Obese Female Rats: Impact on Estrus Cyclicity. Front Endocrinol (Lausanne). 2020;10(January). doi:10.3389/fendo.2019.00942

75. Yi B, Kasai H, Lee HS, Kang Y, Park JY YM. Inhibition by wheat sprout (Triticum aestivum) juice of bisphenol A-induced oxidative stress in young women. Mutat Res. 2011;Sep 18;724(1-2):64-68.

76. Rock CL, Flatt SW, Thomson CA, et al. Effects of a high-fiber, low-fat diet intervention on serum concentrations of reproductive steroid hormones in women with a history of breast cancer. J Clin Oncol. 2004;22(12):2379-2387. doi:10.1200/JCO.2004.09.025

77. Calado A, Neves PM, Santos T, Ravasco P. The Effect of Flaxseed in Breast Cancer: A Literature Review. Front Nutr. 2018;5(February):1-7. doi:10.3389/fnut.2018.00004

78. Phipps R, Slavin L, Kurzer S, Lampe W. of Flax on the Menstrual. 2014;(December):1215-1219.

79. Salehi B, Venditti A, Sharifi-Rad M, et al. The therapeutic potential of Apigenin. Int J Mol Sci. 2019;20(6). doi:10.3390/ijms20061305

80. Schmitz KH, Williams NI, Kontos D, et al. HHS Public Access. 2018;154(2):309-318. doi:10.1007/s10549-015-3604-z.Dose

81. Ticinesi A, Lauretani F, Tana C, Nouvenne A, Ridolo E, Meschi T. Exercise and immune system as modulators of intestinal microbiome: Implications for the gut-muscle axis hypothesis. Exerc Immunol Rev. 2019;25(96):84-95.

82. Mnif W, Hassine AIH, Bouaziz A, Bartegi A, Thomas O, Roig B. Effect of endocrine disruptor pesticides: A review. Int J Environ Res Public Health. 2011;8(6):2265-2303. doi:10.3390/ijerph8062265

83. Colleluori G, Chen R, Turin CG, et al. Aromatase Inhibitors Plus Weight Loss Improves the Hormonal Profile of Obese Hypogonadal Men Without Causing Major Side Effects. Front Endocrinol (Lausanne). 2020;11(May):1-12. doi:10.3389/fendo.2020.00277

84. Morris PG, Hudis CA, Giri D, et al. Breast Tissue of Obese Women With Breast Cancer. Cancer Prev Res (Phila). 2011;4(7):1021-1029. doi:10.1158/1940-6207.CAPR-11-0110.Inflammation

85. Osmers R, Friede M, Liske E, Schnitker J, Freudenstein J HZH. Efficacy and safety of isopropanolic black cohosh extract for climacteric symptoms. Obs Gynecol. 2005;May;105(5 Pt 1):1074-1083.

86. Chen LR, Chen KH. Utilization of isoflavones in soybeans for women with menopausal syndrome: An overview. Int J Mol Sci. 2021;22(6):1-23. doi:10.3390/ijms22063212

87. Dording CM, Schettler PJ, Dalton ED, Parkin SR, Walker RS, Fehling KB, Fava M MD. A double-blind placebo-controlled trial of maca root as treatment for antidepressant-induced sexual dysfunction in women. Evid Based Complement Altern Med. 2015;949036.

88. Suwanvesh N, Manonai J, Sophonsritsuk A, Cherdshewasart W. Comparison of Pueraria mirifica gel and conjugated equine estrogen cream effects on vaginal health in postmenopausal women. Menopause. 2017;24(2):210-215. doi:10.1097/GME.0000000000000742

89. Kim JE, Jeong H, Hur S, Lee J, Kwon O. Efficacy and safety of kudzu flower–mandarin peel on hot flashes and bone markers in women during the menopausal transition: A randomized controlled trial. Nutrients. 2020;12(11):1-13. doi:10.3390/nu12113237

90. Bihlet AR, Byrjalsen I, Andersen JR, et al. The Efficacy and Safety of Multiple Dose Regimens of Kudzu (Pueraria lobata) Root Extract on Bone and Cartilage Turnover and Menopausal Symptoms. Front Pharmacol. 2021;12(October):1-9. doi:10.3389/fphar.2021.760629

91. Myers SP, Vigar V. Effects of a standardised extract of Trifolium pratense (Promensil) at a dosage of 80 mg in the treatment of menopausal hot flushes: A systematic review and meta-analysis. Phytomedicine. 2017;24:141-147. doi:10.1016/j.phymed.2016.12.003

92. So SH, Lee JW, Kim YS, Hyun SH, Han CK. Red ginseng monograph. J Ginseng Res. 2018;42(4):549-561. doi:10.1016/j.jgr.2018.05.002

93. Gopal S, Ajgaonkar A, Kanchi P, Kaundinya A, Thakare V, Chauhan S LD. Effect of an ashwagandha (Withania Somnifera) root extract on climacteric symptoms in women during perimenopause: A randomized, double-blind, placebo-controlled study. J Obs Gynaecol Res. 2021;Dec;47(12):4414-4425.

94. De Franciscis P, Colacurci N, Riemma G, et al. A nutraceutical approach to menopausal complaints. Med. 2019;55(9):16-18. doi:10.3390/medicina55090544

95. Steels E, Steele ML, Harold M CS. Efficacy of a Proprietary Trigonella foenum-graecum L. De-Husked Seed Extract in Reducing Menopausal Symptoms in Otherwise Healthy Women: A Double-Blind, Randomized, Placebo-Controlled Study. Phytother Res. 2017;Sep;31(9):1316-1322.

96. Soleymani M, Siassi F, Qorbani M, et al. Dietary patterns and their association with menopausal symptoms: A cross-sectional study. Menopause. 2019;26(4):365-372. doi:10.1097/GME.0000000000001245

97. Pugliese G. Mediterranean diet as tool to manage obesity in menopause: A narrative review. Nutrition. 2020;Nov-Dec;79(110991).

98. Stojanovska L, Apostolopoulos V, Polman R BE. To exercise, or, not to exercise, during menopause and beyond. Maturitas. 2014;Apr;77(4):318-323.

99. Goldstein KM, Coeytaux RR, Mcduffie JR, et al. Improving vasomotor symptoms; psychological symptoms; and health-related quality of life in peri- or post-menopausal women through yoga: An umbrella systematic review and meta_analysis. Complement Ther Med. Published online 2020:156-164. doi:10.1016/j.ctim.2017.08.011.Improving

100. Guthrie KA, Larson JC, Ensrud KE, et al. Effects of pharmacologic and nonpharmacologic interventions on insomnia symptoms and self-reported sleep quality in women with hot flashes: A pooled analysis of individual participant data from four MsFLASH trials. Sleep. 2018;41(1). doi:10.1093/sleep/zsx190

101. Johnson A, Roberts L, Elkins G. Complementary and Alternative Medicine for Menopause. J Evidence-Based Integr Med. 2019;24:1-14. doi:10.1177/2515690X19829380

102. Carmody JF, Crawford S, Salmoirago-Blotcher E, Leung K, Churchill L ON. Mindfulness training for coping with hot flashes: results of a randomized trial. Menopause. 2011;Jun;18(6):611-620.

103. Chiu HY, Pan CH, Shyu YK, Han BC TP. Effects of acupuncture on menopause-related symptoms and quality of life in women in natural menopause: a meta-analysis of randomized controlled trials. Menopause. 2015;Feb;22(2):234-244.

104. Yonemoto RH. Breast Cancer in Japan and the United States. Arch Surg. 1980;115(9):1056. doi:10.1001/archsurg.1980.01380090036009

105. Stoddard FR, Brooks AD, Eskin BA, Johannes GJ. Iodine alters gene expression in the MCF7 breast cancer cell line: Evidence for an anti-estrogen effect of iodine. Int J Med Sci. 2008;5(4):189-196. doi:10.7150/ijms.5.189

106. Manjer J, Sandsveden M, Borgquist S. Serum iodine and breast cancer risk: A prospective nested case-control study stratified for selenium levels. Cancer Epidemiol Biomarkers Prev. 2020;29(7):1335-1340. doi:10.1158/1055-9965.EPI-20-0122

107. Cann SA, Netten JP Van, Netten C Van, Hospital RJ, Columbia B. cann200. Selen and Iodine in breast cancer prevention .pdf. Published online 2000.

108. Aceves C, Anguiano B, Delgado G. Is iodine a gatekeeper of the integrity of the mammary gland? J Mammary Gland Biol Neoplasia. 2005;10(2):189-196. doi:10.1007/s10911-005-5401-5

109. Farebrother J, Zimmermann MB AM. Excess iodine intake: sources, assessment, and effects on thyroid function. Ann N Y Acad Sci. 2019;Jun(1446(1)):44-65.

110. Bianchi VE, Bresciani E, Meanti R, Rizzi L, Omeljaniuk RJ, Torsello A. The role of androgens in women's health and wellbeing. Pharmacol Res. 2021;171(June):105758. doi:10.1016/j.phrs.2021.105758

111. Jameson JL. Testosterone. In: Endocrinology: Adult and Pediatric. 17th ed. Saunders; 2016.

112. Burger N, Johnson J. Androgen production in women. Androg Reprod Aging. 2005;77(4):1-4. doi:10.1201/b14632-2

113. Clifton S, Macdowall W, Copas AJ, et al. Salivary testosterone levels and health status in men and women in the British general population: Findings from the third national survey of sexual attitudes and lifestyles (natsal-3). J Clin Endocrinol Metab. 2016;101(11):3939-3951. doi:10.1210/jc.2016-1669

114. Chernecky CC. Testosterone. In: Laboratory Tests and Diagnostic Procedures,. 6th ed. Saunders; 2013.

115. Lundy. Testosterone: Drug Information. UpToDate.

116. George P Chrousos. Adrenal Hyperandrogenism. UpToDate.

117. Crawford M, Kennedy L. Testosterone replacement therapy: Role of pituitary and thyroid in diagnosis and treatment. Transl Androl Urol. 2016;5(6):850-858. doi:10.21037/tau.2016.09.01

118. Dos Santos MR, Bhasin S. Benefits and Risks of Testosterone Treatment in Men with Age-Related Decline in Testosterone. Annu Rev Med. 2021;72:75-91. doi:10.1146/annurev-med-050219-034711

119. Dušková M, Kolátorová L, Stárka L. Androgens in women - critical evaluation of the methods for their determination in diagnostics of endocrine disorders. Physiol Res. 2018;67:S379-S390. doi:10.33549/physiolres.933964

120. Matulevicius V, Urbanavicius V, Lukosevicius S, Banisauskaite I, Donielaite G, Galkine A. Importance of dehydroepiandrosterone sulfate assessment with special attention for adrenal tumours and arterial hypertension. Acta Endocrinol (Copenh). 2021;17(1):68-76. doi:10.4183/aeb.2021.68

121. Maninger N, Wolkowitz OM, Reus VI, Epel ES, Synthia H. Neurobiological and Neuropsychiatric Effects of DHEA and DHEA-s. NIH Public Access. 2010;30(1):65-91. doi:10.1016/j.yfrne.2008.11.002. Neurobiological

122. Chernecky C. DHEAS. In: Laboratory Tests and Diagnostic Procedures. 6th ed. Saunders; 2013:432-459.

123. Iliev DI, Braun R, Sánchez-Guijo A, et al. Very High Dehydroepiandrosterone Sulfate (DHEAS) in Serum of an Overweight Female Adolescent Without a Tumor. Front Endocrinol (Lausanne). 2020;11(May):1-7. doi:10.3389/fendo.2020.00240

124. Straub RH, Lehle K, Herfarth H, et al. Dehydroepiandrosterone in relation to other adrenal hormones during an acute inflammatory stressful disease state compared with chronic inflammatory disease: Role of interleukin-6 and tumour necrosis factor. Eur J Endocrinol. 2002;146(3):365-274. doi:10.1530/eje.0.1460365

125. Nemati M, Nemati S, Taheri AM HB. Comparison of metformin and N-acetyl cysteine, as an adjuvant to clomiphene citrate, in clomiphene-resistant women with polycystic ovary syndrome. J Gynecol Obs Hum Reprod. 2017;Sep;46((7)):579-585.

126. Menichini D, Facchinetti F. Effects of vitamin D supplementation in women with polycystic ovary syndrome: a review. Gynecol Endocrinol. 2020;36(1):1-5. doi:10.1080/09513590.2019.1625881

127. Unfer V, Facchinetti F, Orrù B, Giordani B, Nestler J. Myo-inositol effects in women with PCOS: A meta-analysis of randomized controlled trials. Endocr Connect. 2017;6(8):647-658. doi:10.1530/EC-17-0243

128. Pizzo A, Laganà AS, Barbaro L. Comparison between effects of myo-inositol and d-chiro-inositol on ovarian function and metabolic factors in women with PCOS. Gynecol Endocrinol. 2014;30(3):205-208. doi:10.3109/09513590.2013.860120

129. Jamilian M AZ. The Effects of Soy Isoflavones on Metabolic Status of Patients With Polycystic Ovary Syndrome. J Clin Endocrinol Metab. 2016;Sep;101((9)):3386-3394.

130. Li L, Li C, Pan P, et al. A single arm pilot study of effects of berberine on the menstrual pattern, ovulation rate, hormonal and metabolic profiles in anovulatory Chinese women with polycystic ovary syndrome. PLoS One. 2015;10(12):1-11. doi:10.1371/journal.pone.0144072

131. Piotrowska A, Pilch W, Czerwińska-Ledwig O, et al. The Possibilities of Using Chromium Salts as an Agent Supporting Treatment of Polycystic Ovary Syndrome. Biol Trace Elem Res. 2019;192(2):91-97. doi:10.1007/s12011-019-1654-5

132. Szczuko M, Kikut J, Szczuko U, et al. Nutrition strategy and life style in polycystic ovary syndrome—narrative review. Nutrients. 2021;13(7):1-18. doi:10.3390/nu13072452

133. Thomson CA, Chow HHS, Wertheim BC, et al. Breast Cancer Biomarker Modulation in Patients Taking Tamoxifen. 2018;165(1):97-107. doi:10.1007/s10549-017-4292-7.A

134. Haffner SM, Katz MS, Stern MP DJ. The relationship of sex hormones to hyperinsulinemia and hyperglycemia. Metabolism. 1988;Jul;37(7):683-688.

135. Shang Y, Zhou H, Hu M FH. Effect of Diet on Insulin Resistance in Polycystic Ovary Syndrome. . J Clin Endocrinol Metab. 2020;Oct 1;105(10):dgaa425.

136. Kalgaonkar S, Almario RU, Gurusinghe D, et al. Differential effects of walnuts vs almonds on improving metabolic and endocrine parameters in PCOS. Eur J Clin Nutr. 2011;65(3):386-393. doi:10.1038/ejcn.2010.266

137. Yurtdaş G AY. A New Approach to Polycystic Ovary Syndrome: The Gut Microbiota. J Am Coll Nutr. 2020;May-Jun;39(4):371-382.

138. Haidari F, Banaei-Jahromi N, Zakerkish M, Ahmadi K. The effects of flaxseed supplementation on metabolic status in women with polycystic ovary syndrome: A randomized open-labeled controlled clinical trial. Nutr J. 2020;19(1):1-11. doi:10.1186/s12937-020-0524-5

139. Nagata C, Kabuto M SH. Association of coffee, green tea, and caffeine intakes with serum concentrations of estradiol and sex hormone-binding globulin in premenopausal Japanese women. Nutr Cancer. 1998;30(1):21-24.

140. Pino AM, Valladares LE, Palma MA, Mancilla AM, Yáñez M AC. Dietary isoflavones affect sex hormone-binding globulin levels in postmenopausal women. J Clin Endocrinol Metab. 2000;Aug;85(8):2797-2800.

141. Sáez-López C, Soriguer F, Hernandez C, Rojo-Martinez G, Rubio-Martín E, Simó R SD. Oleic acid increases hepatic sex hormone binding globulin production in men. Mol Nutr Food Res. 2014;Apr;58(4):760-767.

142. Friedenreich CM, Woolcott CG, McTiernan A, et al. Alberta physical activity and breast cancer prevention trial: Sex hormone changes in a year-long exercise intervention among postmenopausal women. J Clin Oncol. 2010;28(9):1458-1466. doi:10.1200/JCO.2009.24.9557

143. Smith SJ, Lopresti AL, Teo SYM, Fairchild TJ. Examining the Effects of Herbs on Testosterone Concentrations in Men: A Systematic Review. Adv Nutr. 2021;12(3):744-765. doi:10.1093/advances/nmaa134

144. Wankhede S, Langade D, Joshi K, Sinha SR, Bhattacharyya S. Examining the effect of Withania somnifera supplementation on muscle strength and recovery: A randomized controlled trial. J Int Soc Sports Nutr. 2015;12(1):1-11. doi:10.1186/s12970-015-0104-9

145. Ambiye VR, Langade D, Dongre S, Aptikar P, Kulkarni M, Dongre A. Clinical evaluation of the spermatogenic activity of the root extract of Ashwagandha (Withania somnifera) in oligospermic males: A pilot study. Evidence-based Complement Altern Med. 2013;2013:6-11. doi:10.1155/2013/571420

146. Ahmad MK, Mahdi AA, Shukla KK, et al. Withania somnifera improves semen quality by regulating reproductive hormone levels and oxidative stress in seminal plasma of infertile males. Fertil Steril. 2010;94(3):989-996. doi:10.1016/j.fertnstert.2009.04.046

147. Santos HO, Howell S, Teixeira FJ. Beyond tribulus (Tribulus terrestris L.): The effects of phytotherapics on testosterone, sperm and prostate parameters. J Ethnopharmacol. 2019;235:392-405. doi:10.1016/j.jep.2019.02.033

148. Shukla KK, Mahdi AA, Ahmad MK, Shankhwar SN, Rajender S, Jaiswar SP. Mucuna pruriens improves male fertility by its action on the hypothalamus-pituitary-gonadal axis. Fertil Steril. 2009;92(6):1934-1940. doi:10.1016/j.fertnstert.2008.09.045

149. Jung DH, Lee YJ, Kim CB, Kim JY, Shin SH PJ. Effects of ginseng on peripheral blood mitochondrial DNA copy number and hormones in men with metabolic syndrome: A randomized clinical and pilot study. Complement Ther Med. 2016;Feb(24):40-46.

150. Li Y, Ren J, Li N, Liu J, Tan SC, Low TY MZ. A dose-response and meta-analysis of dehydroepiandrosterone (DHEA) supplementation on testosterone levels: perinatal prediction of randomized clinical trials. Exp Gerontol. 2020;Nov;141(111110).

151. Lo EM, Rodriguez KM, Pastuszak AW, Khera M. Alternatives to Testosterone Therapy: A Review. Sex Med Rev. 2018;6(1):106-113. doi:10.1016/j.sxmr.2017.09.004

152. Raheem OA, Chen T, Akula KP, Greenberg J, Le TV, Chernobylsky D, Sikka SC WT. Efficacy of Non-Testosterone-Based Treatment in Hypogonadal Men: A Review. Sex Med Rev. 2021;Jul;9(3):381-392.

153. Winter AG, Zhao F, Lee RK. Androgen deficiency and metabolic syndrome in men. Transl Androl Urol. 2014;3(1):50-58. doi:10.3978/j.issn.2223-4683.2014.01.04

154. Hembree WC, Cohen-Kettenis PT, Gooren L, et al. Endocrine treatment of gender-dysphoric/ gender-incongruent persons: An endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2017;102(11):3869-3903. doi:10.1210/jc.2017-01658

155. Bakusic J, De Nys S, Creta M, Godderis L, Duca RC. Study of temporal variability of salivary cortisol and cortisone by LC-MS/MS using a new atmospheric pressure ionization source. Sci Rep. 2019;9(1):1-12. doi:10.1038/s41598-019-55571-3

156. Kim YJ, Kim JH, Hong AR, et al. Stimulated Salivary Cortisol as a Noninvasive Diagnostic Tool for Adrenal Insufficiency. Endocrinol Metab. 2020;35(3):628-635. doi:10.3803/enm.2020.707

157. Blair J, Adaway J, Keevil B, Ross R. Salivary cortisol and cortisone in the clinical setting. Curr Opin Endocrinol Diabetes Obes. 2017;24(3):161-168. doi:10.1097/MED.0000000000000328

158. Thau L, Gandhi J SS. Physiology, Cortisol. Stat Pearls Publishing; 2021. https://www.ncbi.nlm.nih.gov/books/NBK538239/#_NBK538239_pubdet_

159. Bae YJ, Reinelt J, Netto J, et al. Salivary cortisone, as a biomarker for psychosocial stress, is associated with state anxiety and heart rate. Psychoneuroendocrinology. 2019;101(October 2018):35-41. doi:10.1016/j.psyneuen.2018.10.015

160. Zhou C, Ye F, Wu H, Ye H, Chen Q. Recent advances in the study of 11β-Hydroxysteroid dehydrogenase type 2 (11β-HSD2)Inhibitors. Environ Toxicol Pharmacol. 2017;52:47-53. doi:10.1016/j.etap.2017.02.021

161. Seckl JR. 11β-hydroxysteroid dehydrogenases: Changing glucocorticoid action. Curr Opin Pharmacol. 2004;4(6):597-602. doi:10.1016/j.coph.2004.09.001

162. Chapman K, Holmes M, Seckl J. 11B-Hydroxysteroid Dehydrogenases Intracellular Gate-Keepers of Tissue Glucocorticoid Action. Physiol Rev. 2013;93(3):1139-1206. doi:10.1152/physrev.00020.2012

163. Woods NF, Mitchell ES, Smith-DiJulio K. Cortisol levels during the menopausal transition and early postmenopause. Menopause. 2009;16(4):708-718. doi:10.1097/gme.0b013e318198d6b2

164. Findling, James, Raff H. Differentiation of Pathologic/Neoplastic Hypercortisolism from Physiologic/Non-neoplastic Hypercortisolism. Eur J Endocrinol. 2017;176(5):R205-R216. doi:10.1530/EJE-16-0946

165. Cannizzaro E, Cirrincione L, Mazzucco W, et al. Night-time shift work and related stress responses: A study on security guards. Int J Environ Res Public Health. 2020;17(2). doi:10.3390/ijerph17020562

166. Chernecky B. Cortisol. In: Laboratory Tests and Diagnostic Procedures. 6th ed. Saunders; 2013.

167. Cservenka A, Lahanas S, Dotson-Bossert J. Marijuana use and hypothalamic pituitary-adrenal axis functioning in humans. Front Psychiatry. 2018;9(OCT). doi:10.3389/fpsyt.2018.00472

168. Favero V, Cremaschi A, Parazzoli C, et al. Pathophysiology of Mild Hypercortisolism: From the Bench to the Bedside. Int J Mol Sci. 2022;23(2):1-27. doi:10.3390/ijms23020673

169. Saxbe DE. A field (researcher's) guide to cortisol: tracking HPA axis functioning in everyday life. Health Psychol Rev. 2008;2(2):163-190. doi:10.1080/17437190802530812

170. Klein M, Weksler N, Gidron Y, Heldman E, Gurski E, Smith OR GG. Do waking salivary cortisol levels correlate with anesthesiologist's job involvement? J Clin Monit Comput. 2012;Dec;26(6):407-413.

171. Mohammadi H, Rezaei M, Sharafkhaneh A, Khazaie H GM. Serum testosterone_cortisol ratio in people with obstructive sleep apnea _ Enhanced Reader.pdf. J Clin Lab Anal. 2020;Jan;34(1):e23011.

172. Bozic J, Galic T, Supe-Domic D, Ivkovic N, Ticinovic Kurir T, Valic Z, Lesko J DZ. Morning cortisol levels and glucose metabolism parameters in moderate and severe obstructive sleep apnea patients. Endocrine. 2016;Sep;53.(3):730-739.

173. Pilger A, Haslacher H, Meyer BM, et al. Midday and nadir salivary cortisol appear superior to cortisol awakening response in burnout assessment and monitoring. Sci Rep. 2018;8(1):1-12. doi:10.1038/s41598-018-27386-1

174. Muscatello R et. a. Comparing the effects of age, pubertal development, and symptom profile on cortisol rhythm in children and adolescents with Autism Spectrum Disorder. Autism Res. 2018;January ;(1):110-120. doi:10.1002/aur.1879.

175. Papanicolaou DA, Mullen N, Kyrou I, Nieman LK. Nighttime salivary cortisol: A useful test for the diagnosis of Cushing's syndrome. J Clin Endocrinol Metab. 2002;87(10):4515-4521. doi:10.1210/jc.2002-020534

176. Fujieda K, Tajima T. Molecular Basis of Adrenal Insufficiency. Pediatr Res. 2005;57(5):62R-69R. doi:10.1203/01.PDR.0000159568.31749.4D

177. Pan X, Wang Z, Wu X, Wen SW, Liu A. Salivary cortisol in post-traumatic stress disorder: A systematic review and meta-analysis. BMC Psychiatry. 2018;18(1):1-10. doi:10.1186/s12888-018-1910-9

178. Aloisi AM, Aurilio C, Bachiocco V, Biasi G FP. Endocrine consequences of opioid therapy. Psychoneuroendocrinology. Dec; 34(Suppl 1:S):162-168.

179. Joseph RM, Ray DW, Keevil B, Van Staa TP, Dixon WG. Low salivary cortisol levels in patients with rheumatoid arthritis exposed to oral glucocorticoids: A cross-sectional study set within UK electronic health records. RMD Open. 2018;4(2):1-8. doi:10.1136/rmdopen-2018-000700

180. Pelewicz K, Miśkiewicz P. Glucocorticoid withdrawal—an overview on when and how to diagnose adrenal insufficiency in clinical practice. Diagnostics. 2021;11(4). doi:10.3390/DIAGNOSTICS11040728

181. Starr LR, Dienes K, Li YI, Shaw ZA. Chronic stress exposure, diurnal cortisol slope, and implications for mood and fatigue: Moderation by multilocus HPA-Axis genetic variation. Psychoneuroendocrinology. 2019;100:156-163. doi:10.1016/j.psyneuen.2018.10.003

182. Vantyghem MC, Marcelli-Tourvieille S, Defrance F WJ. 11beta-hydroxysteroide dehydrogenases. Recent advances. Ann Endocrinol. 2007;Oct;68(5):349-356.

183. Hintzpeter J, Stapelfeld C, Loerz C, Martin HJ, Maser E. Green tea and one of its constituents, epigallocatechine-3-gallate, are potent inhibitors of human 11β-hydroxysteroid dehydrogenase type 1. PLoS One. 2014;9(1):2-10. doi:10.1371/journal.pone.0084468

184. Hu GX, Lin H, Lian QQ, et al. Curcumin as a Potent and Selective Inhibitor of 11β-Hydroxysteroid Dehydrogenase 1: Improving Lipid Profiles in High-Fat-Diet-Treated Rats. PLoS One. 2013;8(3):1-7. doi:10.1371/journal.pone.0049976

185. Marissal-Arvy N, Hamiani R, Richard E, Moisan MP P V. Vitamin A regulates hypothalamic-pituitary-adrenal axis status in LOU/C rats. J Endocrinol. 2013;Sep 6;219(1):21-27.

186. Shearer FJG, Wyrwoll CS, Holmes MC. The Role of 11β-Hydroxy Steroid Dehydrogenase Type 2 in Glucocorticoid Programming of Affective and Cognitive Behaviours. Neuroendocrinology. 2019;109(3):257-265. doi:10.1159/000499660

187. Methlie P, Husebye EE, Hustad S, Lien EA LK. Grapefruit juice and licorice increase cortisol availability in patients with Addison's disease. Eur J Endocrinol. 2011;Nov;165(5):761-769.

188. Dube S, Norby BJ, Pattan V, Carter RE, Basu A BR. 11β-hydroxysteroid dehydrogenase types 1 and 2 activity in subcutaneous adipose tissue in humans: implications in obesity and diabetes. J Clin Endocrinol Metab. 2015;Jan;100(1):E70-6.

189. SELYE H. The general adaptation syndrome and the diseases of adaptation. J Allergy. 1946;17:231.

190. Miller GE, Chen E, Zhou ES. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. Psychol Bull. 2007;133(1):25-45. doi:10.1037/0033-2909.133.1.25

191. Tomas C, Newton J, Watson S. A Review of Hypothalamic-Pituitary-Adrenal Axis Function in Chronic Fatigue Syndrome. ISRN Neurosci. 2013;2013:1-8. doi:10.1155/2013/784520

192. Lopresti AL, Drummond PD, Smith SJ. A Randomized, Double-Blind, Placebo-Controlled, Crossover Study Examining the Hormonal and Vitality Effects of Ashwagandha (Withania somnifera) in Aging, Overweight Males. Am J Mens Health. 2019;13(2). doi:10.1177/1557988319835985

193. Hellhammer J, Hero T, Franz N, Contreras C, Schubert M. Omega-3 fatty acids administered in phosphatidylserine improved certain aspects of high chronic stress in men. Nutr Res. 2012;32(4):241-250. doi:10.1016/j.nutres.2012.03.003

194. White DJ, de Klerk S, Woods W, Gondalia S, Noonan C, Scholey AB. Anti-stress, behavioural and magnetoencephalography effects of an l-theanine-based nutrient drink: A randomised, double-blind, placebo-controlled, crossover trial. Nutrients. 2016;8(1). doi:10.3390/nu8010053

195. Yu JJ, Pei LB, Zhang Y, Wen ZY YJ. Chronic supplementation of curcumin enhances the efficacy of antidepressants in Major Depressive Disorder: a randomized, double-blind, placebo-controlled pilot study. J Clin Psychopharmacol. 2015;Aug;35(4):406-410.

196. Cicero AFG, Sahebkar A, Fogacci F, Bove M, Giovannini M BC. Effects of phytosomal curcumin on anthropometric parameters, insulin resistance, cortisolemia and non-alcoholic fatty liver disease indices: a double-blind, placebo-controlled clinical trial. Eur J Nutr. 2020;Mar;59(2):477-483.

197. Lopresti AL, Smith SJ, Drummond PD. Modulation of the hypothalamic–pituitary–adrenal (HPA) axis by plants and phytonutrients: a systematic review of human trials. Nutr Neurosci. Published online 2021:1-27. doi:10.1080/1028415x.2021.1892253

198. Talbott SM, Talbott JA, Pugh M. Effect of Magnolia officinalis and Phellodendron amurense (Relora®) on cortisol and psychological mood state in moderately stressed subjects. J Int Soc Sports Nutr. 2013;10(1):1. doi:10.1186/1550-2783-10-37

199. Chen H, Jin Z, Sun C, Santos HO, kord Varkaneh H. Effects of dehydroepiandrosterone (DHEA) supplementation on cortisol, leptin, adiponectin, and liver enzyme levels: A systematic review and meta-analysis of randomised clinical trials. Int J Clin Pract. 2021;75(11):1-11. doi:10.1111/ijcp.14698

200. London E CT. Diet and the role of 11beta-hydroxysteroid dehydrogenase-1 on obesity. J Nutr Biochem. Jul;20(7):485-493.

201. Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PWJ. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. Psychopharmacology (Berl). 2015;232(10):1793-1801. doi:10.1007/s00213-014-3810-0

202. Garrido M, Espino J, Gonzalez-Gomez D, Lozano M, Barriga C, Paredes SD et al. The consumption of a Jerte Valley cherry product in humans enhances mood, and increases 5-hydroxyindoleacetic acid but reduces cortisol levels in urine. Exp Gerontol. 2012;Aug;47((8)):573-580.

203. Pascoe M. A Systematic Review of Randomised Control Trials Examining the Effects of Mindfulness on Stress and Anxious Symptomatology. 2016;(March):1-23.

204. Wunsch K, Wurst R, von Dawans B, Strahler J, Kasten N FR. Habitual and acute exercise effects on salivary biomarkers in response to psychosocial stress. Psychoneuroendocrinology. 2019;Aug(106):216-225.

205. Puterman, E., O; Donovan A. Physical activity moderates stressor-induced rumination on cortisol reactivity. Psychosom Med. 2011;73(7):604-611. doi:10.1097/PSY.0b013e318229e1e0.Physical

206. Sanada K, Montero-Marin J, Díez MA, et al. Effects of mindfulness-based interventions on salivary cortisol in healthy adults: A meta-analytical review. Front Physiol. 2016;7(OCT). doi:10.3389/fphys.2016.00471

207. Antonelli, M., Barbieri, G. & Donelli D. Effects of forest bathing (shinrin-yoku) on levels of cortisol as a stress biomarker: a systematic review and meta-analysis. Int J Biometeorol. Published online 2019:1117–1134. doi:/10.1007/s00484-019-01717-x

208. Kotozaki Y, Takeuchi H, Sekiguchi A, et al. Biofeedback-based training for stress management in daily hassles: An intervention study. Brain Behav. 2014;4(4):566-579. doi:10.1002/brb3.241

209. Lu EY, Lee P, Cai S, So WWY, Ng BFL, Jensen MP, Cheung WM TH. Qigong for the treatment of depressive symptoms: Preliminary evidence of neurobiological mechanisms. Int J Geriatr Psychiatry. 2020;Nov;35(11):1393-1401.

210. Hellhammer J, Vogt D, Franz N, Freitas U, Rutenberg D. A soy-based phosphatidylserine/ phosphatidic acid complex (PAS) normalizes the stress reactivity of hypothalamus-pituitary-adrenal-axis in chronically stressed male subjects: A randomized, placebo-controlled study. Lipids Health Dis. 2014;13(1):1-11. doi:10.1186/1476-511X-13-121

211. Nazari S, Rameshrad M HH. Toxicological Effects of Glycyrrhiza glabra (Licorice): A Review. . Phytother Res. 2017;Nov;31(11):1635-1650.

212. Panossian A, Wikman G. Effects of adaptogens on the central nervous system and the molecular mechanisms associated with their stress - Protective activity. Pharmaceuticals. 2010;3(1):188-224. doi:10.3390/ph3010188

213. Black D, Peng C, Sleight A. Mindfulness practice reduces cortisol blunting during chemotherapy: A randomized controlled study of colorectal cancer patients. Cancer. 2017;August 15(16):3088-3096. doi:10.1002/cncr.30698.Mindfulness

214. Spencer RL, Deak T. A users guide to HPA axis research. Physiol Behav. 2017;178:43-65. doi:10.1016/j.physbeh.2016.11.014