

Endo+

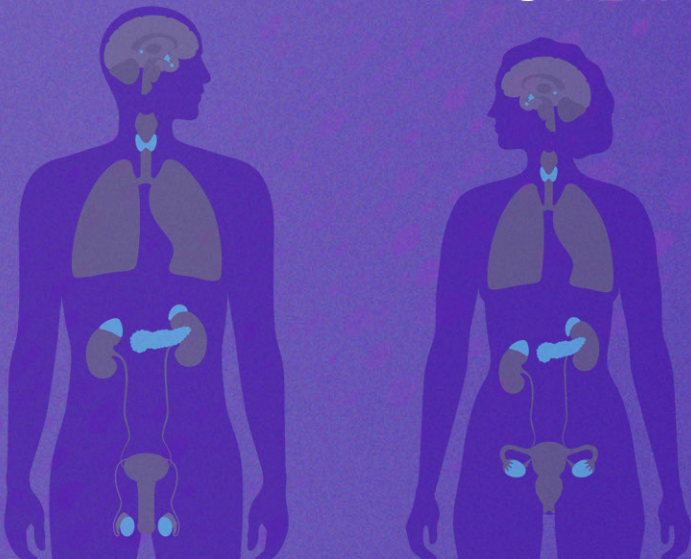
SUPPORT GUIDE



thyroid
ESTROGENS
testosterone
METABOLIC SYNDROME
Inflammation
Cortisol
Adrenal Gland
Diurnal rhythm
weight gain
DHEA
CORTISOL AWAKENING RESPONSE
SALIVA
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Progesterone
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Immune dysfunction
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Depression
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Anabolic hormone
Catabolic hormone
MELATONIN
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HPA axis

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Diurnal rhythm



GENOVA
DIAGNOSTICS®



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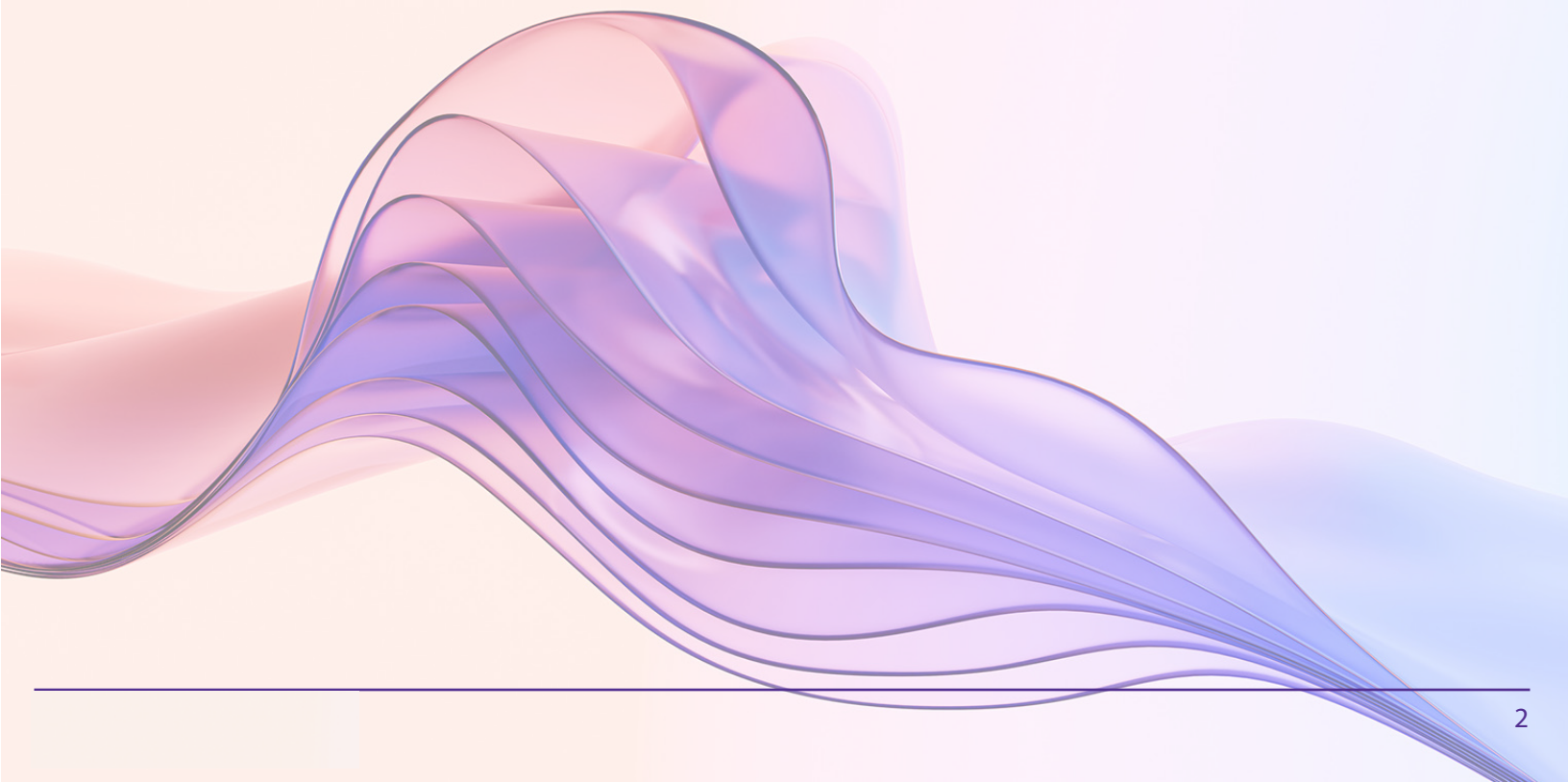
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Introduction

Hormones are chemical messengers released by a cell, gland, or organ into the blood that elicit a cascade of physiologic responses by acting on specific target tissues. Over 50 hormones have been identified. They can be synthesized from cholesterol or peptides and amino acids.

Genova offers a variety of endocrine assessments to meet the testing needs of each patient. Our endocrine products assist the clinician in identifying hormone imbalances, hormone metabolism issues, and disease risk. Reference ranges are based on healthy adult populations. Pediatric reference ranges are not available.

Endo+ is a unique profile that combines multiple hormonal assessments and synthesizes the information so the clinician can quickly identify imbalances. Clinicians can build an individualized hormone assessment for each patient choosing from six component profiles.

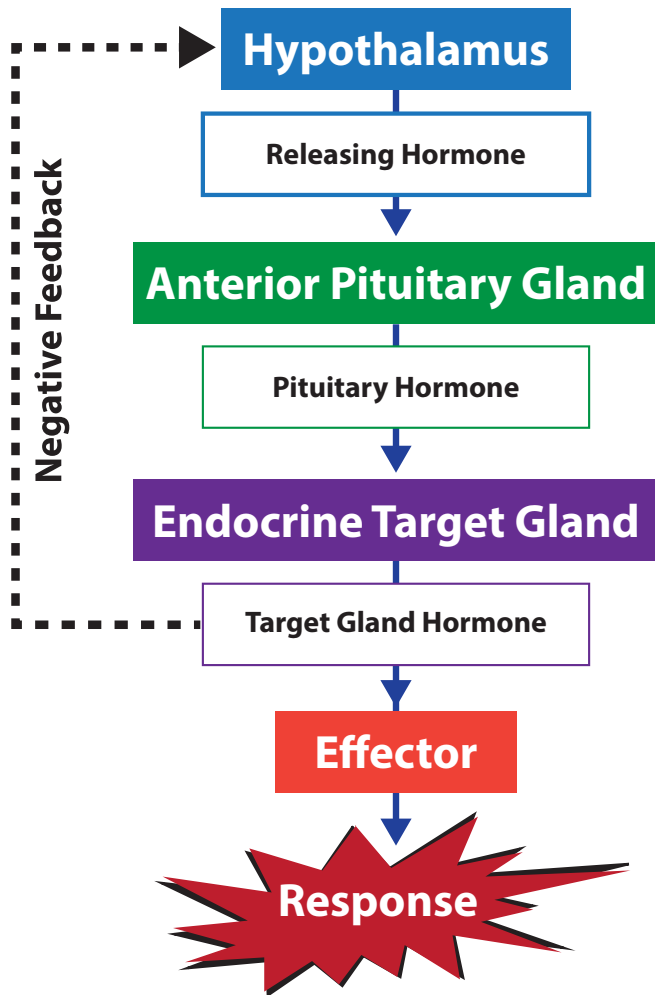
What is included on the Endo+ profile?

- **Sex hormones (saliva):** progesterone, estrone, estradiol, estriol, testosterone
- **Sex hormones (serum):** progesterone, estrone, estradiol, estriol, testosterone, SHBG, DHEA-S
- **Estrogen metabolism (urine):** estrone, estradiol, estriol, 2-hydroxyestradiol, 2-hydroxyestrone, 16a-hydroxyestrone, 4-hydroxyestradiol, 4-hydroxyestrone, 2-methoxyestradiol, 2-methoxyestrone, 4-methoxyestradiol, 4-methoxyestrone
- **Adrenocortex Stress Profile:** cortisol x4, DHEA
- **Thyroid Assessment:** TSH, fT3, fT4, rT3, anti-TG, anti-TPO
- **Melatonin:** melatonin x3
- **Add-ons:**
 - o Serum: LH, FSH, androstenedione, prolactin
 - o Saliva: cortisol awakening response (CAR), secretory IgA
 - o Buccal swab: COMT (V158M), CYP1B1 (L432V + N4535S), MTHFR (A1298C + C677T), VDR (vitamin D receptor) genetic single nucleotide polymorphisms (SNPs)

These smaller component profiles are also available individually.



Hormone Feedback and Regulation



Many hormones have a self-regulating system that involves a negative feedback loop, meaning once the hypothalamus senses that enough of the target hormone is circulating, production shuts down. If levels are too low, the hypothalamus will resume the production cascade (pictured above).

Genova's hormone assays detect endogenous or bioidentical hormones versus non-bioidentical hormones. If a patient is taking non-bioidentical hormones, the negative feedback loop may result in lower levels of endogenous hormones. For this reason, Genova does not recommend testing in patients taking non-bioidentical hormones.

Genova's profiles assess target gland hormones (i.e., cortisol, sex hormones, thyroid hormones), and pituitary hormones (i.e., TSH, LH, FSH). Measuring both levels of the feedback loop helps to distinguish between primary and secondary causes of imbalance.

Patient Population

Hormone imbalances can result in numerous symptoms and conditions. However, many of the clinical manifestations are nonspecific and are also present in non-endocrine disorders. The endocrine system is interconnected to many other organ systems in the body, making it difficult to differentiate the root cause of symptoms. For example, hallmark symptoms of cardiovascular disease may overlap with hormonal imbalances. Testing is important to determine if a hormonal imbalance is contributing to a patient's symptoms. There are many reasons clinicians test hormones including:

- Hormonal symptoms (see chart on following page)
- Hormone supplementation
- Risk assessment for cancers, CVD, etc.

Hormone Balancing Considerations

Every person is unique and treating hormone imbalances must be tailored. Numerous modifiable and non-modifiable factors influence hormone levels:

- Age
- Detoxification capacity and metabolism
- Toxic exposures/endocrine disruptors
- Physical activity
- Medications
- Diet and nutrient status
- Stress
- Infection



Hormone Symptom Comparison Chart

Hormone imbalance symptoms can be associated with multiple hormones.

E Estrogen; **P** Progesterone; **T** Testosterone; **TH** Thyroid; **M** Melatonin; **C** Cortisol

Weight gain	↑↓ E	↓ P	↓ TH	↓ T	↑ C	
Weight loss	↓ C	↑ TH				
Fatigue	↑↓ P	↓ T	↑↓ C	↓ TH	↑↓ E	
Sleep Disturbances/Insomnia	↓ M	↑ C	↑↓ P	↑ TH	↓ E	↑↓ T
Sedation/drowsiness	↑ P	↑ M				
Anxiety/ Feeling Stressed/ Nervous	↓ P	↓ E	↑ TH	↑ C		
Depression	↓ E	↑↓ P	↓ T	↓ TH	↑ C	↓ M
Irritability/ Mood Swings	↑↓ P	↑↓ E	↑ C			
Headaches/Migraine	↑↓ P	↑↓ E	↓ T	↑↓ M		
Infertility	↓ P	↓ E	↑↓ T	↓ TH	↑↓ M	↑ C
Heavy menses	↑ E	↓ TH				
Irregular menses	↓ E	↓ P	↑ T	↑↓ TH		
Breast Tenderness	↑↓ P	↑ E				
Fibrocystic breasts	↑ E	↓ P				
Hot Flashes/ Night Sweats/ Feeling hot	↓ E	↑ P	↑ TH	↓ T	↑↓ C	
Dry Skin/Hair	↓ TH	↓ E				
Feeling cold	↓ TH	↑ M				
Vaginal Dryness	↓ E					
Poor cognition/ concentration / memory	↓ E	↓ T	↑↓ C	↑↓ TH		
Urinary problems / incontinence	↓ E					
Heart palpitations	↓ E	↑ TH				
Bone loss	↓ E	↓ T	↑ TH	↑ C	↓ M	
Joint/muscle pain	↓ E	TH				
Decreased Sex Drive	↑ P	↓ T	↑ C			
Hair Loss	↑ T	↑↓ TH	↑↓ E	↑↓ P		
Hirsutism (females)	↑ T	↑ C				
Acne/ Oily skin	↑ T					
Decreased muscle mass/ strength	↓ T	↑ C				
Gynecomastia (males)	↓ T	↑ E				
Erectile dysfunction	↓ T	↑ C	↓ TH			
Blood pressure irregularity	↑↓ C					



Endo+ Results Overview

The Results Overview page is intended to synthesize all pages of the report into one summary page so the clinician can quickly identify imbalances.

Functional Imbalance Scores

Functional Imbalance Scores												
Key		<div>< 2</div> : Low Need for Support		<div>2-3</div> : Optional Need for Support		<div>4-6</div> : Moderate Need for Support		<div>7-10</div> : High Need for Support				
		Need For HPA Axis Support		Need For Thyroid Support		Need For Sex Hormone Support		Need For Detoxification Support				
		ADRENOCORTEX		THYROID		SEX HORMONES		ESTROGEN METABOLISM				
		<div>10</div>		<div>9</div>		<div>4</div>		<div>2</div>				
Biomarkers	Morning Cortisol		▲	TSH		▲	Estradiol		▼	2/16 Ratio		▲
	Cortisol Curve		▲	FT3		▼	Progesterone		▼	2-OH-E1/E2		▼
	DHEA		▼	rT3		●	Estrone		●	4-OH-E1/E2		●
	DHEA:Cortisol		▼	Thyroid Antibodies		●	Testosterone		●	Methylation Activity		●

The functional imbalance scores are generated using weighted algorithms that incorporate biomarkers belonging to each functional category. The biomarkers that are represented in the algorithm are listed below the score in each functional column. A qualitative indicator of whether the biomarker is normal (green circle), borderline (yellow arrow), or abnormal (red arrow) is located adjacent to the biomarker name. The level of need for support in a functional area is reflected both by the color and score in the circle. Green represents a low need for support and corresponds with scores less than 2, grey represents an optional need for support and corresponds with a score of 2 or 3, yellow indicates moderate need with scores of 4-6, and red indicates high need with scores of 7-10.

For sex hormones, if different sample types are ordered, they will be reported in the “Need For Sex Hormone Support” pillar in the following order: serum>saliva>urine. Therefore, the default sample type is serum. However, the clinician may place more weight on a particular sample type depending on the problem being assessed. This is just a general guideline. For example, if estradiol is ordered in serum, saliva, and urine, the default on the front page would be the serum result, even if there is discrepancy between the sample types. If only salivary and urinary estradiol are ordered, the default on the front page would be the salivary result.

If certain hormones are not ordered, a N/A will populate the pillars and scores will be incomplete.



Therapeutic Support Options

Therapeutic Support Options	<ul style="list-style-type: none"> • Stress Reduction • Mindfulness Training • HRV Biofeedback • Breathwork Training • Exercise/Yoga/Tai Chi • Herbal Adaptogens • Lower Blood Sugar • B-Vitamins • Phosphoserine • Glandulars 	<ul style="list-style-type: none"> • Minerals: Se, Zn, Iron • Tyrosine • Adequate Iodine • Stress Reduction • Reduce Environmental Exposures • Antioxidant Support • Assess Medications • Smoking Cessation • Consider Thyroid HRT 	<ul style="list-style-type: none"> • Address Thyroid Imbalance • Address HPA Axis • Phytoestrogens (for estrogen support) • Phytoandrogens (for testosterone support) • Consider Glandulars • Consider Hormone Precursors • Consider BHRT 	<ul style="list-style-type: none"> • Methylation Support: B-Vitamins, Mg, Betaine • Cruciferous vegetables, berries, rosemary • Soy isoflavones, DIM, I3C • Bioflavonoids, Glutathione • Reduce sugar, stress, environmental toxicity • Reduce adiposity • Physical activity
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Therapeutic support options are listed at the bottom of each column. Therapeutic support options are static on every report and do not change based on a patient's results. These serve as potential treatment ideas. Clinician discretion is advised when selecting appropriate therapeutics for individual patients. More information on therapeutic support options is discussed throughout this guide as they relate to each biomarker.

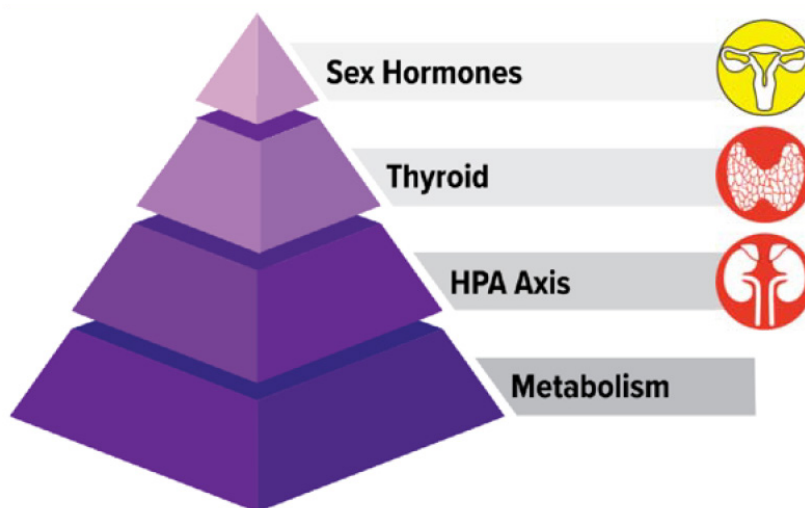
Hormone Pyramid

The hormone pyramid is an illustration demonstrating how hormones influence each other. Hormones related to metabolism (i.e., insulin) make up the base of the triangle. The larger base shows there is a big impact of metabolic hormones on other hormones. The pyramid is bidirectional. Even though the sex hormones make up the smaller apex of the pyramid, sex hormones can influence other hormones in the pyramid.

The colored circles to the right of the pyramid reflect the functional imbalance scores discussed above and show the relative need for therapeutic support in that category. Green represents a low need for support, grey represents an optional need, yellow indicates moderate, and red indicates a high need for support.

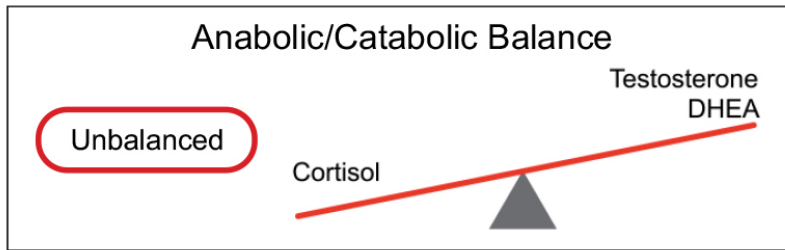
Because hormones influence each other, it is important to cast a wide net when testing, to see where the root cause of imbalance may lie. For example, a patient may have hypothyroidism and thyroid testing is the only assessment ordered. The patient may be treated with thyroid hormones and feel better, but not optimal. If a more comprehensive hormone assessment is ordered, it may show that there is an adrenal imbalance with elevated cortisol that may have been driving the thyroid imbalance all along. Correcting the adrenal imbalance may also correct the thyroid imbalance because the root cause is being addressed. Both adrenal and thyroid imbalances can affect sex hormone levels. Comprehensive panels reveal imbalances in multiple areas.

Please note, Genova's Endo+ does not include hormones related to metabolism – these are generally ordered as part of conventional bloodwork. Hormones that are not ordered will not show an imbalance graphic to the right of the pyramid.





Anabolic/Catabolic Balance

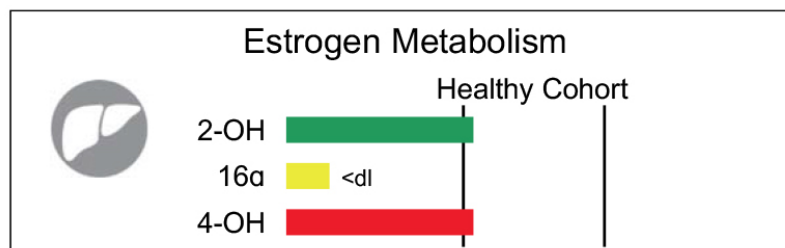


The Anabolic/Catabolic Balance graphic shows the ratio of total cortisol to testosterone and DHEA based on the percentiles of where they fall within their respective reference ranges. The analysis is similar to comparing the relative abundances of testosterone plus DHEA, and total cortisol. When you envision a balance scale, or see-saw, a side that is tipped lower means it is "heavier" in weight compared to the other hormones. For example, looking at the graphic above, cortisol is "heavier", meaning relatively higher compared to testosterone and DHEA. This imbalance is tipped in the direction favoring cortisol, suggesting a more catabolic state.

Cortisol has catabolic properties resulting in wear and tear, whereas testosterone and DHEA are anabolic and promote growth and repair. Excess cortisol can downregulate pathways that produce androgens, creating an imbalance. Adrenal support can be beneficial in balancing the ratio. Less often, the balance will favor the androgens, and this is usually a result of supplementation. There are no known negative consequences of relatively higher androgens with respect to cortisol. It is important to assess individual levels and treat them appropriately.

The information in this graphic will only be displayed if the Adrenocortex Stress Profile is ordered (cortisol, DHEA), and if testosterone is ordered in saliva.

Estrogen Metabolism



The Estrogen Metabolism graphic shows whether a person is metabolizing parent estrogens into healthy or unhealthy metabolites. The horizontal green, yellow, and red bars show how close the results compare to a healthy cohort. 2-hydroxy estrogens (green) are generally considered favorable, whereas increased amounts of 16-alpha (yellow) and 4-hydroxy (red) estrogens tend to be associated with disease risk. These metabolites are often referred to as "the good, the bad, and the ugly", respectively. The information in this graphic will only be displayed if the urine estrogen assessment (Essential Estrogens) is ordered.

Immune dysfunction mood chronic pain Insomnia
Fatigue
STRESS
Resiliency PTSD BURNOUT Anabolic hormone Catabolic
circadian rhythm Depression
HPA axis blood sugar
Cortisol METABOLIC SYNDROME Inflammation
Adrenal Gland
ANXIETY Diurnal rhythm
DHEA weight gain
CORTISOL AWAKENING RESPONSE



ADRENOCORTEX STRESS PROFILE

SUPPORT GUIDE



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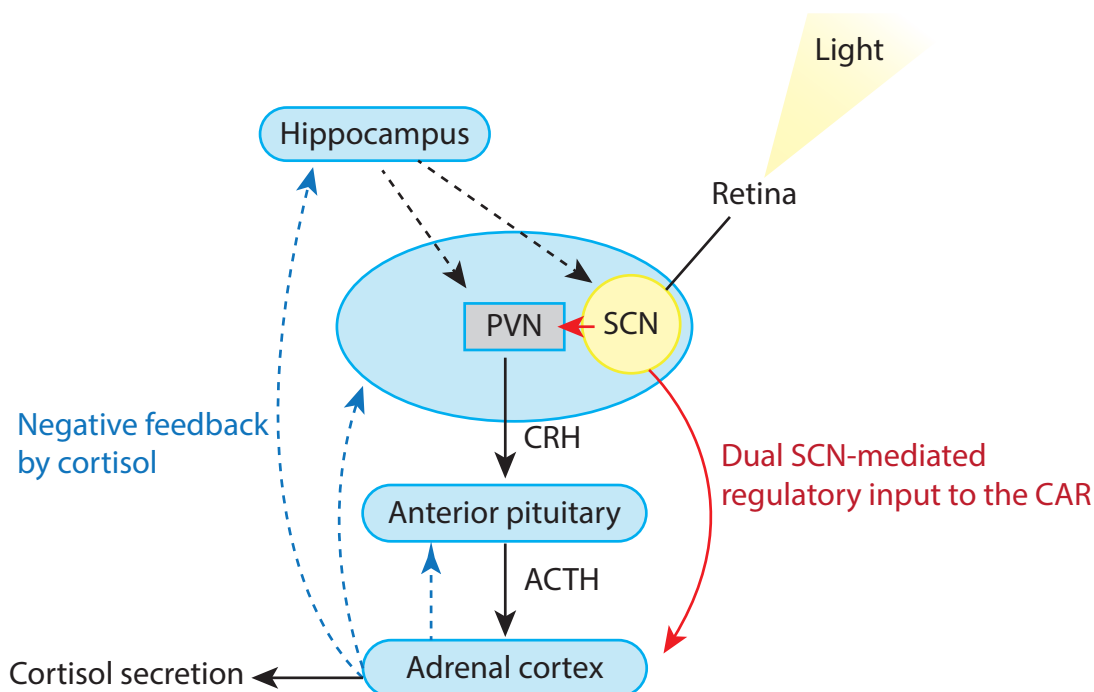
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Hypothalamic-Pituitary-Adrenal (HPA) Axis and the Stress Response:

In response to any stressor, the hypothalamus produces corticotropin releasing hormone (CRH), which stimulates the anterior pituitary to secrete adrenocorticotrophic hormone (ACTH). ACTH triggers the release of glucocorticoids (cortisol) from the adrenal cortex. These glucocorticoids then play an inhibitory role, signaling the system to shut down the stress response via negative feedback. Under optimal conditions, glucocorticoids help the body to maintain homeostasis and play a role in immune activity, growth, reproductive functions, and energy metabolism.



Cortisol shows a strong diurnal rhythm peaking within the first hour after awakening, declining rapidly over the morning hours, and then tapering off over the rest of the day before reaching its lowest point at night.²

In addition to this well-described diurnal cycle, there is a brisk increase of cortisol levels within 30 minutes of awakening in the morning. This phenomenon is termed the **cortisol awakening response (CAR)**.³

CAR is what its name implies: a physiological response to awakening. It is a discreet and distinct component of the cortisol circadian cycle, unrelated to those of cortisol secretion through the rest of the day. CAR represents the momentum of rising cortisol levels that begins several hours prior to awakening and an additional transient increase of up to 50%. The initial cortisol rise begins due to ACTH-mediated normal HPA axis activities with the additional CAR increase caused by supra-chiasmatic nucleus light activation. CAR may play a role in the transition from sleep to full alertness, transcribing a time of day message to the immune system. Therefore, CAR is an independent marker of central biological clock function and overall HPA axis resiliency.⁴



Patient Population:

The symptoms of HPA axis dysfunction can be vague and highly variable. The Adrenocortex Stress Profile may be useful in patients who present with fatigue, insomnia, weight gain, depression, GI complaints, and chronic pain. HPA axis dysfunction is associated with many disease processes, including, but not limited to:

- Hypertension
- Cardiovascular disease
- Gastrointestinal and immune dysregulation
- Diabetes and metabolic syndrome
- Depression
- Chronic fatigue
- Persistent pain
- Neurodegenerative disease and cognitive decline^{2,7-14}

Physical, emotional, and mental stressors, both real and perceived, contribute to overall allostatic load, which leads to adjustments in the body's HPA axis regulatory 'set points.' Daily hassles, chronic pain, blood sugar dysregulation, work overload, and poor relationship quality can alter the HPA axis and can translate into compromised immune function, increased disease risk, psychosocial disturbances, and shortened longevity.²

- The **Adrenocortex Stress Profile (ASP)** offers an assessment of the Hypothalamic-Pituitary-Adrenal (HPA) axis using carefully timed salivary samples of the hormones cortisol and DHEA. Four salivary samples measured throughout the day can give insight into cortisol's natural circadian diurnal rhythm.
- The **Cortisol Awakening Response (CAR)** can be added to the ASP by providing two additional awakening salivary samples to reflect HPA axis resiliency and provide the most comprehensive look at cortisol and the HPA axis.

Cortisol:

It is important to understand the overall pathophysiology of the stress response. A number of mechanisms contribute to disease in chronic stress.

- CRH plays an important role in inhibiting gonadotropin releasing hormone (GnRH) secretion. It also inhibits growth hormone (GH), thyrotropin releasing hormone (TRH) and thyroid stimulating hormone (TSH). Therefore, the stress response includes suppressing reproductive, growth, and thyroid functions.⁵
- Glucocorticoids themselves directly inhibit gonadotropin, GH, and TSH secretion and render their target tissues resistant.⁵
- Glucocorticoids also suppress 5' deiodinase, which converts the inactive tetraiodothyronine (T4) to the active triiodothyronine (T3).⁵
- Glucocorticoids stimulate hepatic gluconeogenesis. They also inhibit insulin actions on skeletal muscle, while potentiating insulin action on adipose tissue. This leads to visceral adiposity and metabolic syndrome. Because of increased hepatic gluconeogenesis and the above described insulin resistance, activation of the HPA axis may contribute to the poor control of diabetic patients during periods of emotional stress or inflammatory diseases.⁵
- Glucocorticoids also have direct effects on the bone by inhibiting osteoblastic activity, which leads to "low turnover" osteoporosis.⁵
- HPA axis dysfunction is also implicated in altering the gastrointestinal mucosa, modulating GI motility, immunity, permeability, and the microbiome.⁶
- Cytokines and other humoral inflammatory mediators are potent activators of the central stress response. Inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α), interleukin-1 β , and interleukin-6 (IL-6), can cause HPA axis stimulation alone, or in synergy with each other. There is evidence that IL-6 plays the major role in HPA axis immune stimulation, especially in chronic inflammatory stress.⁵
- Cortisol inhibits all components of the immune response, which increases infection risk and protects against autoimmune reactions.
- Chronic, maladaptive HPA axis response (low cortisol) mimics a glucocorticoid-deficient state, leading to relative resistance to infections and increased autoimmune susceptibility.⁵

As demonstrated by these systemic effects, HPA axis dysfunction is seen as a root cause for disease; therefore, comprehensive HPA axis evaluation is very important.



DHEA:

Dehydroepiandrosterone (DHEA) is the most abundant circulating steroid hormone in the body. It is made and secreted in the adrenal cortex zona reticularis and can be made in the brain as a neurosteroid. DHEA can be converted downstream in the steroidogenic pathway to create androgens and estrogens. DHEA concentrations peak at around age 25 years and then decline steadily over the following decades.³⁴

DHEA has many neurobiological actions, such as neuroprotection via the blocking of neurotoxic effects, and supporting neurogenesis. DHEA can also influence apoptosis, catecholamine synthesis, and secretion. It has antioxidant and anti-inflammatory properties and can be protective against corticosterone's neurotoxic effects.³⁵

Some labs measure the sulfated form of DHEA as DHEA-s. DHEA-s represents a more stable and larger DHEA pool in the body. DHEA is a neutral steroid and passes rapidly from the blood to the saliva by passive diffusion. DHEA-s is a charged, polar molecule and cannot diffuse easily through lipid membranes into saliva. Salivary DHEA is considered a surrogate marker for DHEA-s.³⁸

DHEA:Cortisol Ratio:

The ratio of DHEA to cortisol is calculated based on collection of the 60 minute post awakening (7:00 AM – 9:00 AM) sample. This calculation represents a measurement of anabolic and catabolic balance. Since DHEA acts not only as an anabolic hormone, but appears to function to down-regulate the cellular effects of cortisol, this measurement can theoretically enhance the predictive value of HPA axis dysfunction.³⁸



The clinical interpretation of the Adrenocortex Stress Profile involves:

1. Timed Cortisol Measurements
2. Overall Diurnal Rhythm/Slope
3. Cortisol Awakening Response
4. DHEA
5. DHEA:Cortisol Ratio

Timed Cortisol Measurements:

Specific cortisol findings throughout a diurnal rhythm may be affected by any number of acute mental, emotional, and physical daily stressors. Abnormal results should be correlated with each patient's clinical presentation and daily routine.^{2,16}

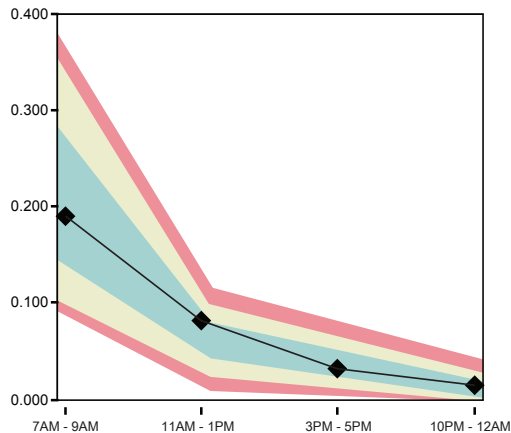
TIMING	SIGNIFICANCE	CONSIDERATIONS WHEN HIGH	CONSIDERATIONS WHEN LOW
Cortisol Awakening Response (CAR) Add-on	HPA axis resiliency Perception of control around chronic stress ¹⁷	Adaptive anticipation of daily stress Stimulation of motor function, immune response, and alertness ^{4,18}	Burnout, depression, PTSD, chronic fatigue syndrome, early loss experiences, material hardship, amnesia, hippocampal damage, non-response, abnormal sampling ^{15,4,18,19}
Morning 7:00AM-9:00AM	Peak ACTH-mediated adrenal gland response	Exercise, blood sugar dysregulation, lifestyle stressors, pain	Inability to mount peak response due to HPA axis dysfunction and/or down regulation from chronic stressors
Midday 11:00AM-1:00PM	Adaptive function of the HPA axis to daily routine	Exercise, blood sugar dysregulation, lifestyle stressors, pain	HPA axis dysfunction
Afternoon 3:00PM-5:00PM	Can reflect glycemic control	Exercise, blood sugar dysregulation, lifestyle stressors, pain	HPA axis dysfunction
Evening 10:00PM- 12:00AM	Baseline HPA axis function	Stress, alcohol, exercise, lifestyle stressors, pain Linked to insomnia and various diseases such as diabetes, cardiovascular disease, hormonally-driven cancers, and osteoporosis ^{18,20-24}	Optimal



Treatment Options:

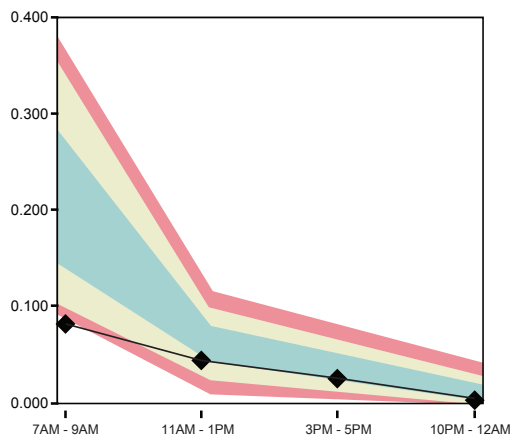
Treatment of abnormal cortisol should be directed at the stressor's root cause. Lifestyle modification with relaxation methods, dietary changes, pain management, and overall HPA axis support with nutrition, adaptogens, and supplements can be helpful.²⁵

Normal Diurnal Slope



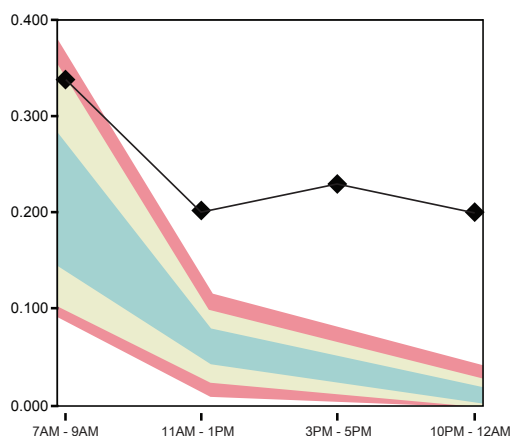
The natural cortisol diurnal rhythm shows a peak within the first hour after awakening, a rapid decline over the morning hours, and then tapering through the rest of the day before reaching its lowest point at night.²

Low Slope



- Chronic stress burden
- Poor psychosocial function
- Lack of HPA axis resiliency
- Lower perceived control over stress
- Post-Traumatic Stress Disorder (PTSD)
- Persistent fatigue, anxiety, and depression
- Predictive of health outcomes, such as increased breast cancer mortality, increased coronary calcifications, and increased body mass index^{2,3,15}

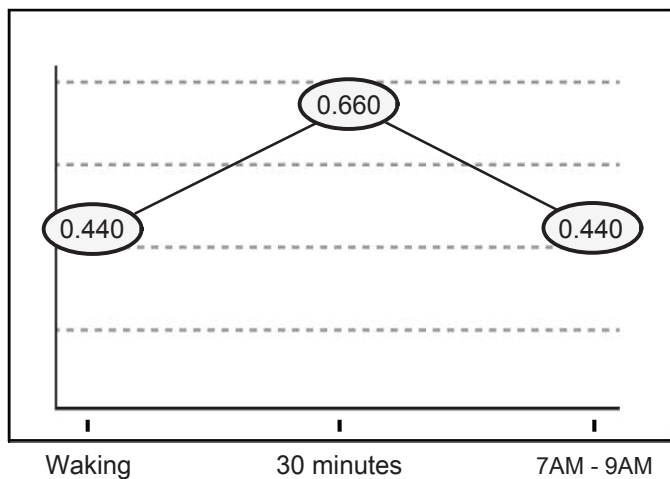
High Slope



- Appropriate response to a major stressor
- Perceived insurmountable challenge¹⁵



Cortisol Awakening Response (CAR) Add-On



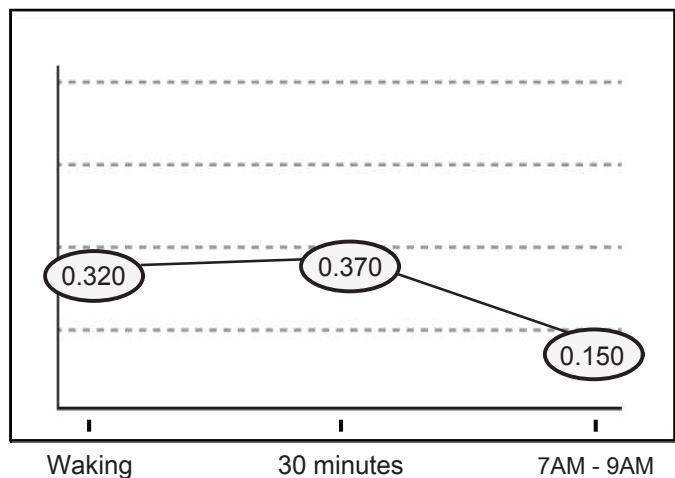
CAR reflects a person's ability to cope with anticipated challenges and the perceptions of control around chronic stress. CAR is calculated as a direct percent increase. A value of at least 50% is expected.^{3,4,17,19,26}

Percent Increase

50

Expected:
>50%

Blunted CAR



- Burnout
- Depression
- PTSD
- Chronic Fatigue Syndrome
- Self-reported health problems
- Early loss experiences
- Material hardship
- Amnesia
- Hippocampal damage^{4,18}

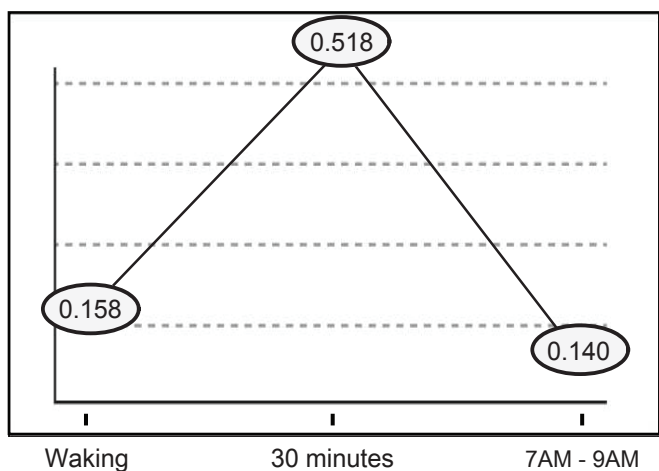
Percent Increase

16

Expected:
>50%



Elevated CAR



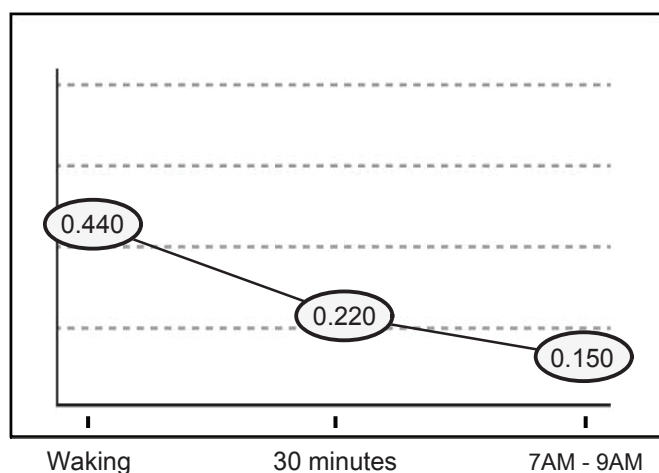
- Adaptive anticipation of daily stressors ("preparing for action")
- Stimulation of motor function, immune response, and alertness^{4,18}

Percent Increase

228

Expected:
>50%

Negative CAR



- Ensure there was no delay between waking and obtaining the first sample
- Literature is evolving regarding clinical implications^{27,28}
- One hypothesis is that a negative CAR percentage may reflect a blunted CAR with further loss of resiliency

Percent Increase

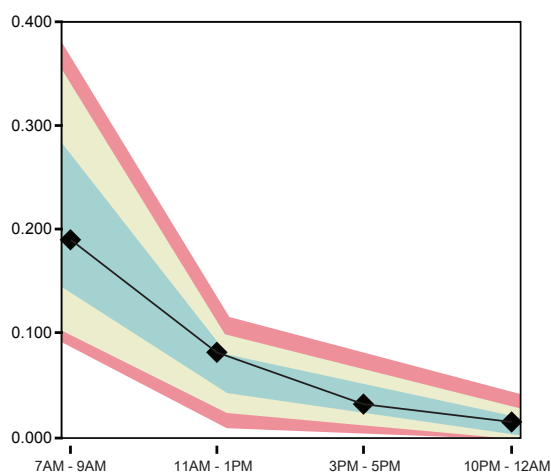
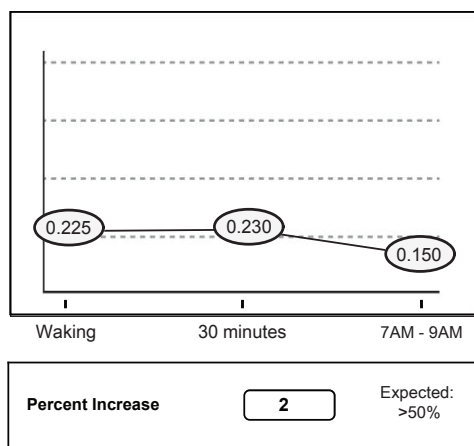
-50

Expected:
>50%



CAR Non-Response

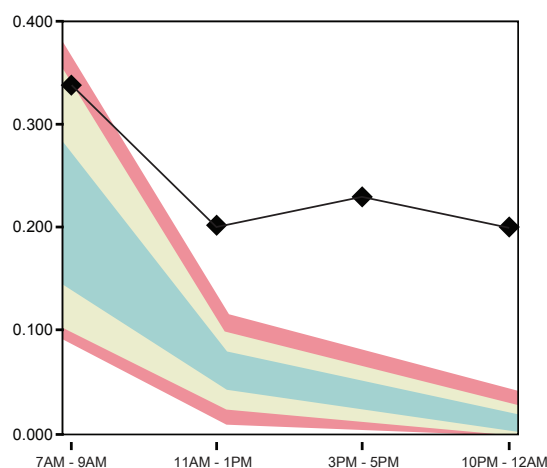
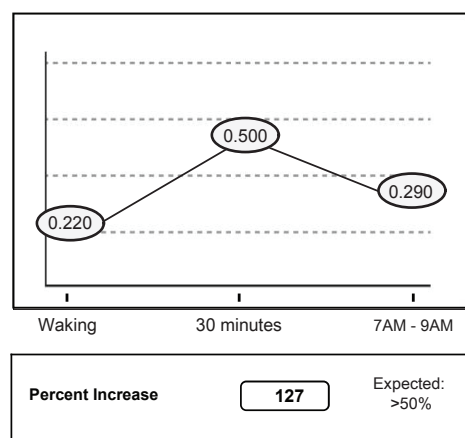
Cortisol Awakening Response



- 25% of healthy adults do not mount a CAR
- Response is defined as an increase of at least $0.09\mu\text{g/dL}$ above individual baseline with otherwise adequate cortisol diurnal curve
- Ensure proper sampling^{17,19}

CAR Elevated with High Slope

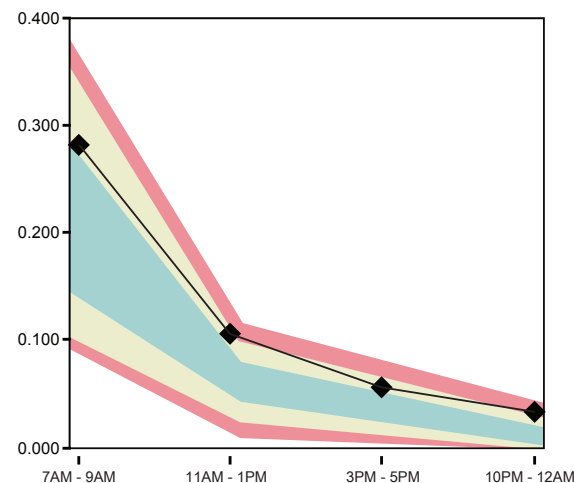
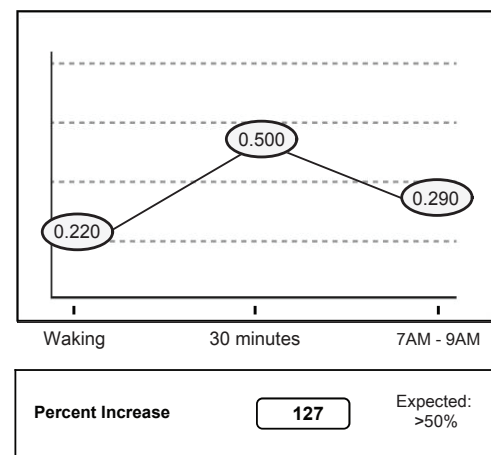
Cortisol Awakening Response



- General HPA axis dysfunction
- Significant stressor, real or perceived^{26,29}

CAR Elevated with Elevated Slope

Cortisol Awakening Response

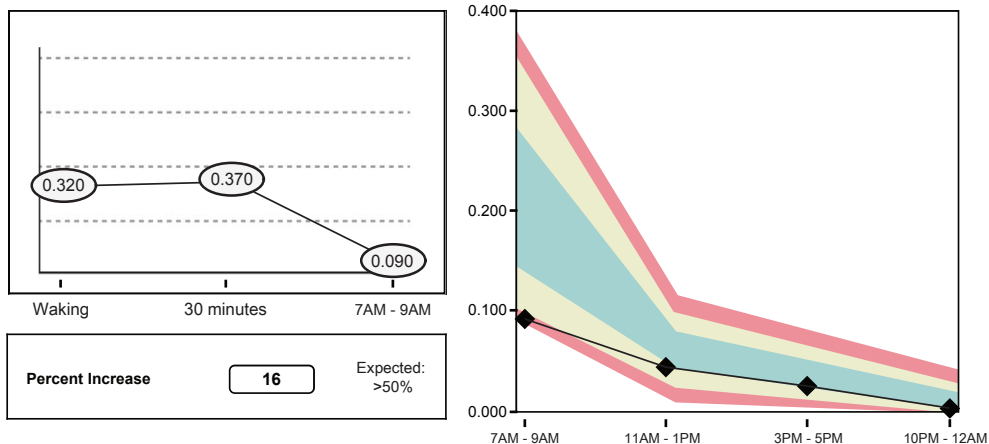


- Anticipation and reflection of daily stress
- Overall total cortisol levels during CAR are predictive of relative mean cortisol levels throughout the day^{26,30}



CAR Blunted with Low Slope

Cortisol Awakening Response



- Generalized HPA axis dysfunction
- Burnout
- Chronic stressor^{26,30}

DHEA

HIGH:	Exogenous exposure, supplementation, polycystic ovary syndrome, adrenal hyperplasia, and adrenal tumors ³¹⁻³⁵
LOW:	<p>Advancing age, chronic stress, HPA axis dysfunction</p> <p>Low DHEA levels have been associated with immune dysregulation, cardiovascular disease, arthritis, osteoporosis, insomnia, declining cognition, depression, fatigue, and decreased libido.³¹⁻³⁵</p>

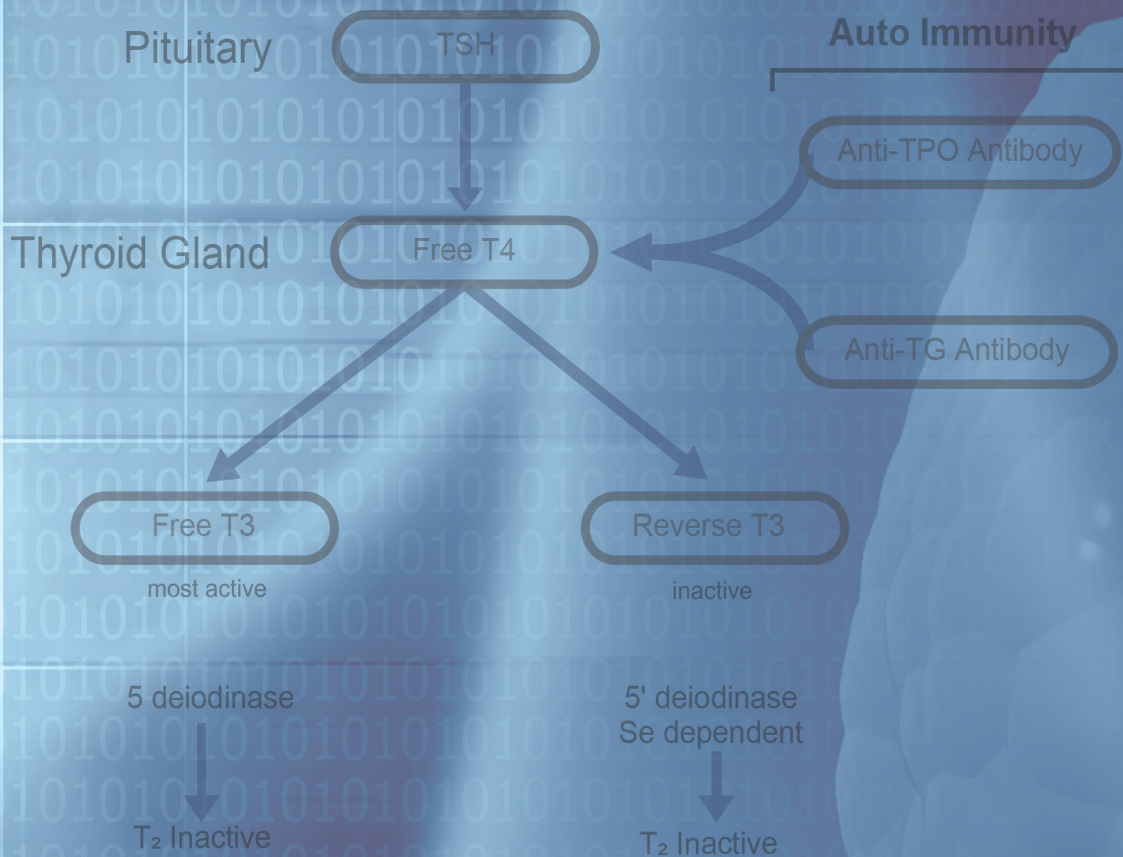
DHEA:Cortisol Ratio

HIGH:	<p>Favors anabolic activity</p> <p>Address specific cortisol and DHEA abnormalities^{32,36-38}</p>
LOW:	<p>Favors catabolic activity</p> <p>Address specific cortisol and DHEA abnormalities^{32,36-38}</p>



References

1. Clow A, Hucklebridge F, Thorn L. The cortisol awakening response in context. *Int Rev Neurobiol*. 2010;93:153-175.
2. Saxbe DE. A field (researcher's) guide to cortisol: tracking HPA axis functioning in everyday life. *Health Psychol Rev*. 2008;2(2):163-190.
3. Fries E, Dettenborn L, Kirschbaum C. The cortisol awakening response (CAR): facts and future directions. *Int J Psychophysiol*. 2009;72(1):67-73.
4. Clow A, Hucklebridge F, Stalder T, Evans P, Thorn L. The cortisol awakening response: more than a measure of HPA axis function. *Neurosci Biobehav Rev*. 2010;35(1):97-103.
5. Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J Psychosom Res*. 2002;53(4):865-871.
6. Carabotti M, Scirocco A, Maselli MA, Severi C. The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann Gastroenterol: quarterly publication of the Hellenic Society of Gastroenterology*. 2015;28(2):203.
7. Ennis GE, Moffat SD, Hertzog C. The cortisol awakening response and cognition across the adult lifespan. *Brain Cogn*. 2016;105:66-77.
8. Heim C, Nater UM, Maloney E, Boneva R, Jones JF, Reeves WC. Childhood trauma and risk for chronic fatigue syndrome: association with neuroendocrine dysfunction. *Arch Gen Psychiatry*. 2009;66(1):72-80.
9. Dedovic K, Ngiam J. The cortisol awakening response and major depression: examining the evidence. *Neuropsychiatr Dis Treat*. 2015;11:1181-1189.
10. Bengtsson I, Lissner L, Ljung T, Rosengren A, Thelle D, Wahrborg P. The cortisol awakening response and the metabolic syndrome in a population-based sample of middle-aged men and women. *Metab*. 2010;59(7):1012-1019.
11. Hackett RA, Steptoe A, Kumari M. Association of diurnal patterns in salivary cortisol with type 2 diabetes in the Whitehall II study. *J Clin Endocrinol Metab*. 2014;99(12):4625-4631.
12. Kern S, Krause I, Horntich A, Thomas K, Aderhold J, Ziemssen T. Cortisol awakening response is linked to disease course and progression in multiple sclerosis. *PLoS One*. 2013;8(4):e60647.
13. Girod JP, Brotman DJ. Does altered glucocorticoid homeostasis increase cardiovascular risk? *Cardiovasc Res*. 2004;64(2):217-226.
14. Hamer M, O'donnell K, Lahiri A, Steptoe A. Salivary cortisol responses to mental stress are associated with coronary artery calcification in healthy men and women. *Eur Heart J*. 2009;31(4):424-429.
15. Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. *Psychoneuroendocrinology*. 2009;34(10):1423-1436.
16. Dickerson SS, Kemeny ME. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psycho Bull*. 2004;130(3):355-391.
17. Stalder T, Kirschbaum C, Kudlelka BM, et al. Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrinology*. 2016;63:414-432.
18. Clow A, Thorn L, Evans P, et al. The awakening cortisol response: methodological issues and significance. *Stress*. 2004;7(1):29-37.
19. Wust S, Wolf J, Hellhammer DH, et al. The cortisol awakening response-normal values and confounds. *Noise Health*. 2000;2(7):79.
20. Levenson JC, Kay DB, Buysse DJ. The pathophysiology of insomnia. *CHEST Journal*. 2015;147(4):1179-1192.
21. Hammer F, Deutschbein T, Marx A, et al. High evening salivary cortisol is an independent predictor of increased mortality risk in patients with systolic heart failure. *Int J Cardiol*. 2016;203:69-73.
22. Hackett RA, Kivimaki M, Kumari M, Steptoe A. Diurnal Cortisol Patterns, Future Diabetes, and Impaired Glucose Metabolism in the Whitehall II Cohort Study. *Int J Clin Endocrinol Metab*. 2016;101(2):619-625.
23. Gonzalez Rodriguez E, Lamy O, Stoll D, et al. High evening cortisol level is associated with low TBS and increased prevalent vertebral fractures. OsteoLaus study. *Int J Clin Endocrinol Metab*. 2017
24. Bower JE, Ganz PA, Dickerson SS, et al. Diurnal cortisol rhythm and fatigue in breast cancer survivors. *Psychoneuroendocrinology*. 2005;30(1):92-100.
25. Panossian A, Wikman G. Effects of Adaptogens on the Central Nervous System and the Molecular Mechanisms Associated with Their Stress-Protective Activity. *Pharmaceuticals*. (Basel, Switzerland). 2010;3(1):188-224.
26. Edwards S, Hucklebridge F, Clow A, Evans P. Components of the diurnal cortisol cycle in relation to upper respiratory symptoms and perceived stress. *Psychosom Med*. 2003;65(2):320-327.
27. Moya-Albiol L, Serrano MA, Salvador A. Job satisfaction and cortisol awakening response in teachers scoring high and low on burnout. *Span J Psychol*. 2010;13(2):629-636.
28. Eek FC, Garde AH, Hansen AM, et al. The cortisol awakening response- an exploration of intraindividual stability and negative responses. *Scand J Work Environ Health*. 2006;32:15-21.
29. Edwards S, Evans P, Hucklebridge F, Clow A. Association between time of awakening and diurnal cortisol secretory activity. *Psychoneuroendocrinology*. 2001;26(6):613-622.
30. Edwards S, Clow A, Evans P, Hucklebridge F. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life sciences*. 2001;68(18):2093-2103.
31. Ohlsson C, Labrie F, Barrett-Connor E, et al. Low Serum Levels of Dehydroepiandrosterone Sulfate Predict All-Cause and Cardiovascular Mortality in Elderly Swedish Men. *Int J Clin Endocrinol Metab*. 2010;95(9):4406-4414.
32. Pluchino N, Drakopoulos P, Bianchi-Demicheli F, et al. Neurobiology of DHEA and effects on sexuality, mood and cognition. *J Steroid Biochem Mol Biol*. 2015;145:273-280.
33. Yasui T, Matsui S, Tani A, Kunimi K, et al. Androgen in postmenopausal women. *J Med Invest*. 2012;59(1):12-27.
34. Maggio M, De Vita F, Fischella A, et al. DHEA and cognitive function in the elderly. *J Steroid Biochem Mol Biol*. 2016;145:281-292.
35. Maninger N, Wolkowitz OM, Reus VI, et al. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Front Neuroendocrinol*. 2009;30(1):65-91.
36. Moraes H, Deslandes A, Maciel-Pinheiro PdT, et al. Cortisol, DHEA, and depression in the elderly: the influence of physical capacity. *Arquivos de neuro-psiquiatria*. 2016;74(6):456-461.
37. Daskalakis NP, McGill MA, Lehrner A, Yehuda R. Endocrine aspects of PTSD: Hypothalamic-Pituitary-Adrenal (HPA) axis and beyond. *Comprehensive Guide to Post-Traumatic Stress Disorders*. 2016:245-260.
38. Guillems TG, Edwards L. Chronic stress and the HPA axis. *The Standard*. 2010;9(2):1-12.




Comprehensive Thyroid Assessment


SUPPORT GUIDE

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**Comprehensive
Thyroid Assessment**
 63 Zillioas Street
 Asheville, NC 28801
 © Genova Diagnostics


Patient: **SAMPLE PATIENT**







DOB:

Sex:

MRN:

4400 Comprehensive Thyroid Assessment - Serum
Methodology: Chemiluminescent, RIA



Comprehensive Thyroid Assessment					
Central Regulation					
	Reference Range		Reference Range		
Thyroid Stimulating Hormone (TSH)	 1.90	0.40-2.50 microIU/mL	Free Thyroxine (T4)	 0.80	0.61-1.12 ng/dL
Peripheral Regulation					
	Reference Range		Reference Range		
Free Triiodothyronine (FT3)	 2.7	2.5-3.9 pg/mL	Reverse T3 (rT3) *	 20.0	9.0-35.0 ng/dL
Thyroid Antibodies					
	Reference Range		Reference Range		
Anti-Thyroglobulin Antibody (Anti-TG)	 2.9	<4.0 IU/mL	Anti-Thyroid Peroxidase (Anti-TPO)	 7	<9 IU/mL

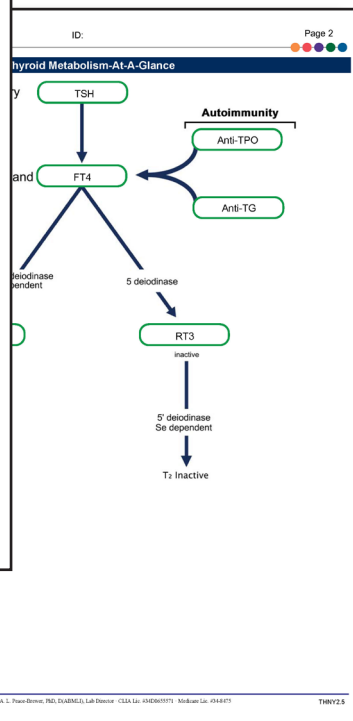
*The performance characteristics of all assays have been verified by Genova Diagnostics Inc. Assays are cleared by the U.S. Food and Drug Administration, unless otherwise noted with *.

Thyroid Metabolism Summary

Thyroid hormone production is centrally regulated (hypothalamus-pituitary-thyroid axis) but thyroxine (T4) from the thyroid gland is peripherally transformed in liver and kidney cells into T3 and reverse T3 (rT3). Ultimately, the site of action for thyroid hormones is at cell nuclei throughout the body, where T3 is five times as potent as T4, and rT3 is completely inert. Thyroid dysfunction may occur even when the hypothalamus-pituitary-thyroid axis is operating adequately. Problems with peripheral conversion (reflected by T3 and rT3 levels) and/or with immune system interference in the form of auto-antibodies (reflected by anti-thyroglobulin and anti-thyroidal peroxidase antibodies) may still affect thyroid hormone production or its action at the cellular level. Thus to achieve a comprehensive assessment of thyroid adequacy, central regulation, peripheral conversion, and auto-immune involvement must be thoroughly evaluated.

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THYR2.4



The National Academy of Clinical Biochemistry (NACB) suggests a serum FT4 concentration in the upper third of

Thyroid Physiology and Metabolism

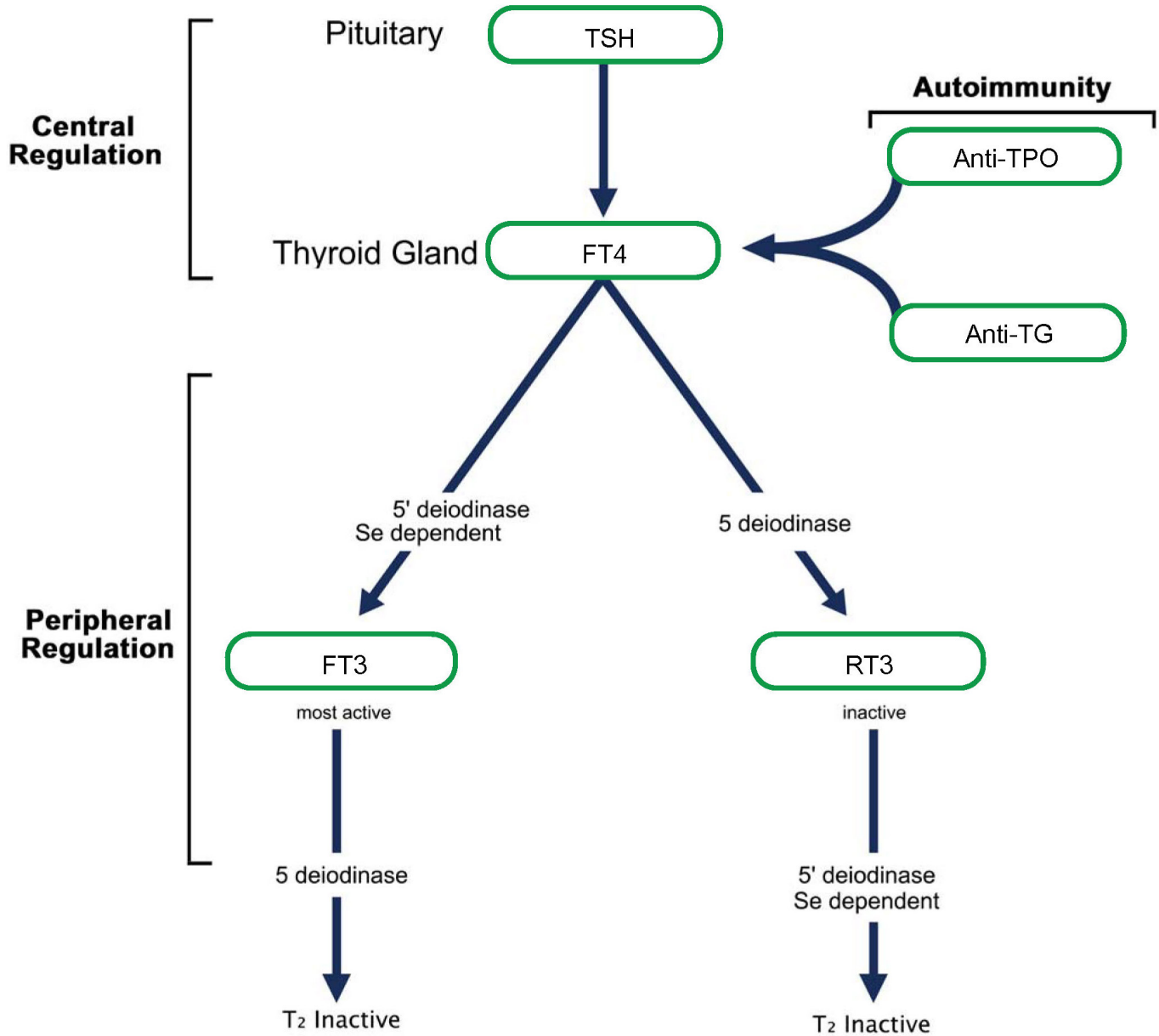
The thyroid gland is a butterfly-shaped organ in the anterior trachea. It is sometimes referred to as the 'thermostat' of the body since thyroid hormones are involved in metabolism, thermogenesis, energy expenditure, growth, and development. Thyroid hormone production affects many organ systems including its regulation of cardiac output, stroke volume, heart rate, and cardiac contractility. Any dysregulation can result in abnormalities of the nervous system and present with numbness, tingling, pain, or burning sensation. Thyroid hormone can also affect mood and gastrointestinal motility. Additionally, it increases basal metabolic rate, which then signals increased oxygen consumption, heat production, glucose absorption, glycogenolysis, gluconeogenesis, lipolysis, protein synthesis, and catabolic protein degradation.¹ With this, any abnormality within thyroid production and metabolism has far reaching effects.

The thyroid is mainly regulated by the Hypothalamic-Pituitary-Thyroid axis (HPT axis). The hypothalamus secretes thyrotropin-releasing hormone (TRH) which then signals the anterior pituitary to release thyroid-stimulating hormone (TSH). TSH works directly on the cells of the thyroid gland to secrete two different hormones. The main output is the prohormone thyroxine (T4) – which contains four iodine atoms – and to a lesser extent the more biologically active triiodothyronine (T3). Most T3 is created peripherally by removing one iodine molecule from T4 using deiodinase enzymes. In circulation, thyroid hormones are protein bound to thyroid binding globulin, pre-albumin, and albumin. Only the free components have the ability to bind to their respective tissue-specific receptors.²

Although T3 is more biologically active, it also has a biologically inert form called reverse T3. Reverse T3 (rT3) is created by type 1 and type 3 deiodinase enzymes and may block free T3 binding sites. Reverse T3 is created based on the metabolic needs of the body.

The HPT axis is influenced by thyroid hormone levels producing a negative feedback signal to the hypothalamus and pituitary to maintain homeostasis. TSH release from the hypothalamus is very sensitive to any minor change in free T4, such that abnormal TSH levels can be detected much earlier in hyper- or hypo-thyroid conditions. Very minor changes in T3 and/or T4 can result in large changes in TSH, making it an important screening tool.^{1,2}

Thyroid Metabolism-At-A-Glance



Important Nutrients and Thyroid Metabolism Disruptors

The synthesis and metabolism of thyroid hormones require several key nutrients. Additionally, there are factors that can interfere in each step. The processed foods in the standard American diet, topsoil erosion, changes in farming practices, and toxic exposures can all contribute to underlying micronutrient deficiencies or other interferents leading to thyroid dysfunction. Although not an exhaustive list, below are a few key considerations:

1. **Iodine** is an essential nutrient for the production of T3 and T4. Deficiencies are seen world-wide and are a common cause of thyroid disease. Many developed countries developed fortification of foods (such as iodized salt) which has vastly improved the incidence of adverse effects and thyroid disease. Other countries routinely use iodine supplementation. However, this can be a double-edged sword since excess iodine can also be problematic. Excess iodine from things like supplementation, iodized salt, iodinated contrast media, large amounts of dietary seaweed, and medications like amiodarone can precipitate both hypo- and hyperthyroidism, mainly among those with pre-existing thyroid disease or other risk factors.³
2. **Selenium** is particularly abundant in the thyroid gland and is the main component of selenoproteins – the enzymes responsible for thyroid hormone synthesis, deiodinase enzymes, and antioxidant protection. There are studies which suggest a role for selenium supplementation in autoimmune thyroiditis, however the optimal range of selenium in plasma is narrow and needs to be further defined.⁴⁻⁷
3. **Zinc** plays an important role in the metabolism of thyroid hormone. It functions as a critical cofactor on the activity of deiodinase enzymes. The deiodinase enzymes are responsible for removing iodine from thyroid hormone to convert T4 to the more biologically active T3, as well as creating the inactive reverse T3. Zinc may also regulate TRH, TSH, and transcription factors involved in synthesizing thyroid hormones.⁸
4. **Iron** is an important cofactor in the activity of the heme-dependent enzyme thyroid peroxidase, which helps in thyroid hormone synthesis. Iron deficiency can also lower serum T3 and T4 levels, as well as decrease the utilization of thyroid hormones by reducing binding capacity.⁹ It has been shown that deficiencies in iron, selenium, and zinc can also blunt the efficacy of iodine supplementation.¹⁰
5. **Tyrosine**, along with many amino acids, are integral in the formation and metabolism of thyroid hormone. Tyrosine, a nonessential amino acid, is a necessary precursor within the thyroglobulin protein. Thyroglobulin contains 5000 amino acids including a high amount of tyrosine.¹¹ Amino acid deficiencies can downregulate the HPT axis, which is assumed to be an energy-saving mechanism.¹²
6. **Fat soluble vitamins** like vitamin A, E, and D are also important influences in thyroid metabolism. Vitamin A can inhibit TSH secretion and affect the pituitary-thyroid axis and affect peripheral hormone metabolism. Vitamin A supplementation is being studied as a means to reduce subclinical hypothyroidism.¹³ Thyroid hormones are often associated with oxidative stress status, therefore vitamin E has been studied in its ability to mitigate oxidative stress associated with deiodinase enzymes and thyroid hormone synthesis.^{14,15} Vitamin D's role is still being elucidated but may show more promise in its ability to trigger innate and adaptive immunity in autoimmune thyroid response.¹⁶
7. There are many **lifestyle and environmental factors** which can disrupt normal thyroid hormone production and metabolism. Endocrine disrupting toxins like fluoride, Bisphenol A, phthalates, cadmium, and pesticides can bind to peripheral binding sites preventing proper utilization of thyroid hormone. They can also affect iodine uptake, trigger autoimmune responses, and be a risk factor in various cancers, including cancer of the thyroid gland.¹⁷ Stress and the cortisol response have also been implicated in altering the conversion of T4 to T3 as well as decreasing production of TSH.¹⁸ Other environmental factors which can influence thyroid function include fasting and over-feeding, temperature extremes, and a growing list of prescription medications.¹⁹

Thyroid Clinical Conditions

Autoimmune Thyroid Disease

As with all autoimmune diseases, thyroid autoimmune disorders can develop within the triad of increased intestinal permeability, genetic predisposition, and an environmental trigger.

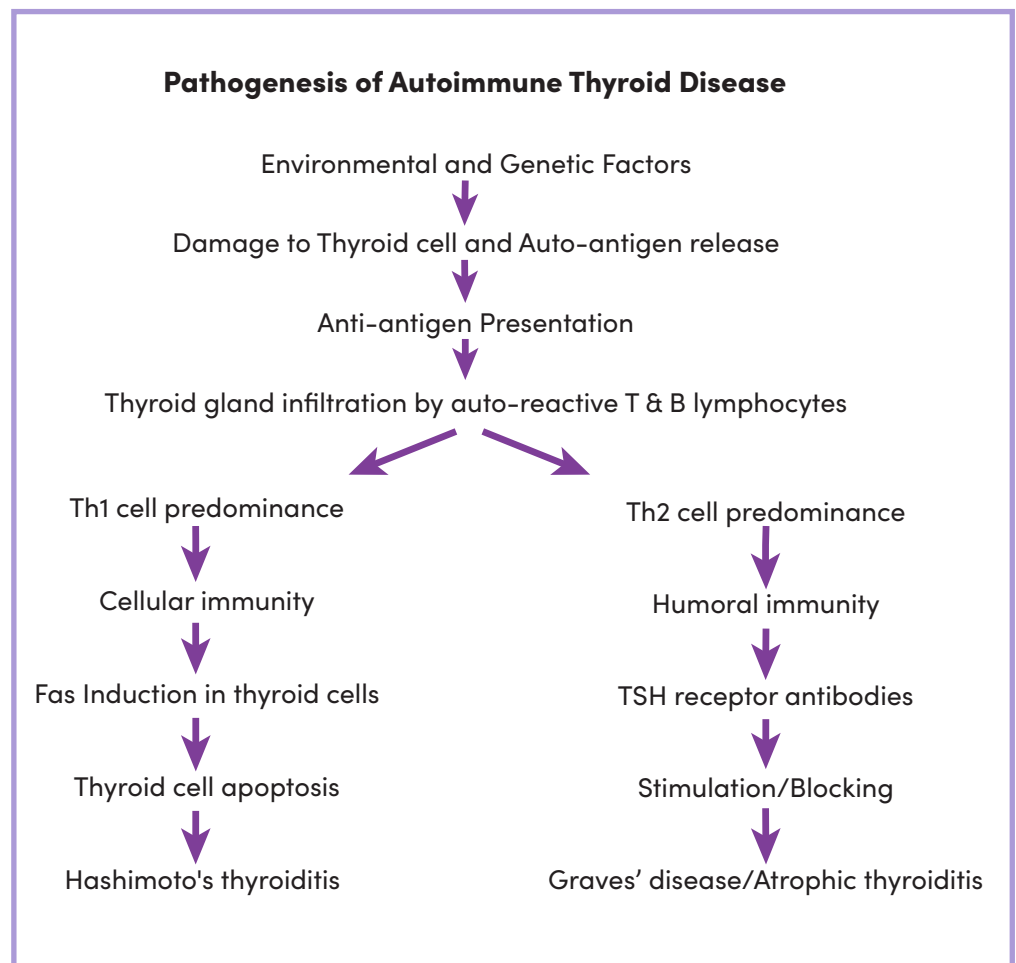
Thyroid autoimmunity is far more prevalent in females at a ratio of 5-10:1. The main thyroid autoimmune disorders are **Graves' disease** and **Hashimoto's thyroiditis**. Both conditions share the pathologic characteristics of T and B cell alterations of the thyroid gland. Other variants include post-partum thyroiditis, drug-induced thyroiditis, and those associated with polyglandular autoimmune syndromes.²⁰ Genetic predisposition accounts for a large percentage of disease, though environmental factors such as viral infection, smoking, stress, and medications such as amiodarone, interferon, and iodine can trigger the autoimmune cascade.²⁰

There are three main thyroid autoantibodies: Anti-Thyroid Stimulating Hormone Receptor (Anti-TSHR), Anti-Thyroid Peroxidase (Anti-TPO), and Anti-Thyroglobulin (Anti-Tg). Autoimmune thyroid diseases can show overlap between these autoantibodies with one or more being present in each condition.

Hashimoto's thyroiditis is characterized by the formation of autoantibodies (anti-thyroglobulin and anti-thyroid peroxidase) causing T and B lymphocyte infiltration of the thyroid gland with subsequent thyroid gland enlargement and/or goiter formation.²¹ This infiltration eventually destroys thyroid cells by

cell and antibody-mediated responses. Clinically, it presents as a typical hypothyroid state.

Graves' disease is characterized by thyrotropin receptor antibodies stimulating the TSH receptor resulting in a hyperthyroid presentation. Graves' disease is not considered destructive since lymphocytes are less likely to infiltrate the follicular cells due to humoral immunity and a predominantly B-lymphocyte response.



There are mixed T helper cell inflammatory responses in both Hashimoto's and Graves' disease with different clinical phenotypic expression. They also may in fact, coexist in the same gland and progress from one to the other. Transition from Graves' hyperthyroidism to Hashimoto's hypothyroidism can occur, while transition from Hashimoto's to Graves' is rare.²²⁻²⁴

Hypothyroidism

Hypothyroidism, or decreased thyroid function, is the most common clinical thyroid disorder and more prevalent in women and the elderly.¹⁵ It can be divided into two categories: primary and secondary hypothyroidism. Primary hypothyroidism lies within the thyroid gland itself as the inability to produce and secrete sufficient amounts of thyroid hormone. Secondary hypothyroidism refers to decreased thyroid gland stimulation from pituitary or hypothalamic causes. There are instances where laboratory abnormalities might precede overt physical manifestations which is often termed 'subclinical hypothyroidism.'

Symptoms of hypothyroidism include:²⁶

- fatigue
- cold intolerance
- paresthesia
- constipation
- decreased heart rate
- voice hoarseness
- dull facial expression
- periorbital swelling
- slowing of deep tendon reflexes
- dry skin
- weight gain
- depression
- menorrhagia
- slow speech

Severe and long-standing hypothyroidism can cause myxedema coma with blatant, life-threatening symptoms like coma, hypothermia, bradycardia, seizures, and respiratory depression.²⁷

The most common cause of hypothyroidism in developed countries is the autoimmune disease Hashimoto's thyroiditis. However, world-wide, iodine deficiency and excess play a significant role as precipitating causes.³ Radiation therapies, surgical treatments, certain medications, thyroid cancers, and benign nodular diseases can also precipitate primary hypothyroidism. Secondary causes are rare but include pituitary or hypothalamic tumors, inflammatory or infiltrative central nervous system diseases, hemorrhagic necrosis, or surgical and radiation treatment of hypothalamic and pituitary disease.

Hyperthyroidism

Hyperthyroidism, or inappropriately overactive thyroid function, is sometimes referred to as 'thyrotoxicosis.' High synthesis and secretion of thyroid hormone by the thyroid gland leads to elevated tissue levels.

Symptoms of hyperthyroidism can be overt or subclinical. When present, they include:²⁸

- tachycardia and palpitations
- weight loss
- tremor
- sweating
- exophthalmos
- oligomenorrhea
- extraocular muscle dysfunction
- atrial fibrillation
- osteoporosis
- anxiety
- hair thinning
- hyperreflexia

'Thyroid Storm' is a rare endocrine emergency whereby patients exhibit exaggerated signs of hyperthyroidism with varying degrees of organ decompensation.²⁹

Much like hypothyroidism, iodine deficiency and excess may contribute to hyperthyroidism. More commonly, it is caused by autoimmunity (Graves' disease), toxic nodular goiters, and/or hyper-functioning thyroid nodules.^{3,30} Other causes include medications, infection, radiation therapies, and iatrogenic or factitious ingestion of exogenous thyroid hormone.³⁰ Rare TSH- secreting pituitary adenomas or pituitary resistance to thyroid hormone can be a cause of secondary hyperthyroidism.³¹

Thyroid Cancer and Thyroid Nodules

Over 90% of thyroid nodules are small, benign lesions that will never become clinically significant. Some lesions are malignant and as with most thyroid disease, are more prevalent in women. Thyroid cancer is the fifth most common cancer in women in the United States and incidence continues to rise. It can be either be derived from the thyroid follicle or derived from the parafollicular neuroendocrine cells of the thyroid.

Differentiated thyroid cancer is the most common type accounting for over 95% of all cases and arises from the thyroid follicular epithelial cells. The term 'differentiated' refers to the process where immature cells become mature with specific functions and describes whether tumor tissue looks like normal tissue. Well-differentiated cells look more normal, and they grow and spread slowly. Poorly differentiated thyroid cancer is far more aggressive.

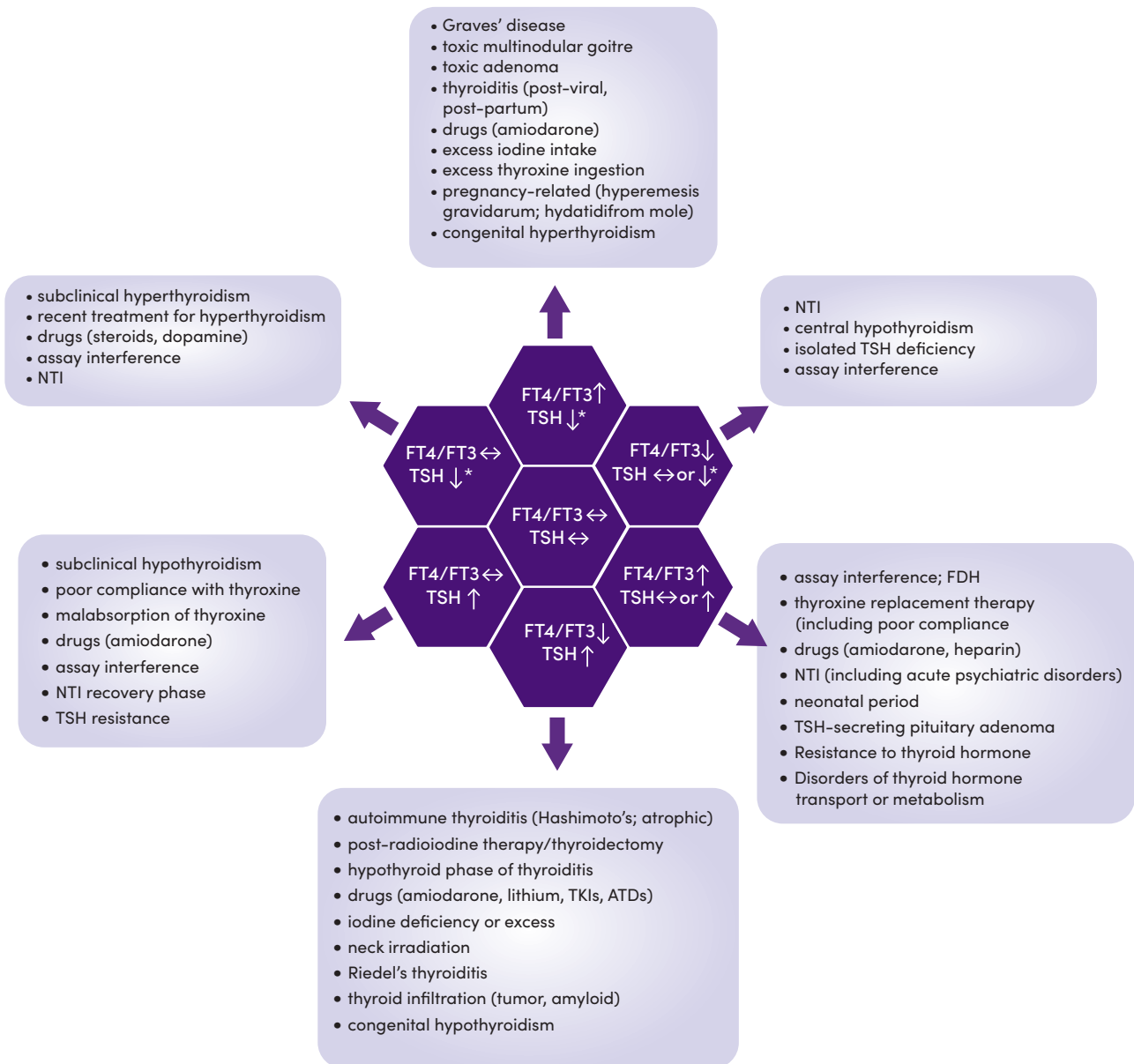
Well-differentiated follicular thyroid cancers include several subtypes: papillary, follicular, and Hurthle cell thyroid cancers. Anaplastic thyroid cancer is a follicular type though can arise from and coexist with differentiated types or occur de novo. Medullary thyroid cancer is derived from the neuroendocrine C-cells and is fairly rare.³²

Nonthyroidal Illness (NTI)

It is common to have abnormal levels of thyroid hormones with any acute critical or inflammatory illness unrelated to the thyroid gland. This is sometimes called 'euthyroid sick syndrome'. The most common abnormality is a reduction in T3.³³ Additionally, higher reverse T3 is sometimes seen, along with normal or suboptimal TSH. Significant abnormalities in free thyroid hormones in the absence of typical clinical symptoms or goiter formation, should raise suspicion of nonthyroidal illness as an etiology. Both central and peripheral components of the hypothalamic-pituitary-thyroid axis can show robust changes during critical illness and prolonged fasting. This may be a beneficial adaptive response in an attempt to reduce energy expenditures, prevent protein breakdown, and promote survival.³⁴

Laboratory Assessment

Results of thyroid function testing should be interpreted in light of a patient's clinical presentation. Nonthyroidal illness, medications, nutrition, and lifestyle factors should also be taken into consideration and addressed. Rather than focusing on one specific analyte to evaluate thyroid function, comprehensive panels are helpful to evaluate thyroid hormone synthesis and metabolism. Commonly encountered patterns of thyroid function tests should be used by clinicians to help target treatment strategies and further investigative studies.³⁵⁻³⁸



Schematic representation of different patterns of thyroid function tests and their causes.

ATDs: Antithyroid drugs
 FDH: Familial dysalbuminaemic hyperthyroxinaemia
 FT3: Free triiodothyronine
 FT4: Free thyroxine
 NTI: Non-thyroid illness
 TH: Thyroid hormone
 TKIs: Tyrosine kinase inhibitors
 TSH: Thyroid-stimulating hormone (thyrotropin)

*Signifies that TSH can be either fully suppressed (eg, as seen in classical primary hyperthyroidism) or partially suppressed (ie, measurable, but below the lower limit of normal)

Thyroid Stimulating Hormone (TSH)

Thyroid stimulating hormone (TSH) is released by the anterior pituitary in response to thyrotropin releasing hormone (TRH) signaling by the hypothalamus.

TSH binds directly to the membrane receptors on thyroid follicles to secrete 2 thyroid hormones: the prohormone thyroxine (T4) and to a lesser extent the more biologically active triiodothyronine (T3).

TSH release from the hypothalamus is very sensitive to any minor change in circulating free T4, such that abnormal TSH levels can be detected much earlier in hyper- or hypo-thyroid conditions. Very minor changes in T3 and/or T4 can result in large changes in TSH, making it an important screening tool.^{1,2}

Measurement of TSH has been shown to be the most useful screening test in a vast majority of patients, though clinical decisions should not be made based on this single value. The 'normal reference range' for TSH has been a focus of debate with most governing bodies agreeing that a tighter range than had been previously accepted is optimal. 'Normal' TSH levels can vary based on ethnicity, age, and pregnancy. Clinical correlation, nutritional and lifestyle factors, and patterns of other thyroid hormones should be used to guide therapeutic decisions.^{39,40}

High Levels:

TSH is high in states of hypothyroidism including Hashimoto's thyroiditis. Iodine deficiency and excess as well as certain medications like amiodarone and lithium can lead to elevations of TSH. High TSH is also seen in infiltrative diseases of the thyroid or thyroidectomies, nonthyroidal illnesses, and subclinical hypothyroidism. It is important to interpret findings in the context of other thyroid biomarkers.³⁵

Low Levels:

Low TSH is seen with hyperthyroidism including Graves' autoimmune thyroiditis. Toxic multinodular goiter and toxic adenomas can secrete thyroid hormone and lower TSH levels. Patients taking excess thyroid supplementation, or drugs such as steroids, dopamine, and amiodarone can have low TSH levels. Nonthyroidal illness and iodine deficiency

and excess can also cause low levels. It is important to interpret findings in the context of other thyroid biomarkers.³⁵

[\(Follow this link for clinical considerations of commonly encountered thyroid hormone patterns\)](#)

Free Thyroxine (FT4)

Thyroxine is secreted by the thyroid gland in response to TSH. It is a prohormone that contains four iodine molecules and must be peripherally converted via deiodinase enzymes to the biologically active T3. Thyroxine circulates bound to protein carriers such as thyroid binding globulin, pre-albumin, and albumin. Only unbound, free hormones can act on receptor sites and enter tissues based on metabolic demands. TSH secretion is regulated by levels of T4 and T3, though appears to be highly sensitive to circulating T4 levels.⁴¹ With that, TSH and Free T4 are often used together as indicators of hyper- and hypothyroidism. A low TSH with high T4 is found in hyperthyroidism, while a high TSH and low T4 can indicate hypothyroidism.

High Levels:

Elevated FT4 levels with a low TSH can be found in hyperthyroidism or in patients supplementing with thyroxine. High FT4 is also seen in Graves' disease, various types of thyroiditis, euthyroid sick syndrome, nonthyroidal illness, toxic multinodular goiter, and adenomas. It is important to interpret findings in the context of other thyroid biomarkers.³⁵

Low Levels:

Decreased free thyroxine is seen in hypothyroidism as well as Hashimoto's autoimmune thyroiditis. Iodine deficiency and excess may result in low levels as well nonthyroidal illnesses. Medications and assay interferences can also cause lower levels of free T4. It is important to interpret findings in the context of other thyroid biomarkers.³⁵

[\(Follow this link for clinical considerations of commonly encountered thyroid hormone patterns\)](#)

Free Triiodothyronine (FT3)

Free Triiodothyronine is the biologically active form of thyroid hormone. Most T3 is made peripherally by removing an iodine molecule from T4 using deiodinase enzymes. A small percentage (approximately 10-20%) is directly secreted by the thyroid gland itself.^{42,43}

Serum FT3 levels are remarkably stable over periods of time since the HPT axis is wired to preserve FT3 levels. It tolerates an elevated T4 in order to preserve T3 function and abilities. There are conditions where the hypothalamus-pituitary axis allows T3 levels to fall such as fasting, nonthyroidal illnesses, and hypothalamic hyperthyroidism. In fact, it can be common to see low T3 with normal TSH and T4 in nonthyroidal illness due to the adaptive and protective mechanisms of the HPT axis.^{34,41}

High Levels:

FT3 is elevated in states of hyperthyroidism such as Graves' disease and other types of thyroiditis. Excess iodine intake or supplementation with thyroid hormone replacement therapy can increase FT3. Toxic multinodular goiter and toxic adenomas can excrete excess FT3 and increase levels. It is important to interpret findings in the context of other thyroid biomarkers.³⁵

Low Levels:

Low FT3 is seen in hypothyroidism and Hashimoto's thyroiditis. Iodine excess and deficiency, nonthyroidal illness, and infiltrative thyroid diseases or thyroidectomies can all contribute to low levels. It is important to interpret findings in the context of other thyroid biomarkers.³⁵

[\(Follow this link for clinical considerations of commonly encountered thyroid hormone patterns\)](#)

Reverse T3 (rT3)

Reverse T3 is created peripherally from T4 by deiodinase enzymes. It is biologically inactive and can block T3 receptor sites limiting the action of thyroid hormone. T4 can be metabolized into either T3 or rT3 depending on metabolic need. As outlined previously, the thyroid continually senses metabolic demand and may downregulate as an adaptive, protective mechanism to reduce energy expenditure and catabolism. As part of this downregulation, it may begin peripherally producing rT3. Although rT3 itself is biologically inactive, there are clinical associations to consider given its receptor blocking capacity.

High Levels:

Elevations in the inactive rT3 are seen in advancing age, fasting, catabolism, infection, inflammation, and stress. Some medications have also been shown to increase rT3 such as propranolol, levothyroxine, cimetidine, and some steroids.⁴⁴⁻⁴⁷ Nutritional inadequacies such as iron deficiency, as well as toxic exposures, should also be considered as causes of rT3 elevation.⁴⁸ Addressing these underlying factors may help to reduce inactive rT3 levels. In the elderly population, higher levels of rT3 are associated with lower levels of function.^{18,49} Additionally, since the conversion of T4 to T3 happens in the liver, high T3 levels are also seen in advanced liver disease.⁵⁰

Low Levels:

Any condition which contributes to lowering T4 levels can by default lower rT3 since it requires T4 as a precursor. Evaluation of the underlying cause of low T4 may be warranted. In the absence of hypothyroid symptoms and a normal T4, low levels of reverse T3 are not clinically significant.

Anti-Thyroglobulin Antibody (Anti-TG)

The thyroglobulin (Tg) protein is made in the follicular cells of the thyroid and combines with iodine molecules to form T3 and T4 upon stimulation by TSH. Thyroglobulin can leak into systemic circulation naturally, or with any thyroid tissue damage or enlargement of the thyroid gland. Circulating Tg does not necessarily induce auto-antibody production and can perhaps induce some self-tolerance at low levels.²⁴ However, antibodies can be made against Tg and in genetically predisposed patients result in autoimmune thyroid disease. Anti-Tg antibodies do not fix complement nor cause thyroid cell destruction. Their function in circulation is still being debated as to whether or not it is clinically active. Although at times anti-Tg though useful in autoimmunity, it's also used both pre- and post-operatively in thyroid cancer monitoring.^{24,51}

It should be noted that anti-Tg antibodies are present in a percentage of healthy patients with no thyroid disease present. Elevated levels should be correlated with clinical presentation, medical history, and other thyroid serologies. Anti-Tg levels are associated with several autoimmune diseases outlined below.

High Levels:

There is significant overlap in auto-antibody production in autoimmune thyroid disease. Elevations of anti-Tg can be seen in both Hashimoto's thyroiditis and Grave's disease. Evaluation of levels of other thyroid hormones and clinical presentation should be taken into consideration. Ultrasound and radioactive uptake studies are sometimes used in conjunction with serologies for definitive diagnosis.

There is also overlapping of anti-Tg and other thyroid antibodies in several autoimmune diseases and clinical conditions such as cancer,⁵¹ vitiligo,⁵² Sjogren's syndrome⁵³, rheumatic diseases⁵⁴, polycystic ovary syndrome⁵⁵, type 1 diabetes mellitus⁵⁶, and others.⁵⁷⁻⁶¹

Low Levels:

Low or undetectable levels are optimal.

Anti-Thyroid Peroxidase Antibody (Anti-TPO)

Thyroid peroxidase is a heme-containing enzyme responsible for adding iodine molecules to tyrosine residues in thyroglobulin to produce thyroid hormones. As an intracellular enzyme, there are several areas on the molecule which can provoke an autoimmune response. Both the iodine-combining sites and epitopes related to the tyrosyl residue combining sites have been proposed as the most antigenic in genetically predisposed individuals.⁶²

Anti-TPO antibodies are more common and indicative of autoimmune thyroid disease than anti-Tg. Anti-TPO antibodies act differently than anti-Tg. Although they can be present in healthy populations, in genetically predisposed patients they can activate complement and cause damage to thyroid cells due to antibody-dependent cell cytotoxicity. They are present in nearly all (>90%) of Hashimoto's thyroiditis patients and fair percentage of those with Graves' disease.⁶³ While they act as cytotoxic in Hashimoto's thyroiditis, their role in Graves' disease has not been well established.²⁴

High Levels:

Elevated anti-TPO antibodies should be correlated with clinical presentation and other thyroid serologies. They are prevalent in Hashimoto's thyroiditis and Graves' disease but can also be found in healthy subjects.^{62,63} Much like anti-Tg, there is cross reactivity and correlation with other non-thyroidal autoimmune conditions, such as rheumatic illnesses, systemic lupus erythematosus, type 1 diabetes mellitus, celiac disease, and others.^{24,64} The proposed mechanisms for cross-reactivity with other autoimmune illnesses is still being studied though common genetic backgrounds, and exposure to an environmental or viral trigger are possible.⁶⁴

Low Levels:

Low or undetectable levels are optimal.

Treatment Resources

In a patient with abnormal thyroid function testing, addressing nutritional inadequacy and environmental factors is important when creating a treatment protocol. Additionally, there are many resources available to help guide prescriptive medication therapies.

[American Thyroid Association](#)

[American Association of Clinical Endocrinology](#)

[European Society of Endocrinology](#)

[British Thyroid Foundation](#)

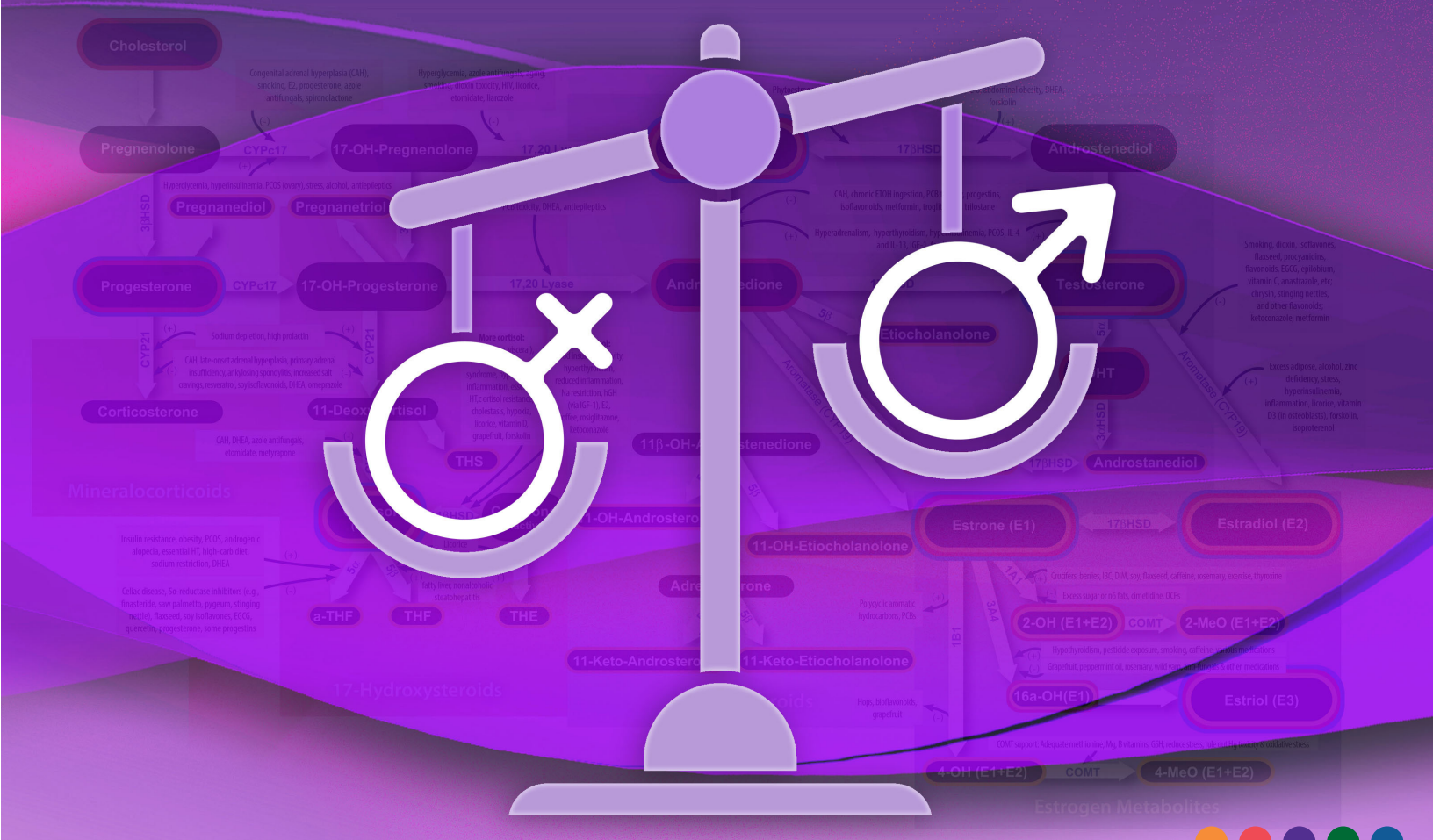
[Institute for Functional Medicine](#)

[American Academy of Anti-Aging Medicine](#)

References

1. Pirahanchi Y, Tariq MA, Jialal I. Physiology, thyroid. StatPearls [Internet]. 2021.
2. Sheehan MT. Biochemical Testing of the Thyroid: TSH is the Best and, Oftentimes, Only Test Needed – A Review for Primary Care. Clin Med Res. 2016;14(2):83-92.
3. Leung AM, Braverman LE. Iodine-induced thyroid dysfunction. Curr Opin Endocrinol Diabetes Obes. 2012;19(5):414-419.
4. Ventura M, Melo M, Carrilho F. Selenium and Thyroid Disease: From Pathophysiology to Treatment. Int J Endocrinol. 2017;2017:1297658.
5. Lacka K, Szeliga A. Significance of selenium in thyroid physiology and pathology. Pol Merkuri Lekarski. 2015;38(228):348-353.
6. Gorini F, Sabatino L, Pingitore A, Vassalle C. Selenium: An Element of Life Essential for Thyroid Function. Molecules. 2021;26(23):7084.
7. Winther KH, Bonnema SJ, Cold F, et al. Does selenium supplementation affect thyroid function? Results from a randomized, controlled, double-blinded trial in a Danish population. Eur J Endocrinol. 2015;172(6):657-667.
8. Severo JS, Morais JBS, de Freitas TEC, et al. The role of zinc in thyroid hormones metabolism. Int J Vit Nutr Res. 2019.
9. Zimmermann MB, Köhrle J. The impact of iron and selenium deficiencies on iodine and thyroid metabolism: biochemistry and relevance to public health. Thyroid. 2002;12(10):867-878.
10. O’Kane SM, Mulhern MS, Pourshahidi LK, Strain J, Yeates AJ. Micronutrients, iodine status and concentrations of thyroid hormones: a systematic review. Nutr Rev. 2018;76(6):418-431.
11. Mansourian A. Metabolic pathways of tetraiodothyronine and triiodothyronine production by thyroid gland: a review of articles. Pakist J Biol Sci. 2011;14(1):1.
12. Pałkowska-Goździk E, Lachowicz K, Rosołowska-Huszcz D. Effects of dietary protein on thyroid axis activity. Nutrients. 2018;10(1):5.
13. Farhangi MA, Keshavarz SA, Eshraghian M, Ostadrahimi A, Saboor-Yaraghi AA. The effect of vitamin A supplementation on thyroid function in premenopausal women. J Am Coll Nutr. 2012;31(4):268-274.
14. Sarandöl E, Taş S, Dirican M, Serdar Z. Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: effect of vitamin E supplementation. Cell Biochem Function. 2005;23(1):1-8.
15. Shokri Z, Khoshbin M, Koohpayeh A, et al. Thyroid diseases: Pathophysiology and new hopes in treatment with medicinal plants and natural antioxidants. Int J Green Pharm. 2018;12(3).
16. Muscogiuri G, Tirabassi G, Bizzaro G, et al. Vitamin D and thyroid disease: to D or not to D? Eur J Clin Nutr. 2015;69(3):291-296.
17. Oliveira KJ, Chiamolera MI, Giannocco G, Pazos-Moura CC, Ortiga-Carvalho TM. Thyroid function disruptors: from nature to chemicals. J Mol Endocrinol. 2019;62(1):R1-R19.
18. Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res. 2002;53(4):865-871.
19. Sarne D. Effects of the Environment, Chemicals and Drugs on Thyroid Function. MDText.com, Inc., South Dartmouth (MA); 2000.
20. Tomer Y, Huber A. The etiology of autoimmune thyroid disease: a story of genes and environment. J Autoimmun. 2009;32(3-4):231-239.
21. Ralli M, Angeletti D, Fiore M, et al. Hashimoto’s thyroiditis: An update on pathogenic mechanisms, diagnostic protocols, therapeutic strategies, and potential malignant transformation. Autoimm Rev. 2020:102649.
22. LiVolsi VA, Baloch ZW. The Pathology of Hyperthyroidism. Front Endocrinol. 2018;9:737-737.
23. Franco J-S, Amaya-Amaya J, Anaya J-M. Thyroid disease and autoimmune diseases. In: Autoimmunity: From Bench to Bedside. El Rosario University Press; 2013.
24. Fröhlich E, Wahl R. Thyroid Autoimmunity: Role of Anti-thyroid Antibodies in Thyroid and Extra-Thyroidal Diseases. Front Immunol. 2017;8:521-521.
25. Dong Y, Fu D. Autoimmune thyroid disease: mechanism, genetics and current knowledge. Eur Rev Med Pharmacol Sci. 2014;18(23):3611-3618.
26. Clinical Practice Guidelines for Hypothyroidism in Adults: Cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Thyroid. 2012;22(12):1200-1235.
27. Mathew V, Misgar RA, Ghosh S, et al. Myxedema coma: a new look into an old crisis. J Thyroid Res. 2011;2011.

28. Sharma A, Stan MN. Thyrotoxicosis: diagnosis and management. Paper presented at: Mayo Clinic Proceedings 2019.
29. Nayak B, Burman K. Thyrotoxicosis and thyroid storm. *Endocrinol Metab Clin*. 2006;35(4):663-686.
30. LiVolsi VA, Baloch ZW. The Pathology of Hyperthyroidism. *Front Endocrinol*. 2018;9(737).
31. Gilbert J. Thyrotoxicosis - investigation and management *Clin Med*. 2017;17(3):274-277.
32. Cabanillas ME, McFadden DG, Durante C. Thyroid cancer. *Lancet*. 2016;388(10061):2783-2795.
33. Ganesan K, Wadud K. Euthyroid sick syndrome. *StatPearls*. 2020.
34. Fliers E, Boelen A. An update on non-thyroidal illness syndrome. *J Endocrinol Investig*. 2021;44(8):1597-1607.
35. Koulouri O, Gurnell M. How to interpret thyroid function tests. *Clin Med*. 2013;13(3):282-286.
36. Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. *Best Pract Res Clin Endocrinol Metab*. 2013;27(6):745-762.
37. Soh S-B, Aw T-C. Laboratory Testing in Thyroid Conditions - Pitfalls and Clinical Utility. *alm*. 2018;39(1):3-14.
38. Gurnell M, Halsall DJ, Chatterjee VK. What should be done when thyroid function tests do not make sense? *Clin Endocrinol*. 2011;74(6):673-678.
39. Kratzsch J, Fiedler GM, Leichtle A, et al. New reference intervals for thyrotropin and thyroid hormones based on National Academy of Clinical Biochemistry criteria and regular ultrasonography of the thyroid. *Clin Chem*. 2005;51(8):1480-1486.
40. Biondi B. The Normal TSH Reference Range: What Has Changed in the Last Decade? *J Clin Endocrinol Metab*. 2013;98(9):3584-3587.
41. Abdalla SM, Bianco AC. Defending plasma T3 is a biological priority. *Clin Endocrinol*. 2014;81(5):633-641.
42. Jonklaas J, Bianco AC, Bauer AJ, et al. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. *Thyroid*. 2014;24(12):1670-1751.
43. Armstrong M, Asuka E, Fingeret A. Physiology, Thyroid Function. In: *StatPearls*. Treasure Island StatPearls Publishing LLC.; 2022.
44. Verburg FA, Smit JW, Grelle I, Visser TJ, Peeters RP, Reiners C. Changes within the thyroid axis after long-term TSH-suppressive levothyroxine therapy. *Clin Endocrinol*. 2012;76(4):577-581.
45. Kallner G, Ljunggren JG, Tryselius M. The effect of propranolol on serum levels of T4, T3 and reverse-T3 in hyperthyroidism. *Acta Med Scand*. 1978;204(1-2):35-37.
46. Perret G, Hugues JN, Louchahi M, Varoquaux O, Modigliani E. Effect of a short-term oral administration of cimetidine and ranitidine on the basal and thyrotropin-releasing hormone-stimulated serum concentrations of prolactin, thyrotropin and thyroid hormones in healthy volunteers. A double-blind cross-over study. *Pharmacology*. 1986;32(2):101-108.
47. Chopra IJ, Williams DE, Orgiazzi J, Solomon DH. Opposite effects of dexamethasone on serum concentrations of 3,3',5'-triiodothyronine (reverse T3) and 3,3',5'-triiodothyronine (T3). *J Clin Endocrinol Metab*. 1975;41(5):911-920.
48. Eftekhari MH, Eshraghian MR, Mozaffari-Khosravi H, Saadat N, Shidfar F. Effect of iron repletion and correction of iron deficiency on thyroid function in iron-deficient Iranian adolescent girls. *Pakist J Biol Sci*. 2007;10(2):255-260.
49. van den Beld AW, Visser TJ, Feelders RA, Grobbee DE, Lamberts SWJ. Thyroid Hormone Concentrations, Disease, Physical Function, and Mortality in Elderly Men. *J Clin Endocrinol Metab*. 2005;90(12):6403-6409.
50. Kabadi UM, Premachandra BN. Serum T3 and reverse T3 levels in hepatic cirrhosis: relation to hepatocellular damage and normalization on improvement in liver dysfunction. *Am J Gastroenterol*. 1983;78(11):750-755.
51. Jo K, Lim D-J. Clinical implications of anti-thyroglobulin antibody measurement before surgery in thyroid cancer. *Korean J Intern Med*. 2018;33(6):1050-1057.
52. Kasumagic-Halilovic E, Ovcina-Kurtovic N, Helppikangas H. Anti-thyroglobulin Antibody and Vitiligo: A controlled study. *Our Dermatol Online*. 2015;6(2):145.
53. Baldini C, Ferro F, Mosca M, Fallahi P, Antonelli A. The Association of Sjögren Syndrome and Autoimmune Thyroid Disorders. *Front Endocrinol*. 2018;9:121-121.
54. Bourji K, Gatto M, Cozzi F, Doria A, Punzi L. Rheumatic and autoimmune thyroid disorders: a causal or casual relationship? *Autoimmun Rev*. 2015;14(1):57-63.
55. Janssen OE, Mehlmauer N, Hahn S, Offner AH, Gärtner R. High prevalence of autoimmune thyroiditis in patients with polycystic ovary syndrome. *Eur J Endocrinol*. 2004;150(3):363-369.
56. Basu M, Pandit K, Banerjee M, Mondal SA, Mukhopadhyay P, Ghosh S. Profile of auto-antibodies (Disease related and other) in children with type 1 diabetes. *Ind J Endocrinol Metab*. 2020;24(3):256.
57. Long Y, Zheng Y, Chen M, et al. Serum thyroid-stimulating hormone and anti-thyroglobulin antibody are independently associated with lesions in spinal cord in central nervous system demyelinating diseases. *PLoS One*. 2014;9(8):e100672.
58. Ali H, Baig JA, Alam JM. Frequency distribution of anti-thyroid peroxidase and anti-thyroglobulin antibodies in relation to TSH, T3 and T4 levels. *Pak J Physiol*. 2017;13(3):32-35.
59. Pereira WLdCJ, Reiche EMV, Kallaur AP, et al. Frequency of autoimmune disorders and autoantibodies in patients with neuromyelitis optica. *Acta Neuropsych*. 2017;29(3):170-178.
60. Wan K-S, Wu C-S. The essential role of anti-thyroid antibodies in chronic idiopathic urticaria. *Endo Res*. 2013;38(2):85-88.
61. Bansal S, Hayman G. 9 Graves Disease Associated with Chronic Idiopathic Urticaria: 2 Case Reports. *J Invest Allergol Clin Immunol*. 2009;19(1):54.
62. Kohno Y, Yamaguchi F, Saito K, Niimi H, Nishikawa T, Hosoya T. Anti-thyroid peroxidase antibodies in sera from healthy subjects and from patients with chronic thyroiditis: differences in the ability to inhibit thyroid peroxidase activities. *Clin Exp Immunol*. 1991;85(3):459-463.
63. Zaletel K, Gaberšček S. Hashimoto's Thyroiditis: From Genes to the Disease. *Curr Genomics*. 2011;12(8):576-588.
64. Hussein SAE, Mansour HE, Hussein MS, Abdel Aziz ASM, Aziz NN. Thyroid Autoantibodies in Egyptian Patients with Autoimmune Rheumatic Diseases: Relation to Disease Activity and Functional Impairment. *Egypt J Hosp Med*. 2020;80(2):936-942.



Saliva • Urine • Blood

Sex Hormones

SUPPORT GUIDE



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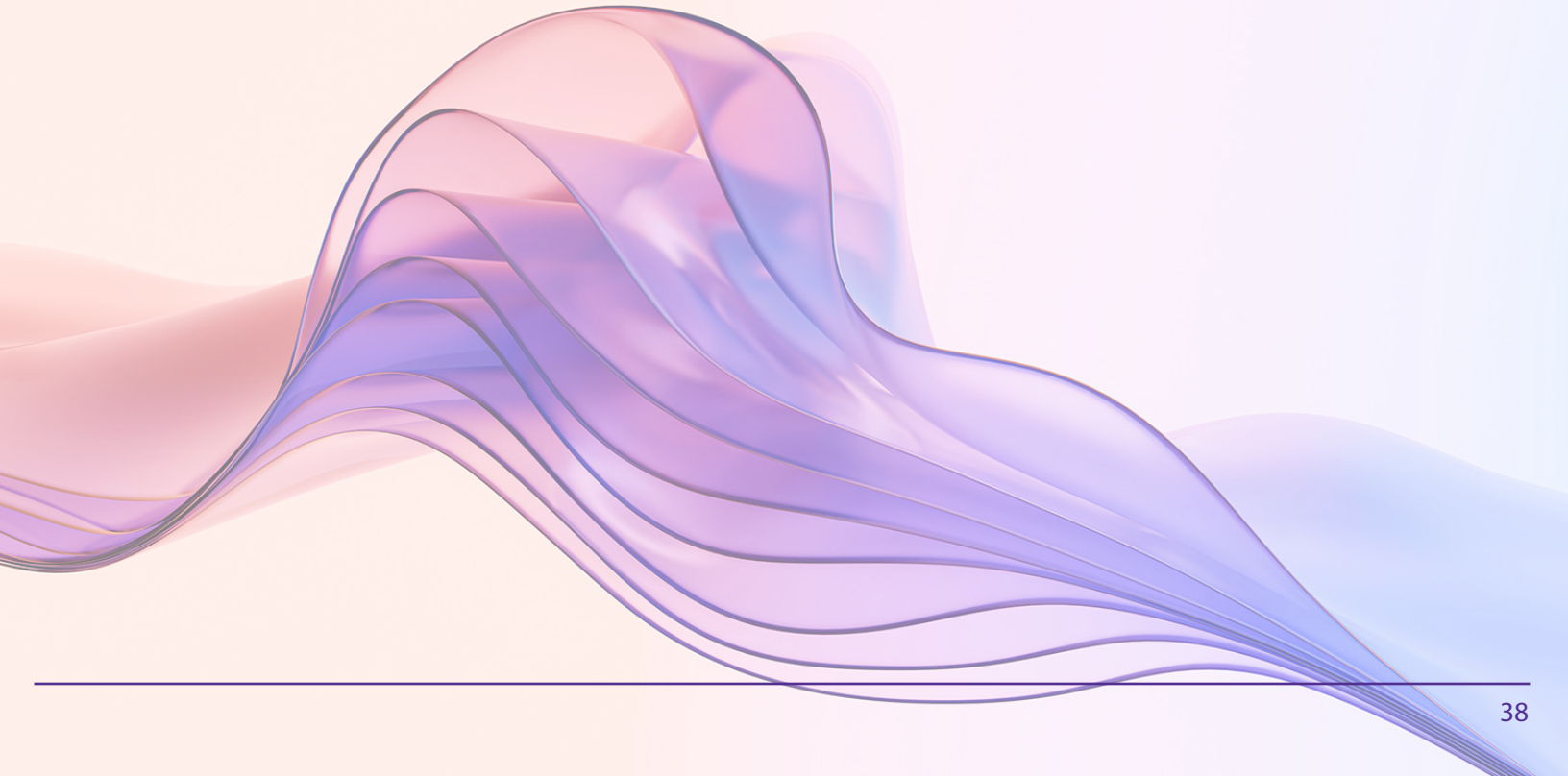
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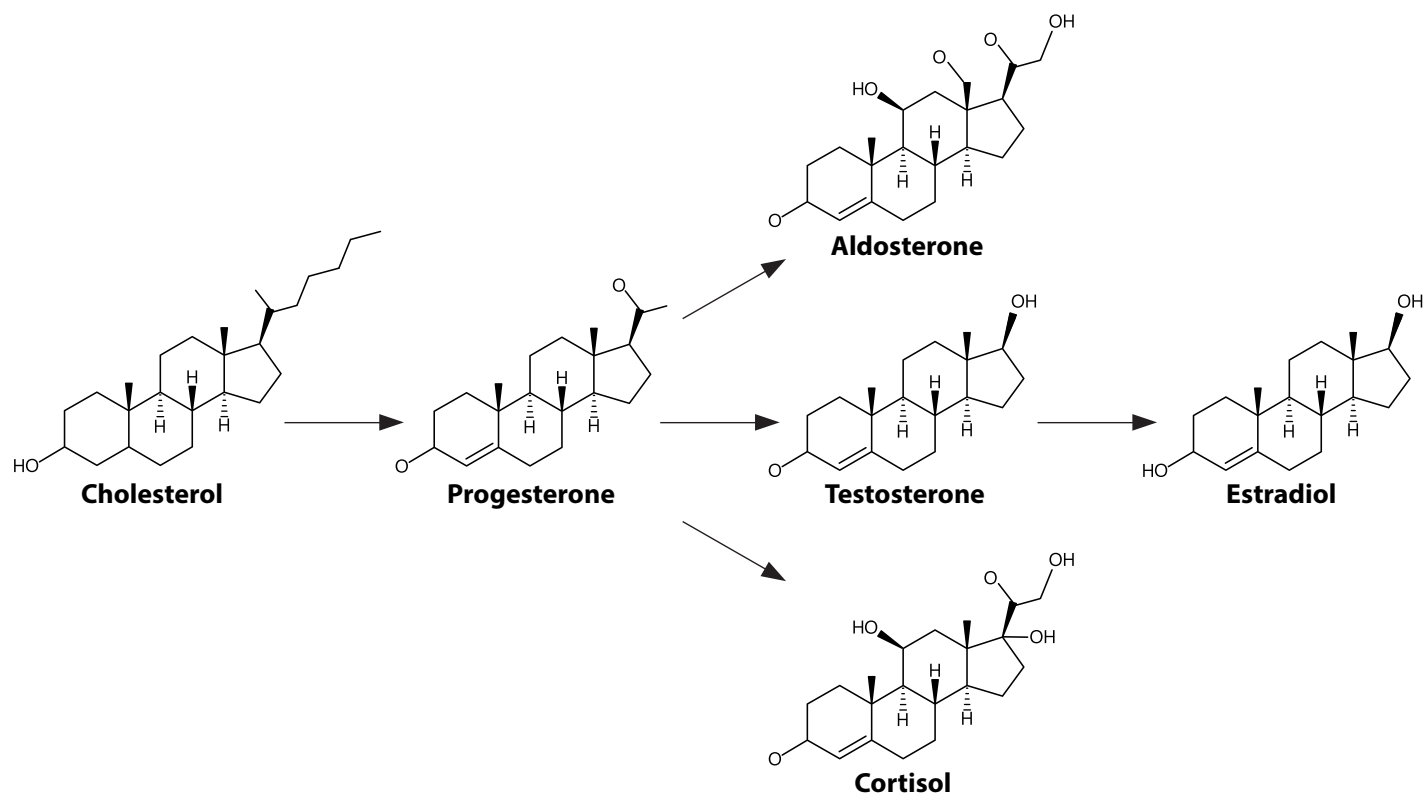
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 Steroidogenic Pathways Chart.....94



Introduction

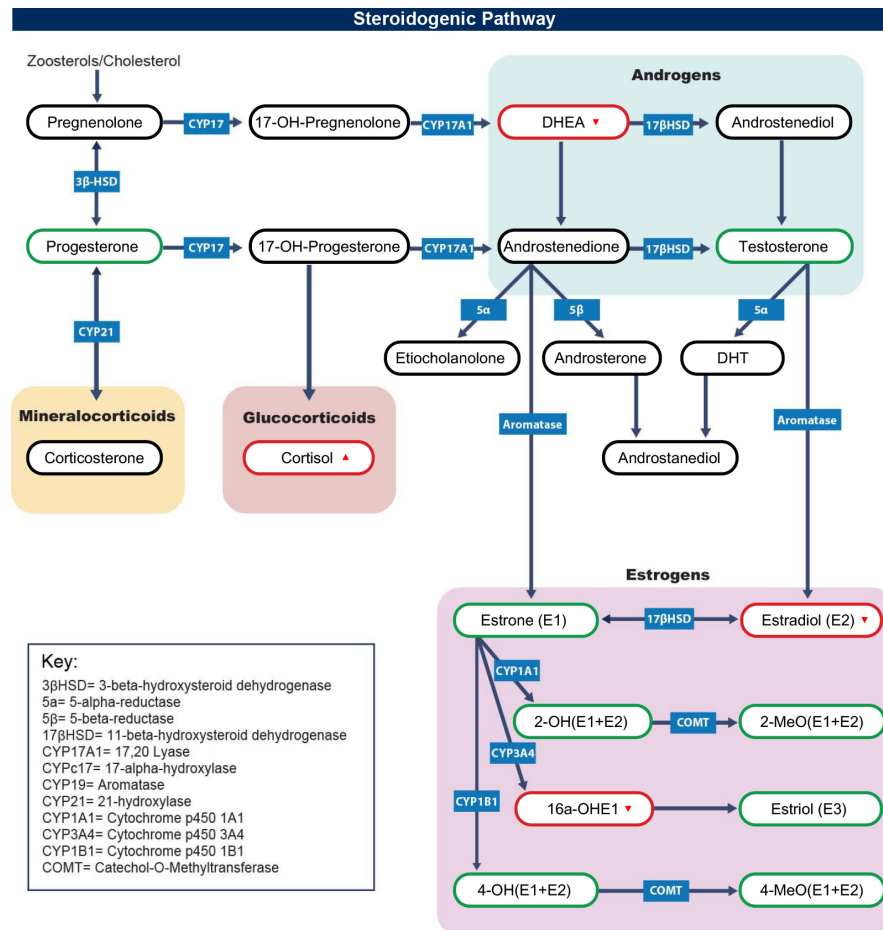
Genova offers a variety of assessments for sex hormones, including serum, saliva, and urine testing for adult males and pre- or postmenopausal females. Parent hormone levels and hormone metabolites provide information about possible causes of a patient's symptoms and health conditions. Measuring hormones as a comprehensive panel is favored over single markers and is better for understanding steroid hormone metabolism.¹



Steroidogenic Pathway

Cholesterol is the common precursor of all steroid hormones, in particular LDL cholesterol. Steroid hormones share a basic structure of a polycyclic complex of 17 carbon atoms forming a four-ring system. According to the number of carbon atoms, the steroids form different groups including progestogens (21-carbon atoms), glucocorticoids (21-carbon atoms), mineralocorticoids (21-carbon atoms), androgens (19-carbon atoms), and estrogens (18-carbon atoms).²

The steroidogenic pathway is the same in every organ (ovary, testis, adrenal cortex, brain, placenta), however, the type and amount of synthesized and secreted hormone depends on the expression of enzymes specific to each organ. For example, the ovaries do not contain the enzyme 21 α -hydroxylase or 11 α -hydroxylase to synthesize glucocorticoids or mineralocorticoids.²



The enzymes that make up the steroidogenic pathways are influenced by multiple factors, including diet, lifestyle, hormones, certain pathologies, medication, supplements, environmental toxins, and genetics. See the [Steroidogenic Pathways Chart](#) for information on factors that up and down-regulate enzymes.

After hormones are synthesized, they can be carried in the bloodstream either bound or unbound to their respective receptors. When bound to proteins like albumin or sex hormone binding globulin (SHBG), they are not bioavailable. Free, unbound hormones are considered bioavailable and ready to be used in tissues.

- Unbound = Bioavailable
- Bound = Not bioavailable

Furthermore, hormones then need to be detoxified and made water-soluble for safe excretion in urine or stool. This happens in the liver during phase 1 and phase 2 detoxification.

Hormone Detoxification

Phase 1

In phase 1 detoxification, the liver primarily uses a group of enzymes called cytochrome p450 (CYP450) to initiate the breakdown by modifying the chemical structure and exposing functional groups now ready for further detoxification. These modifications involve processes such as oxidation, reduction, and hydroxylation. These intermediates are reactive and potentially toxic and therefore must undergo phase 2 detoxification to make the molecules water-soluble for safe excretion.

Phase 2

In phase 2, these reactive intermediates undergo conjugation reactions – meaning they combine with other molecules to become water-soluble. These molecules/reactions include glucuronidation, sulfation, methylation, acetylation, and glutathione conjugation. These newly conjugated molecules are now water-soluble and safe for excretion.

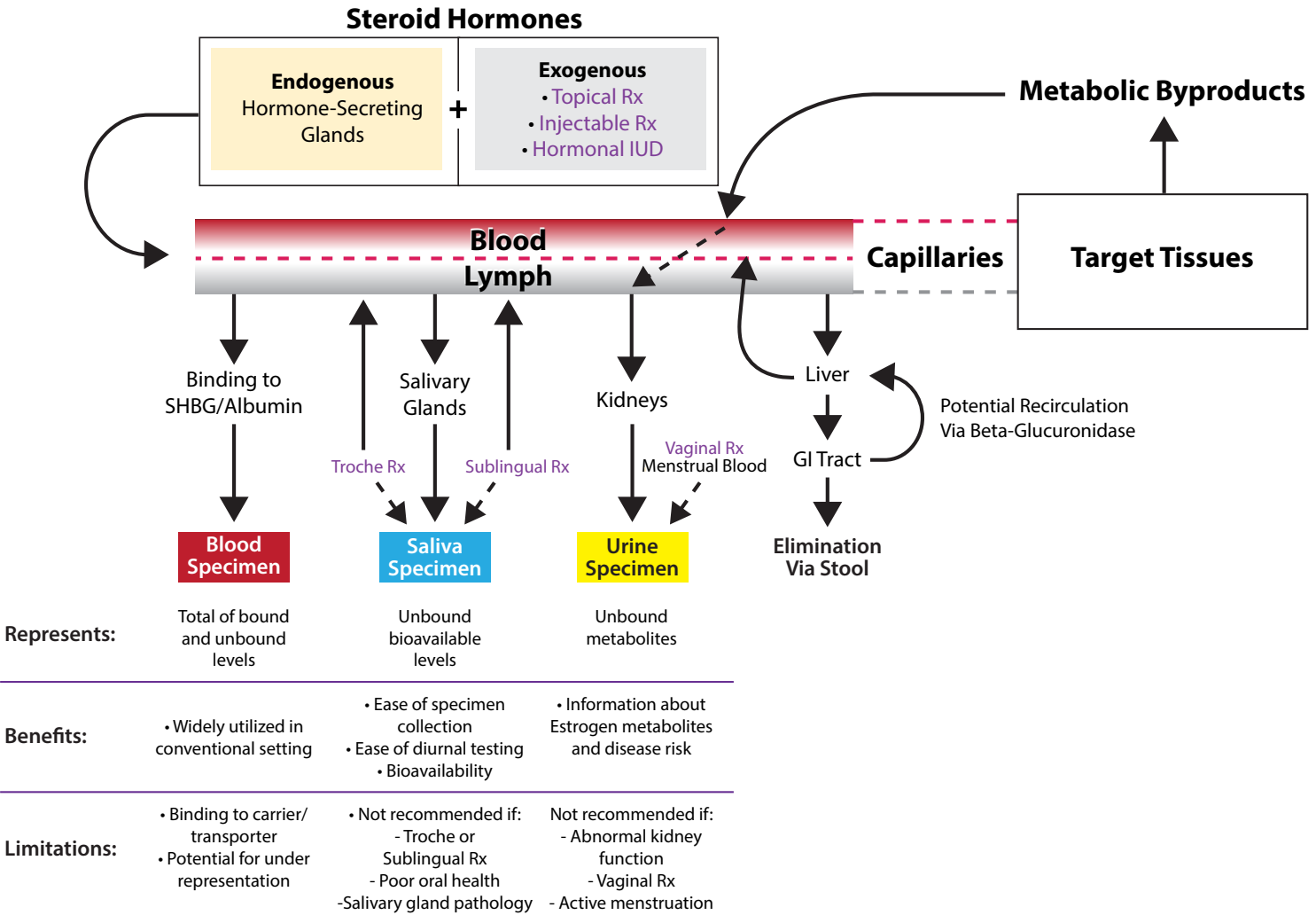
- Conjugated hormones = molecule attached making it water-soluble for excretion
- Unconjugated hormones = not attached to molecules and not yet made water-soluble



Sample Type Comparison

Hormones can be measured in multiple sample types including blood, urine, and saliva. Serum or plasma has been the standard of care for hormone testing however, other sample types have clinical relevance and convenient collection.

- Possible factors accounting for different results in different sample types include steroid binding proteins, genetic factors, salivary secretory mechanics, steroid metabolism, and methodology.^{3,4} *For this reason, when monitoring hormone levels, it is important to choose the same sample type each time for comparison.*
- Not only is it important to choose the same sample type for monitoring hormones, but it is also important to collect at the same time for subsequent collections. Sex hormones exhibit an endogenous circadian rhythm, and it is important to follow Genova’s suggested collection times for an accurate comparison.⁵





What sample type should I use to measure sex hormones?

Serum

- In-office collection
- Evaluate baseline primary sex hormone levels
- Measures bound + unbound circulating hormones (E1, E2, E3, progesterone, DHEA) and bioavailable testosterone
- HRT considerations*:
 - Oral, sublingual, pellets, injectables likely accurately represented
 - Transdermal creams may be underrepresented

Saliva

- At-home collection
- Evaluate baseline primary sex hormone levels
- Measures unbound, bioavailable hormones
- Optimal choice to evaluate hormones requiring multiple samples
 - Diurnal cortisol and melatonin levels
 - E2 and progesterone throughout the menstrual cycle
- Blood from bleeding gums can contaminate sample resulting in false elevations
- HRT considerations*:
 - Oral preparations likely accurately represented
 - Transdermal creams may be overrepresented
 - Sublingual preparations may contaminate sample
 - Results include a therapeutic cohort range

Urine

- At-home collection
- Evaluate baseline primary sex hormone levels (estrogens only)
- Optimal choice to evaluate estrogen metabolism for risk
- 24-hour collection for patients on HRT or first morning void (FMV) for non-supplemented patients; (note: Endo+ FMV only)
- Not recommended in patients with abnormal kidney function since results are ratioed to creatinine
- HRT considerations*:
 - Oral, sublingual preparations likely accurately represented
 - Vaginal preparations may contaminate sample

*Disclaimer:

There is no industry standard by which to monitor HRT. Guidelines are lacking regarding HRT preparations and ideal sample types, timing, etc. Genova will continue to follow literature as it evolves. Genova's assays detect endogenous and bioidentical hormones only. Testing is not recommended for patients taking non-bioidentical hormones.



ENDO+ HORMONE SAMPLE TYPE TESTING OPTIONS

	SERUM	SALIVA	URINE
Estrogens (E1, E2, E3)	●	●	●
Estrogen metabolites			●
Progesterone	●	●	
Testosterone	●	●	
SHBG	●		
Cortisol		●	
DHEA		●	
DHEA-S	●		
Thyroid hormones	●		
Melatonin		●	

URINE

Urine hormones provide insight into hormone metabolism and risk. Estrogen and metabolites are measured using Liquid Chromatography with Tandem Mass Spectrometry (LC/MS-MS), a widely accepted methodology in the literature for hormone testing.^{21,22} Urinary steroids are mostly inactive and include hydrophilic metabolites including glucuronidated and sulfated conjugates.¹⁴

There is modest correlation with blood and urine estrogens which may be explained by how estrogens are metabolized and/or excreted, though it is unclear what mechanism drives this discrepancy.^{4,23-27} Urinary estrogen levels tend to lag a day behind serum levels. Despite modest correlation with blood, urine has important clinical utility. Urine is a preferred sample type for

assessing metabolism and patterns.²⁷ Having appropriate reference ranges for each sample type is important for analyzing data and assessing risk.

First morning urine specimens are more consistently seen in recent studies on urinary estrogens and their metabolites. While some studies assess urinary hormone levels over 24 hours, a FMV spot urine is sufficient to determine estrogen metabolism since there is not a lot of diurnal variability. Since urinary estrogens are adjusted to creatinine, a FMV is comparable to a 24-hour sample in those not supplementing.²⁸⁻³¹

Urine hormone results may be altered in patients with kidney disease since results are ratioed to creatine concentration. Vaginal hormone applications prior to testing may contaminate samples.

More information on methodology and clinical utility can be found in the section [“Important Interpretation Considerations for Urine Hormones”](#).

BLOOD

Genova’s Hormonal Health serum hormone profile assesses parent hormone levels as well as SHBG. Blood hormone testing has served as the standard of care for hormone testing. However, saliva and urine testing are gaining interest due to the non-invasive collection. Blood assesses total hormone levels which includes both bound and unbound hormones. Frequent serum sampling is invasive and inconvenient making saliva a more convenient sample type for multiple sample collections.



SALIVA

Genova offers a variety of salivary hormone profiles designed to fit the population being tested, with one-day profiles for patients with relatively stable levels (men, postmenopausal females) to month-long assessments for cycling females. Salivary testing offers the convenience of at-home non-invasive collection.⁶ It is very stable and can be stored in a freezer and subjected to repeated freezing and thawing without any adverse assay effects.⁷ There is generally good consistency between saliva and blood hormone levels.⁸⁻¹³

Saliva is an ultrafiltrate of blood, reflecting the biologically active (free) fraction of hormones in the bloodstream, which would account for sex hormone activity at the tissue level.^{3,6,14,15} Only free, unbound hormone passes into saliva due to molecular weights of binding proteins albumin and sex hormone binding globulin (SHBG). Salivary hormone concentrations are typically 1-10% of blood hormone concentrations.¹⁴⁻¹⁶ The low concentration may be problematic for detection, so sensitive methods must be used.¹⁴

Hormones pass through the lipophilic layers of the capillaries and glandular epithelial cells into the saliva. Genova assesses unconjugated steroid levels in salivary assessments.

- Unconjugated steroids are lipophilic, highly membrane permeable, and show a better association to serum/plasma hormone levels as compared to conjugated steroids.¹⁴
- Conjugated steroids, such as sulfated dehydroepiandrosterone (DHEA-S), have limited lipid solubility and are not able to permeate the membrane. Instead, these conjugated hormones may enter the saliva via the tight junctions of the acinar cells.¹⁴

Certain factors may cause inaccurate salivary test results.

- Blood contamination of a saliva sample may result in falsely elevated levels if a patient has gingivitis or bleeding gums.^{14,17-19}
- Salivary gland dysfunction caused by a large number of diseases and medications may prevent proper release of hormones into saliva resulting in low levels.²⁰
- Sublingual supplementation just prior to testing may contaminate the sample.

Troubleshooting Salivary Hormone Results

There are a variety of factors that can explain abnormal salivary hormone results. Salivary hormone testing is more prone to factors affecting levels as compared to serum testing.

Factor	Potential Impact	Notes
Timing of hormone Rx	<ul style="list-style-type: none"> • An absorption peak or trough may be observed, depending on when the Rx was taken prior to specimen collection 	<ul style="list-style-type: none"> • Example: night-time progesterone dosing prior to a morning specimen collection may result in a progesterone absorption peak
Dose of hormone Rx	<ul style="list-style-type: none"> • Ensure proper dose was prescribed • Ensure dosing instructions are being followed properly by the patient • Consider that steroid hormones are lipophilic and accumulation may occur over time 	<ul style="list-style-type: none"> • Example: if dose is 0.1mg topical, ensure the patient isn't applying 1mg
Application site of hormone Rx	<ul style="list-style-type: none"> • Sublingual or troche hormones may result in extremely high levels in salivary specimen, due to direct entry into saliva • Topical hormones applied to upper body, face, or non-fatty tissues may result in uncharacteristically high levels in saliva 	<ul style="list-style-type: none"> • Hormone Rx application in close proximity to salivary glands or directly over lymphatics may introduce concentrated levels into the salivary specimen
Synthetic hormone Rx	<ul style="list-style-type: none"> • Synthetic preparations (progestins, AndroGel, etc.) may result in spurious findings (Genova internal analysis) 	<ul style="list-style-type: none"> • Genova hormone assays measure either the bioidentical form of the hormone or its metabolite

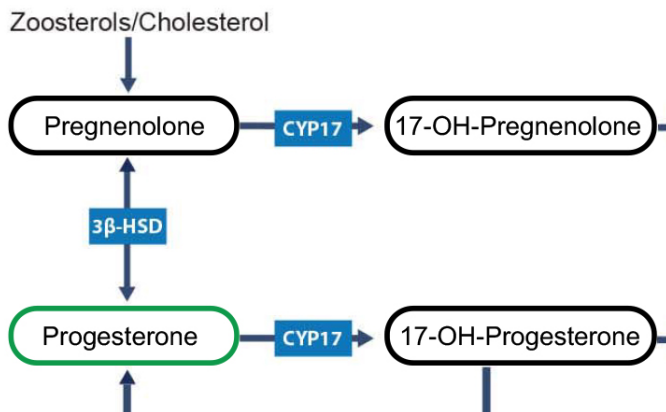
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Factor	Potential Impact	Notes
Unknown exposures to hormones	<ul style="list-style-type: none"> • Use of personal care products, creams, lotions, contaminated supplements, may result in suspiciously elevated levels • Transference from someone else using topical hormones: skin-to-skin or skin-to-surface-to-skin contact 	<ul style="list-style-type: none"> • Higher-than-expected levels of hormones may result
Impaired detoxification or excessive recirculation	<ul style="list-style-type: none"> • Poor liver health (impaired methylation, sulfation, glucuronidation, or conjugation) • Constipation • Elevated beta-glucuronidase levels in the gut can lead to excessive recirculation of steroid hormones 	<ul style="list-style-type: none"> • Higher-than-expected levels of hormones may result
Poor oral health	<ul style="list-style-type: none"> • Gingival blood contamination in saliva may result in uncharacteristically high levels in saliva specimen • Salivary gland degradation may prevent proper release of hormones into saliva resulting in low levels²⁰ 	<ul style="list-style-type: none"> • The health and quality of the saliva are somewhat dependent upon that of the mouth
Overweight / obesity	<ul style="list-style-type: none"> • Adipose tissue contains high levels of the aromatase enzyme, which can readily convert androgens to estrogens • High amounts of adipose tissue may have increased ability to act as a reservoir for lipophilic hormones, either lowering measurable levels initially or slowly releasing excess hormones over time 	<ul style="list-style-type: none"> • Progesterone is highly lipophilic and may accumulate in subcutaneous fat cells
High secretors of hormones	<ul style="list-style-type: none"> • Some individuals may possess a high- or low- ability to release hormones into the saliva^{3,14} 	<ul style="list-style-type: none"> • Due to individual variation and possibly other unknown factors, it may be best to utilize another specimen type if the result doesn't match the clinical presentation



Progesterone



Progesterone belongs to the group of steroid hormones called 'progestogens'. Synthetic hormones that have a similar action to progesterone but differ structurally are called 'progestins'. Supplementation with progesterone versus synthetic progestins can result in different physiologic responses and side effects.³²

Progesterone is mainly secreted by the corpus luteum in the ovary and plays an important role in the menstrual cycle and maintaining early pregnancy.³³ Progesterone is also produced in smaller quantities by the ovaries themselves, the adrenal glands, the brain and in the placenta during pregnancy.³⁴

Progesterone Functions:^{2,34,35}

- Reproduction – implantation and maintaining uterine lining
- Menstrual cycle regulation
- Mood
- Cognition
- Neuroprotection
- Myelin generation
- Diuresis through the renin-angiotensin system
- Body temperature
- Smooth muscle relaxation
- Sedative and analgesic effects
- Osteoblast proliferation
- Immunosuppressive effects

Although thought of as a female hormone, progesterone is also produced in the testes and adrenal glands in males, and is involved in testosterone production and sperm function.^{36,37}

The action of progesterone and other hormones are mediated via their receptors.^{34,38} The progesterone receptor (PR) is expressed in the female reproductive tract, mammary gland, brain, pituitary gland, and immune cells.³⁴

Progesterone exhibits an endogenous circadian rhythm depending on menstrual phase.⁵ For information about progesterone's role in the menstrual cycle, visit the section on "[Rhythm Profile and the Menstrual Cycle](#)".

Progesterone Metabolism

Progesterone's circulating half-life in the body is relatively short, at approximately five minutes.² Progesterone circulates in the bloodstream almost entirely bound to plasma proteins; only unbound, free progesterone is biologically active.³³ Genova's serum progesterone assesses total progesterone which includes both bound and unbound progesterone. Approximately 2.4% of circulating progesterone is free. Progesterone binds with low affinity but high capacity to albumin (approximately 79.3%), and a smaller amount is bound with high affinity and low capacity to corticosteroid-binding globulin (approximately 17.7%). Alterations in binding protein concentrations may account for the kinetic variability of progesterone.³⁴

Progesterone has multiple metabolic fates with the liver being the major site for progesterone metabolism. Progesterone can be:

- hydroxylated by cytochrome P450 enzymes and subsequently sulfated and glucuronidated, then excreted in urine or stool^{1,2,34}
- converted into corticosterone via CYP21 which then becomes aldosterone
- converted to the intermediate 17-OH Progesterone via 17-hydroxylase and become various other sex hormones (androgens, cortisol)
- converted to neuroactive allopregnanolone via the enzyme 5α-reductase and 3α-hydroxysteroid dehydrogenase³⁹⁻⁴¹
- conjugated with glutathione and excreted¹



High Levels:

Progesterone Excess Signs & Symptoms^{32,42-49}

Breast tenderness	Depression
Low libido	Irritability
Fatigue	Sleep issues
Sedation/drowsiness	Higher body temperature
Headache	Feeling stressed
Blood sugar dysregulation	

Clinical Associations with High Progesterone

There are no known serious consequences caused by elevated progesterone, however, understanding why progesterone is elevated is important. Elevated progesterone is a consequence of certain conditions, versus a cause. These include:

- Congenital adrenal hyperplasia, along with elevated androgens and lower cortisol levels^{50,51}
- Endometriosis and Infertility
 - (Some infertile women with endometriosis can see progesterone spikes during the follicular phase^{52,53})
- Pregnancy⁵⁴
- Certain types of ovarian cancer^{55,56} and ovarian cysts⁵⁷

Considerations and causes for elevated progesterone:^{6,44,58-64}

- Caffeine
- Smoking
- Vitamin C (supplementation or dietary excess)
- Cow's milk
- Stress
- Pregnancy
- Supplementation with progesterone
 - Transdermal progesterone creams may result in significantly elevated levels with salivary testing.^{65,66}
- Supplementation with high doses of DHEA
 - May result in a falsely elevated progesterone level due to assay interference.
 - (See the following section on "[Progesterone Supplementation and Testing](#)" for more information.)
- Blood contamination of a saliva sample due to gingivitis or bleeding gums.
 - Blood concentrations of steroid hormones are several-fold higher than saliva levels.^{14,17-19,67}
- Up- or downregulation of enzymes within the steroidogenic pathway

- There are many medications, supplements, nutrients, exposures, and lifestyle factors that can influence hormone levels as well as the enzymes required for steroid production. (Refer to the [steroidogenic pathways chart](#) and other hormone levels to assess whether pathways are up or down regulated.)

Therapeutic Options:

- Addressing the above factors may help to optimize progesterone.
- Fiber, exercise, and vitamin D may reduce levels.⁶⁸⁻⁷²

Low Levels:

Progesterone Deficiency Signs & Symptoms^{46,47,73-77}

Breast tenderness	Mood swings (rapidly fluctuating levels)
Fibrocystic breasts	Insomnia
Fatigue	Infertility
Anxiety	Irritability
Depression	Bloating
Irregular menses	Migraine

Low progesterone during the luteal phase may indicate an anovulatory cycle. The literature cites luteal serum progesterone levels of less than 1 ng/mL as a cut point to determine an anovulatory cycle.^{78,79}

Clinical Associations with Low Progesterone:

Luteal phase deficiency (LPD) is a condition of low progesterone during the luteal phase of the menstrual cycle (biochemical LPD), and/or a short luteal phase of less than 10 days (clinical LPD).⁷⁸ The cut point for biochemical LPD is a peak luteal serum progesterone of less than 10 ng/mL.⁷⁹

LPD symptoms and associations:⁷⁸⁻⁸²

- Shorter menstrual cycles
- Lighter menstrual bleeding
- Miscarriage
- Infertility
- Lower serum estradiol (follicular and luteal menstrual phases)
- Hypothalamic amenorrhea
- Anorexia
- Extreme exercise



- Hyperprolactinemia
- Obesity
- Ovulatory women with PCOS
- Endometriosis
- Thyroid disorders

Addressing these conditions may correct a luteal phase defect.

The translocation of lipopolysaccharide (LPS), a bacterial endotoxin, from the gut lumen into the circulation may promote ovarian inflammation and decrease progesterone production resulting in luteal deficiency.⁸³

A common drug prescribed during fertility treatments, clomiphene citrate, is used to normalize progesterone secretion in luteal phase deficiency.⁵³

In addition to LPD, low serum progesterone is associated with:

- epilepsy⁸⁴
- obesity^{85,86}
- endometrial cancer⁸⁷
- ovarian cancer⁸⁸
- chronic fatigue syndrome⁸⁹
- menopause³⁵

“Progesterone steal” or “pregnenolone steal” is a concept often referred to in the functional medicine space, claiming that during stress, hormone precursors will be shunted towards cortisol and away from progesterone, resulting in low progesterone. On the contrary, studies show that progesterone, along with cortisol, increase due to stress.^{6,44,62-64}

There are some primate studies showing elevated cortisol and decreased progesterone and altered follicular and luteal function following a stress challenge.^{90,91} However, human studies are lacking to demonstrate this connection. Studies are needed to determine if there is a change in sex hormone levels over time with chronic stress, which is often what is suggested with progesterone steal.

One theory on why this concept is misunderstood is that low progesterone is associated with anxiety and may predispose patients to feeling stressed more easily. This may in turn result in elevated cortisol levels, giving the impression that cortisol is “stealing” precursors for its production. In general, stress management is important for hormonal homeostasis.

Considerations and causes of low progesterone:^{80,92,93}

- Alcohol intake
- Obesity
- Thyroid disease
- Menopause
- Low cholesterol
- Dietary factors
 - Selenium insufficiency
 - Mediterranean diet, vegetable protein, fiber, and isoflavone intake:

This may seem surprising, since many of these factors are considered healthy. A suggested mechanism may be that decreased levels of microbial B-glucuronidase activity in patients with higher fiber diets may result in decreased deconjugation and less reabsorption of sex hormones in the colon.⁷⁹
- Supplementation with synthetic, non-bioidentical forms of hormones
 - The negative feedback loop from these synthetic hormones causes decreased endogenous production of hormones. Although, certain synthetic formulas cause unpredictable levels of hormones. (See the following section on [“Progesterone Supplementation and Testing”](#) for more information.)
- Up- or downregulation of enzymes within the steroidogenic pathway

There are many medications, supplements, nutrients, exposures, and lifestyle factors that can influence hormone levels as well as the enzymes required for steroid production. (Refer to the [steroidogenic pathways chart](#) and other hormone levels to assess whether pathways are up or down regulated.)

Therapeutic Options:

- Weight loss, reducing alcohol, exercise (not extreme), improving insulin sensitivity, and lowering TSH levels may help to restore progesterone levels.⁹⁴⁻⁹⁷
- Chaste Tree Berry (Vitex) and vitamin C have been shown to improve luteal phase progesterone.^{60,79,98,99}
- In males, low progesterone may result in low testosterone. Check testosterone levels.



Progesterone Supplementation and Testing

Progesterone is commonly prescribed to treat amenorrhea, infertility, endometriosis, fibrocystic breasts, prevention of pregnancy loss and PMS, and is used in combination with estrogen hormone therapy (HT) to prevent estrogen-induced endometrial hyperplasia.^{34,35}

The antiestrogenic effect of progesterone on the endometrium is associated with a suppression of the estrogen receptor, activation of the 17 β -HSD type 2 enzyme that converts E2 to E1, and the estrone sulfotransferase that causes estrone conjugation.¹⁰⁰

In addition to its antiestrogen effects, it also exerts sedative effects. After oral progesterone administration, metabolites may interact with the GABA receptors.¹⁰⁰

In contrast to the endometrium, progesterone can enhance the proliferative effect of estrogens on breast epithelium.¹⁰⁰ Most clinicians aim for luteal progesterone levels for therapeutic benefit and endometrial protection.

Oral Progesterone

The bioavailability of progesterone is impacted by how it is formulated.¹⁰¹ The most common route of progesterone supplementation is oral. A maximum concentration is generally reached within 1-3 hours, with a half-life of approximately 16-18 hours. Less than 5% of orally administered progesterone is bioavailable, as compared to several of the synthetic progestins. Pharmacokinetics of supplementation varies among individuals based on many factors including age, liver and kidney function, route of delivery, and other factors.³⁴

Oral micronized progesterone is available as a standardized FDA-approved product. It tends to be accurately represented in both serum and saliva and has many studies showing safety and efficacy.

Micronization of progesterone increases the half-life and decreases destruction in the GI tract. Absorption of oral micronized progesterone is enhanced twofold when taken with food.⁴² Salivary and serum progesterone levels correlate and peak simultaneously after oral micronized progesterone administration.¹⁰²

Transdermal Progesterone

Transdermal delivery via topical creams or gels tends to result in very high, supraphysiological salivary progesterone levels. Serum levels do not change significantly with transdermal delivery.¹⁰³ However, even if serum levels don't rise, it has been shown that antiproliferative effects on the endometrium are still achieved.^{34,66}

A widely held assumption is that serum levels must be at luteal levels, higher than 5 ng/mL in order to inhibit estrogen-stimulated endometrial proliferation.¹⁰³ If serum is used for monitoring, it may result in overdosing of progesterone and if saliva is used for monitoring, it may result in a dose reduction.¹⁰³

Progesterone is very lipophilic and along with lipophilic ingredients of creams, may saturate the fatty layer below the dermis.¹⁰⁴ Red blood cells passing through the capillaries may play a role in transporting progesterone to salivary glands and other tissues, however this theory is debated.^{65,104} Progesterone may also be carried through lymph vessels to target tissues and other tissues including salivary glands.¹⁰³ The accumulation of topical progesterone in tissues in the absence of a change in serum levels suggests that salivary levels are more clinically relevant than serum in use of topical progesterone cream.^{101,105}

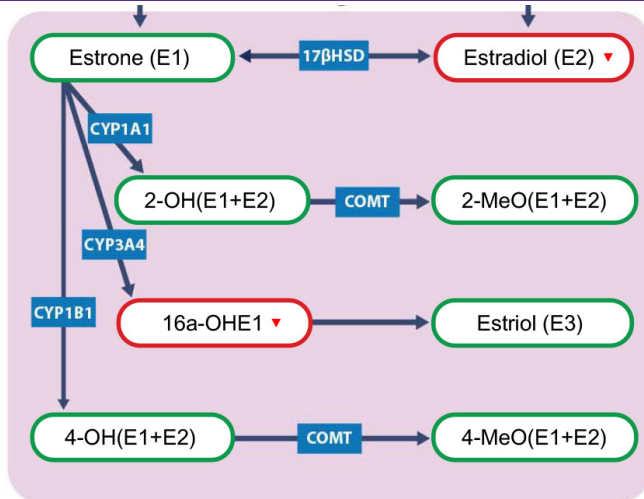
Topical progesterone creams are only available through custom compounded methods versus regulated products and may vary substantially from batch to batch. There is a paucity of literature assessing hormone levels of patients taking compounded formulations, and no known studies assessing long-term safety.^{101,105} A study using compounded progesterone cream or gel with verified doses showed wide interindividual variability which may be attributed to skin absorption kinetics, bathing habits, and differences in subcutaneous fat affecting the rate of progesterone absorption and clearance.¹⁰³

Being that progesterone cream is available via compounding and OTC only – there are no FDA-approved formulations, and thus no standardization or studies that support an optimal sample type for monitoring.

A study comparing oral progesterone to an over-the-counter (OTC) topical progesterone showed similar serum levels when a smaller dosage of cream was used.¹⁰⁶ This suggests that oral dosing and transdermal dosing are not equal and should be taken into consideration when prescribing. This study also shows the potency of unregulated OTC products.

More information can be found in the section entitled [“Hormone Therapy and Testing Impact”](#) at the end of this guide.

Estrogens



Human estrogens include estrone (E1), estradiol (E2) (or 17β-estradiol), and estriol (E3).

- **Estrone (E1):** During menopause, a dramatic drop in E2 production leaves E1 as the predominant circulating estrogen.
 - E1 may have a pro-inflammatory role.^{22,107,108}
 - E1 is a weak estrogen with only 4% of the estrogenic activity of E2.¹⁰⁰
 - E1 can interconvert to E2 via the 17β-HSD type 1 enzyme, thus increasing its estrogenic potency.¹⁰⁰
 - E1 is also produced by adrenal androstenedione via aromatization.¹⁰⁸
 - Unlike E2 and E3, E1 does not accumulate in target tissues.¹⁰⁰
 - The most abundant circulating estrogen is estrone sulfate (E1S), at about 10-fold higher than unconjugated estrone.¹⁰⁹ E1S serves as a biologically inactive reservoir for the generation of active estrogens in target tissues.¹
- **Estradiol (E2):** E2 is the most biologically active form of estrogen and is predominant in nonpregnant women prior to menopause.^{22,107}
 - It is thought to have more anti-inflammatory actions, compared to E1.¹⁰⁸
 - Estradiol exhibits an endogenous circadian rhythm depending on menstrual phase.⁵ For information about estradiol's role in the menstrual cycle, visit the section on ["Rhythm Profile and The Menstrual Cycle"](#).

- **Estriol (E3):** E3 predominates during pregnancy.^{22,107}

- E3 acts as a weak estrogen in most tissues, but is more estrogenic in the vaginal epithelium.¹⁰⁰
- E3 is the least potent of the three estrogens and is formed through 16α-hydroxylation of E1 and E2 in the liver.
- E3 can be reconverted to 16α-OHE1 or conjugated and excreted.^{1,100,110}
- E3 is rapidly excreted in urine, and serum and saliva levels are low to undetectable.¹

While estrogen is thought of as a female hormone, both males and females synthesize estrogen, but in varying amounts. Estrogens are produced in the growing ovarian follicles and corpus luteum, the placenta, adrenals, Leydig cells, liver, endometrium, brain, muscle, and adipose tissue.¹⁰⁰ Abdominal white adipose tissue is the major site for estrogen production in the postmenopausal female.^{111,112}



Estrogen Functions:^{107,113}

- Stimulates growth, blood flow, and water retention in sexual organs
- Increases lipoprotein receptors resulting in decreased LDL cholesterol
- Promotes glucose homeostasis
- Increases turgor and collagen production in skin
- Inhibits the function of osteoclasts
- Neuroprotection and vasoprotective effects

Estradiol concentration is associated with cognition, mood, and memory in women.⁶ Estrogen increases the potential for coagulation.¹⁰⁷ Estrogens are known to play a causal role in breast, endometrial, and ovarian cancers.^{4,21}

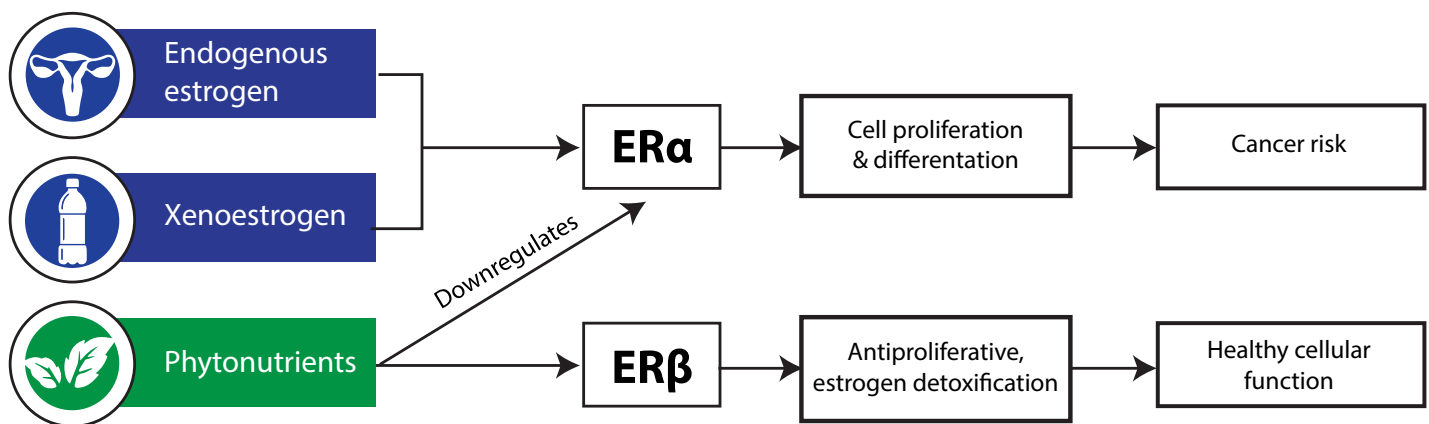
In males, estrogens play a role in healthy sperm function.¹¹⁰ E2 is important for normal development and function of reproductive and nonreproductive organs. The testes produce approximately 20% of circulating estrogens with the remainder produced by adipose, brain, skin, and bone. Androgens have significant effects on male bone, however estrogens are more important for bone growth and maintenance.¹¹⁴ E2 and testosterone are important for the preservation of memory and cognitive function in men.⁶ Estrogens are known to play a causal role in testicular and prostate cancers.^{4,21}

Estrogen Circulation and Receptor Binding

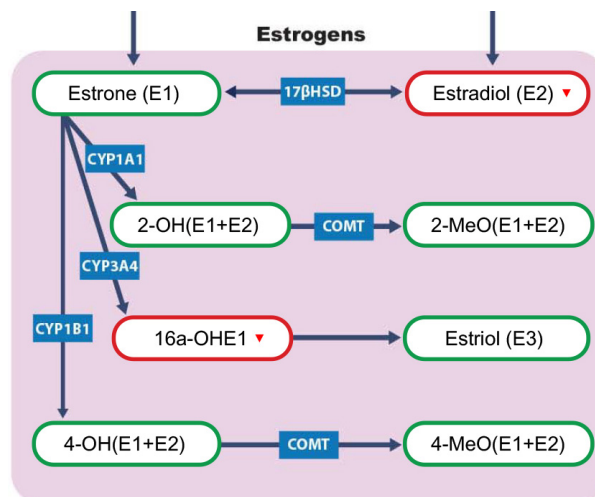
Estrogen circulates in the bloodstream almost entirely bound to plasma proteins; only unbound estrogen is biologically active and able to diffuse into target tissues to exert its specific effect.^{33,107} Circulating estradiol is mostly bound to SHBG, with about 30% loosely bound to albumin and about 1-3% unbound and free.^{107,112} Serum estrogen represents total estrogen including both bound and unbound levels.¹⁰⁷ The most abundant circulating estrogen is the sulfate conjugate of estrone, at about 10-fold higher than unconjugated estrone.¹⁰⁹

The action of estrogen and other hormones are mediated via their receptors.^{34,38} Estrogen receptors (ER), ER α and ER β are expressed in different tissues and the various estrogens differ in their binding affinity to each receptor. The hormonal potency of the various estrogens is not only determined by their interactions with ERs, but also by the concentration of the estrogen.¹⁰⁰ Estrogen receptors are also influenced by xenoestrogens and phytonutrients.

Estrogens & Estrogen Receptor Sensitivity



Aromatase is a cytochrome P450 enzyme (CYP19) responsible for the biosynthesis of estradiol and estrone from androgens.¹¹¹ Aromatase is expressed in the brain, ovary, testes, blood vessels, liver, bone, skin, adipose and endometrium.¹¹⁰ Aromatase levels increase as a function of age and obesity, with levels correlating with an increasing BMI in postmenopausal women and men.^{87,115}

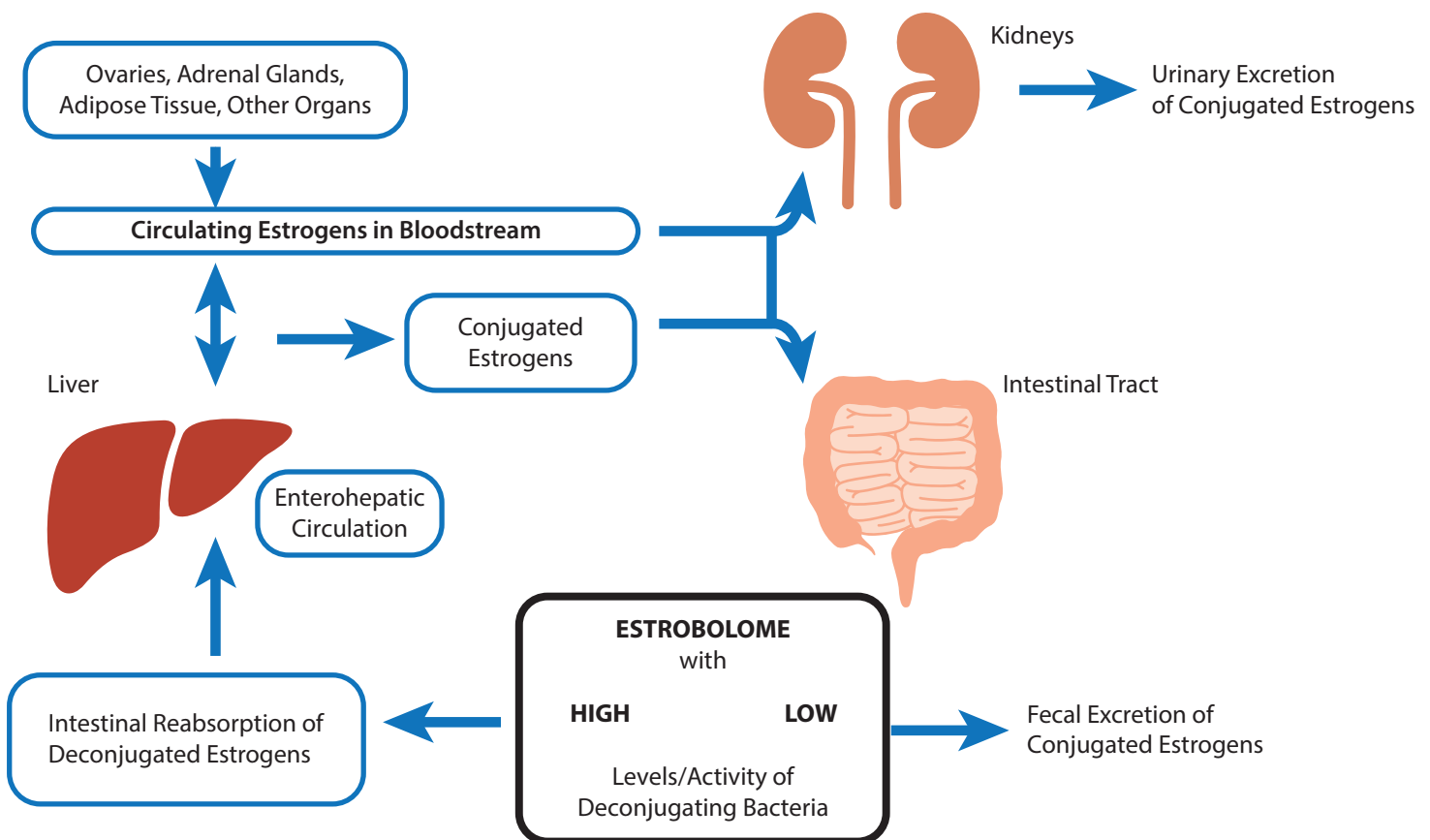


In **phase 2 metabolism**, estrogens and metabolites need to be conjugated to become water-soluble for safe excretion. This process of conjugating and detoxifying estrogen occurs via sulfation, glucuronidation, methylation, or glutathione conjugation.^{21,116} Conjugated estrogens are not ligands for estrogen receptors and do not promote estrogen receptor-mediated activity.¹⁰⁹ The following graphic shows pathways of estrogen conjugation:¹⁰⁹



- **Methylation:** Estrogen methylation is catalyzed by the enzyme catechol-O-methyltransferase (COMT) and represents the major detoxification pathway for estrogen. COMT genetic polymorphisms can impair COMT function, slowing down methylation capability and increasing risk of circulating phase 1 intermediates.¹⁰⁹
- **Sulfation:** The most abundant circulating estrogen conjugates are the sulfates, followed by the glucuronides.¹⁰⁹ Estrogen sulfates are inactive and are catalyzed by the estrogen sulfotransferase (SULT) enzyme.^{114,117} Estrogen sulfates can be desulfated in the tissues and converted back to active estrone and estradiol.¹⁰⁹ The gut microbiome also contains sulfatases capable of reactivating hormones for potential reabsorption and systemic recirculation.¹¹⁸ Polychlorinated biphenyls (PCBs), phthalates, and parabens are potent inhibitors of the human SULT enzyme, which inhibits sulfate conjugation thus enhancing endogenous estrogen activity.^{117,119,120}
- **Glutathione conjugation:** Glutathione S-transferase P1 is the enzyme involved in detoxifying estrogen quinones. Glutathione conjugation is important because estrogen quinones can form DNA adducts which can result in damage and may lead to the initiation of cancer.¹²²
- **Glucuronidation:** Enzymes of the UGT-glucuronosyltransferase superfamily (UGTs) are expressed in the liver and extrahepatic tissues and couple glucuronic acid with a steroid hydroxy group.¹ Estrogen glucuronide conjugates are readily excreted in both urine and bile.¹⁰⁹ Once excreted into the bile and GI tract, the microbial-produced enzyme beta-glucuronidase can deconjugate the estrogen-glucuronide, resulting in free estrogen and potential reabsorption and systemic recirculation.¹¹⁸ Increased β -glucuronidase activity has been reported in high fat and protein diets and is decreased with fiber consumption. Some studies have shown that increased microbial diversity is associated with healthier estrogen metabolism. The intestinal bacterial microbiome related to estrogen metabolism is collectively called the 'estrobolome' and is illustrated in the figure below.¹²¹

- The [GI Effects Comprehensive stool profile](#) assesses beta-glucuronidase levels and can be helpful to determine if elevated estrogen could be a result of microbiome reactivation.





High Levels:

Estrogen Excess Signs & Symptoms^{48,75,76,113,123-134}

Mood swings/PMS	Fibrocystic breasts
Breast tenderness/ swelling	Uterine fibroids
Water retention	Weight gain
Nervous/ irritable/anxious	Vaginal yeast infections
Irregular menses	Cold body temperature
Heavy periods	Gynecomastia (males)
Sleep disturbances	Benign prostatic hyperplasia (males)
Migraine (fluctuating levels)	Erectile dysfunction (males)
Headaches	

Clinical Associations with High Estrogen^{87,108,112-114,116,135-142}

- Liver disease and cholestasis
- Hyperthyroidism
- PCOS
- Endometriosis
- Multiple sclerosis
- Ovarian, endometrial, breast, prostate, and other cancers
- Systemic lupus erythematosus
- Obesity
- Schizophrenia

There appears to be a bidirectional role between elevated estradiol and elevated bile acids or cholestasis, although the mechanisms are still being studied. It is thought that the downregulation of sulfotransferases results in increased estradiol as well as bile acids. Elevated estradiol can also lead to decreased bile acid flow and toxic bile accumulation leading to cholestasis. Excess estrogens can induce oxidative stress and pro-inflammatory cytokine expression in the liver.^{143,144} Glucuronides of estradiol formed during glucuronidation in the liver are also known to induce cholestasis.¹⁰⁹

PCOS is a condition characterized by excess androgens, however, androgens are converted to estrogen in peripheral tissues which can lead to endometrial hyperplasia and cancer.¹³⁰ In PCOS, E2 tends to be normal in serum, however may be elevated in saliva since saliva represents unbound E2. Elevated androgens decrease SHBG allowing for higher free androgen and E2 levels.¹³

Endometriosis and other conditions are associated with increased estrogen or estrogen dominance, with increased production in local tissues, but often normal circulating levels.¹⁴⁵

In men, higher serum E2 levels were seen with coronary disease and sudden cardiac arrest.¹¹⁴

Considerations and causes of elevated estrogen:^{14,60,61,67,87,93,113,117,119-121,137,140,146-149}

- Obesity/adiposity
- Alcohol intake
- Sedentary lifestyle
- Aromatase enzyme upregulation
- Bioidentical hormone replacement therapy:
 - It is important to consider dose, metabolic pathways, and other factors that influence estrogen levels. See the following section on "[Estrogen Supplementation and Testing](#)" for more information.
- Downregulation of estrogen detoxification pathways
- Upregulation of fecal β -glucuronidase causing increased enterohepatic circulation
- Exposure to endocrine disrupting environmental chemicals: PCBs, phthalates, parabens, and others
- Medications that are metabolized using the CYP450 enzymes in the liver may inhibit the metabolism of estrogen causing higher levels.^{113,150,151}
- Nutritional factors:
 - Vitamin C
 - Cow's milk
 - High fat/low fiber Western diets: This may be due to higher bacterial fecal β -glucuronidase levels associated with higher fat and protein diets whereas fiber consumption decreases its activity.^{152,153}
- Blood contamination of salivary sample due to gingivitis or bleeding gums
- Up- or downregulation of enzymes within the steroidogenic pathway:
 - There are many medications, supplements, nutrients, exposures, and lifestyle factors that can influence hormone levels as well as the enzymes required for steroid production. (Refer to the [steroidogenic pathways chart](#) and other hormone levels to assess whether pathways are up or down regulated.)



Therapeutic Options:

- Fiber, calorie reduction, weight management, exercise, alcohol reduction, and vitamin D may reduce estrogen levels and decrease breast cancer risk.^{68-72,87,112,137,140,154}
- Consider checking SHBG and thyroid hormone levels: Lower SHBG may be associated with increased estrogen as well as testosterone.¹⁴⁰ There are many factors that influence SHBG levels including thyroid conditions. In hypothyroidism, SHBG is lower, causing a reduction in total circulating hormones, but an increase in the free fraction. The opposite is true with hyperthyroidism. Both hypo- and hyperthyroidism can result in a reduction of the rate of metabolic clearance of sex steroids.¹⁵⁵
- Physical activity may increase SHBG levels, which helps to bind excess E2.¹¹²
- Evaluate for, and support, genetic single nucleotide polymorphisms (SNPs) of enzymes within the estrogen detoxification pathways:
 - The enzyme Catechol-O-methyltransferase (COMT) is one of the enzymes that helps detoxify estrogen. SNPs may slow this enzyme down resulting in higher estrogen levels in patients on estrogen therapy.¹⁵⁶
 - Methylation support in the form of B vitamins, magnesium, and trimethylglycine (TMG) may be beneficial to support COMT and estrogen detoxification.^{157,158}

Nutritional support of the beneficial pathways of estrogen detoxification can help normalize estrogen balance. See the chart below:¹⁵⁹

Detox Strategy	Nutrients
Promote 2-hydroxylation over 4- and 16 α -hydroxylation of estrogens	Cruciferous vegetables, I3C, DIM, xanthohumol, rosemary, isoflavones (soy, kudzu, clover)
Reduce oxidation of catechol estrogens (2-OH and 4-OH)	Vitamins A, E, C, NAC, SOD, turmeric, green tea, lycopene, α -lipoic acid, flavonoids
Promote methylation of catechol estrogens (2-OH and 4-OH)	Folate, vitamins B2, B6, B12, TMG, magnesium
Increase SHBG to reduce unbound active estrogen	Fiber, lignans (flax), isoflavones (soy, kudzu, clover)
Inhibit aromatase, reducing androgen conversion to estrogens	Lignans (flax), flavonoids (chrysin)
Upregulate Phase I and II detoxification of estrogens	Turmeric/curcumin, milk thistle, D-limonene, magnesium, vitamins B2, B6, B12, flavonoids
Inhibit or lower fecal β -glucuronidase levels to prevent intestinal estrogen reabsorption	Fiber, probiotics, calcium D-glucarate, milk thistle
Modify estrogen receptor activity	Isoflavones (soy, kudzu), lignans (flax), I3C, DIM, xanthohumol, resveratrol



Low Levels:

Estrogen Deficiency Signs & Symptoms^{43,46,110,123-125,160-168}

Hot flashes	Depression
Night Sweats	Insomnia
Vaginal dryness/atrophy	Heart palpitations
Cognitive decline	Bone loss
Irregular menses	Alopecia
Incontinence	Migraine (fluctuations from higher to lower E)
Low libido	Anxiety
Arthralgia	Infertility
Urinary/vaginal infections	Irritability/mood swings
Weight gain (waist)	

Clinical Associations with Low Estrogen^{147,155,169-172}

- Menopause
- Primary ovarian insufficiency (i.e., premature ovarian failure, hypergonadotropic hypogonadism)
- Secondary hypogonadism (i.e., hypogonadotropic hypogonadism)
- Kallmann syndrome
- Thyroid disorders
- Severely underweight
- Stress
- Obesity
- Pituitary dysfunction

(LH, FSH, and prolactin levels can help distinguish primary versus secondary hypogonadism.)

While luteal phase deficiency (LPD) is often attributed to low progesterone, both follicular and luteal serum E2 concentrations tend to be significantly lower in women with LPD. Associated symptoms include shorter menstrual cycles, lighter menstrual bleeding, miscarriage, and infertility.⁷⁸

Reduction of endogenous estrogen levels increases risk of bone fracture, cardiovascular disease, and Alzheimer's disease in postmenopausal women.¹¹⁰ Epidemiological studies have shown that the development of atherosclerosis is potentiated by a long-term estrogen deficiency.¹⁰⁰

In men, low E2 and testosterone is associated with osteoporosis, although E2 levels show a stronger correlation.^{114,173}

Considerations and causes of low estrogen:^{110,113,115,147,172,174-178}

- Advancing age
- Menopause
 - Serum E2 levels decrease by 85%-90% and serum E1 by 65-75% from premenopausal levels.
 - Estrogen levels begin to drop in the first 5 years following menopause, then decline steeply beyond 5 years.
- Hysterectomy
- Aromatase inhibitors
- Analgesic medication and NSAID use
- Supplementation with synthetic, non-bioidentical forms of hormones
 - The negative feedback loop from these synthetic hormones causes decreased endogenous production of hormones. See the following section on "[Estrogen Supplementation and Testing](#)" for more information.
- Low fat, high fiber diets
 - Vegetarians and Asian immigrants eating low fat, high fiber diets tend to have lower estrogen levels. One study showed higher estrogen excretion in stool and lower urinary excretion with higher fiber diets, demonstrating its influence of enterohepatic circulation on plasma estrogen levels.¹⁷⁹
- Excessive exercise
- Severely underweight
- Stress
- Thyroid disorders
- Smoking
- Pituitary dysfunction
- Up- or downregulation of enzymes within the steroidogenic pathway
 - There are many medications, supplements, nutrients, exposures, and lifestyle factors that can influence hormone levels as well as the enzymes required for steroid production. (Refer to the [steroidogenic pathways chart](#) and other hormone levels to assess whether pathways are up or down regulated.)

******Regarding estriol, serum and saliva levels of E3 tend to be low to undetectable as it is rapidly excreted in urine. It is therefore relatively abundant in urine compared to other urinary metabolites.^{1,23}



Therapeutic Considerations:

- Investigate and address any lifestyle factors, medications, or supplements that may be contributing.
- Evaluate thyroid, hypothalamic-pituitary-adrenal axis (HPA axis), and pituitary status.
- Vitamin C may help with increasing estradiol as well as progesterone levels.⁶⁰
- Botanical and herbal supplementation for symptom relief such as soy/isoflavones, black cohosh, maca, red clover, dong quai, wild yam, and others.^{180,181}
- Supplementation with hormone replacement therapy (See next section regarding supplementation and testing)

Estrogen Supplementation and Testing

There are many forms of estrogen supplementation that have varying impacts on testing. Each form has its own pharmacokinetics and bioavailability making it challenging to understand the exact effects on lab testing.

- **Buccal:** The oral mucosa is highly vascularized, resulting in rapidly absorbed estradiol that enters directly into the circulation.¹⁰⁰
- **Oral:** Serum levels of estradiol are 10-fold higher after oral estradiol and the bioavailability is approximately 5 times higher.¹⁰⁰ Concurrent alcohol ingestion with oral estradiol causes a threefold rise in serum E2 levels, by slowing estrogen metabolism.¹⁴⁶
- **Vaginal:** After vaginal cream, the serum concentrations are 10 to 20-fold higher than that of the same dose of oral estradiol. However, very low doses vaginally result in a transient smaller increase in serum levels that returns to baseline at 24 hours.¹⁰⁰
- **Transdermal:** Transdermal estradiol can result in large intra- and interindividual fluctuations in serum levels.¹⁰⁰ Transdermal estrogen leads to a higher estradiol/estrone ratio, whereas oral estrogens lead to a lower estradiol/estrone ratio.^{182,183} Alcohol ingestion may decrease E2 clearance after removal of transdermal E2 patches, thus prolonging the half-life of circulating estradiol.¹⁸⁴
- **Pellets:** E2 pellets are associated with relatively small fluctuations during the months after implantation.¹⁰⁰

The following table illustrates the changes in serum estradiol and estrone after supplementing.¹⁰⁰

Table 6 Ratio between the serum concentrations of estrone and estradiol in premenopausal and postmenopausal women as well as in postmenopausal patients treated with estradiol, depending on the route of administration. The values do not reflect the efficacy or tolerability of the therapies

	Estrone : Estradiol ratio
Premenopause	1 : 2
Postmenopause	2 : 1
Oral estrone sulfate	5 : 1
Oral estradiol	5 : 1
Transdermal estradiol (patch)	1 : 1
Transdermal estradiol (gel)	1 : 1
Intranasal estradiol	2 : 1
Sublingual estradiol	1 : 3
Vaginal estradiol	1 : 5
Subcutaneous estradiol (implant)	1 : 1.5
Intramuscular estradiol	1 : 2

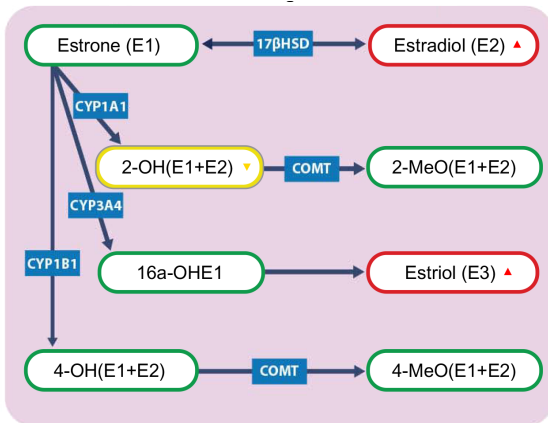
Estrogen administration causes an increase in serum thyroxine-binding globulin and may affect thyroid hormone levels; it is important to assess thyroid function in patients taking hormone therapy, especially estrogen.¹⁸⁵

When supplementing estrogen, it is important to consider progesterone supplementation if the patient still has a uterus. Estrogen primes the endometrial glands, and progesterone controls this by decreasing the number of estrogen receptors, thus preventing endometrial cancer.⁴²

More information can be found in the section entitled [“Hormone Therapy and Testing Impact”](#) at the end of this guide.



Essential Estrogens - Urine Estrogens and Metabolites



Genova's urinary Essential Estrogens and Endo+ profiles measure parent estrogens and metabolites. These measurements are used to assess hormonally mediated disease risk and to monitor therapies that reduce risk.

Estrogen detoxification takes place in the liver.
Detoxification metabolites are then excreted in stool or urine.

Phase 1 involves the CYP450 enzymes which hydroxylate estrogen into an active or potentially toxic metabolite.^{21,142,186}

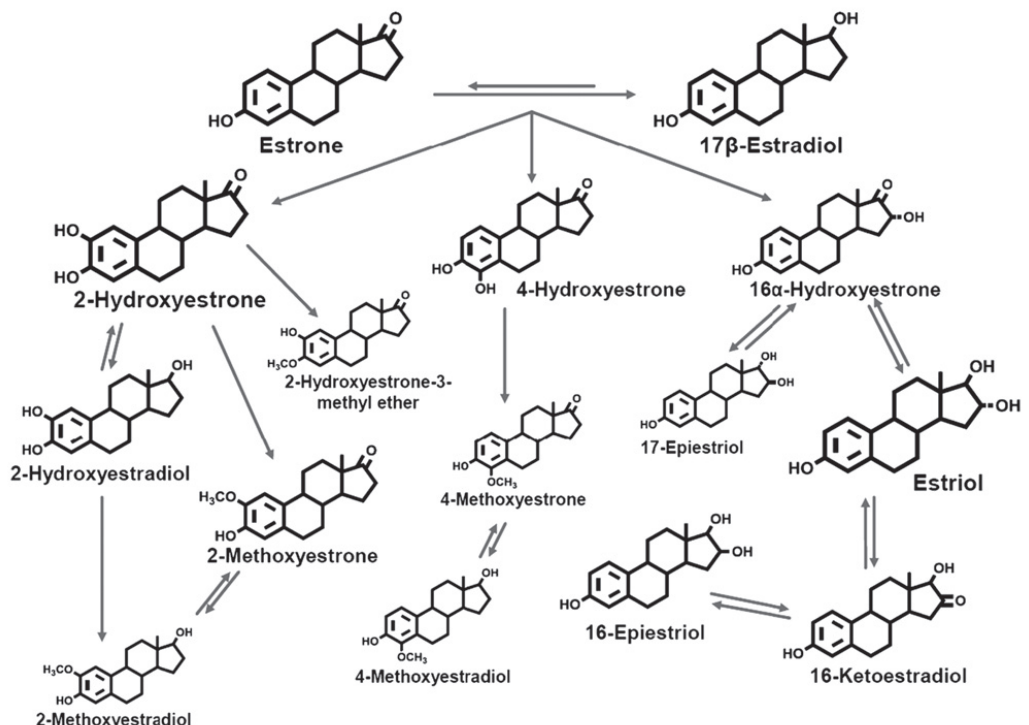
- If phase 1 hydroxylation occurs at estrogen's carbon position C2, the enzyme CYP1A1 acts and irreversibly yields the metabolites 2-hydroxyestrone and 2-hydroxyestradiol (2-OH-E1+E2), also called catechol estrogens.
- If the hydroxylation occurs at carbon position C16, the enzyme CYP3A4 works to irreversibly yield the metabolite 16-hydroxyestrone.

- If the hydroxylation occurs at carbon position C4, the CYP1B1 enzyme creates the metabolites 4-hydroxyestrone and 4-hydroxyestradiol (4-OH-E1+E2), also called catechol estrogens.

Phase 2 requires the active phase 1 estrogen intermediates to be conjugated with a molecule to make them water-soluble for excretion – (glucuronidation, glutathione conjugation, sulfation, and methylation.) 2-methylestrogens are the major metabolite of estrone and estradiol in the urine, thus methylation using the COMT enzyme represents a major pathway for estrogen detoxification.¹⁰⁹

**Certain metabolites may be harmful or protective and therefore are important for assessing risk.*

The figure at right, taken from Sampson et. al., is another depiction that shows unidirectional and irreversible conversions to metabolites shown by a single arrow, while double arrows indicate the ability of the metabolites to interconvert. Sizes of the chemical structures are based on the relative abundance of the estrogen or metabolite.¹⁸⁷





FMV Versus 24-hour Collection

While some studies assess urinary hormone levels over 24 hours, a first morning void (FMV) spot urine is sufficient to determine estrogen metabolism since there is not a lot of diurnal variability. First morning urine specimens are more consistently seen in recent studies on urinary estrogens and their metabolites, although these studies include unsupplemented individuals. A 24-hour sample may be preferred in patients taking hormone therapies, to account for peak and trough levels seen with supplementation, although data is lacking to support this. Since both the 24-hour and FMV profiles are ratioed to creatinine, and urinary assessment is best studied for metabolism and ratios versus absolute levels, a FMV sample may be appropriate in patients utilizing hormone therapies. It is unclear how hormone therapies influence estrogen metabolism pathways.

Important Interpretation Considerations for Urine Hormones

Methodology: Since the early studies on estrogen metabolism using tissue and cell culture and less sensitive/specific methodologies, there has been much debate about the clinical utility of measuring circulating metabolites due to conflicting findings in the literature.^{22,116,188}

More recently, there have been larger epidemiologic studies that help to solidify earlier theories that certain estrogen metabolites may be associated with risk.¹⁸⁷ When reviewing the literature, Genova has considered methodology (EIA, LC-MS/MS, etc.), conjugated versus unconjugated estrogens and metabolites, sample type (urine versus serum), menopausal status (pre- versus postmenopausal), cycle phase, and has followed the studies that more closely reflect the current Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS) platform.

Estrogen and metabolites are measured using LC-MS/MS, a widely accepted and preferred methodology in the literature for hormone testing, often regarded by some as the gold standard.^{4,21,22,116,142} Assessing studies that are similar to Genova's urinary estrogen assessment is important for meaningful clinical associations. Research is ongoing and Genova will continue to monitor the literature.

- Genova's methodology assesses the total of the conjugated and unconjugated forms of parent estrogens and metabolites. A specific process is applied using sulfatases and glucuronidases to liberate the estrogens

and metabolites for assessment. Urine represents unbound estrogen levels as compared to blood which has both free circulating estrogens as well as protein-bound estrogens. A higher concentration of estrogen metabolites can be measured in urine versus blood.¹⁸⁹

Comparing Circulating with Urine Levels:

Urinary levels can be influenced by metabolism. Urine is a filtrate of circulating blood levels and is therefore thought to correlate with blood. However, it is helpful to measure both sample types to assess potential metabolism issues. For example, is a low urinary estrogen or estrogen metabolite level due to low serum circulating levels, or low urinary excretion and liver metabolism/conjugation? Having both sample types offers a deeper layer of insight.

Population Studied and Menopausal Status:

Literature on urine hormones and their metabolites as it relates to cancer risk is at times somewhat contradictory. The reason for this appears to be the population group studied. For example, two landmark studies on urine hormone metabolism and breast cancer risk were the US-based Nurses' Health Study II and the Shanghai Women's Health Study.^{23,190}

The Nurses' Health Study II found that high urinary excretion of estrogen decreased breast cancer risk. This study was in a premenopausal – luteal phase population. The Shanghai Women's Health Study found the opposite to be true, that lower urinary levels are preferred. This study was in postmenopausal females with high circulating levels. Menopausal status can change interpretation as per the literature.

There can also be actual differences in how quickly or efficiently certain cultural populations metabolize parent estrogens.¹⁹¹

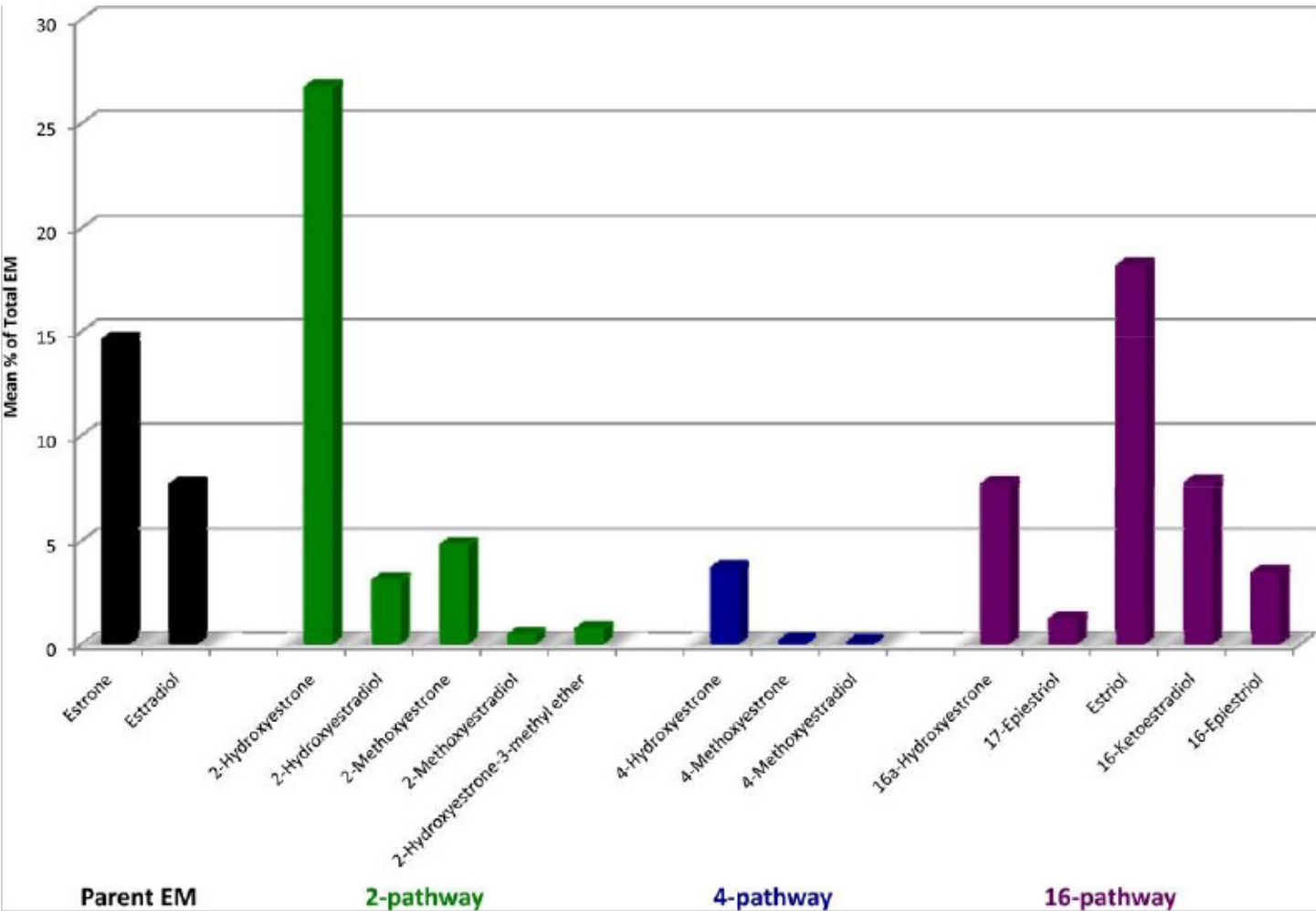
Furthermore, consider the diet and culture of the population being studied. For example, literature shows that those with higher fiber diets excrete more estrogen in stool and therefore have lower urinary excretion, likely due to less enterohepatic recirculation and healthy estrobolome.^{152,179,192} Another example is that Asian cultures generally consume lower fat, higher fiber diets, also high in soy isoflavones and have lower estrogen levels overall.¹⁹³

**In summary, patient menopausal status must be taken into consideration when interpreting urinary hormone results. Results should also be viewed in context of the patient's cultural background and/or diet, and other metabolites, as well as circulating levels assessed in blood.*



Relative Metabolite Abundance:

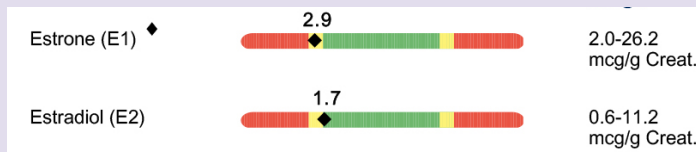
Undetectable or low levels of some metabolites can be common in a healthy population. The figure below, from Eliassen et. al., is showing the percentages of total of estrogens and metabolites among a control group of premenopausal females.²³ These proportions are similar to healthy postmenopausal women and controls, although the percentages of certain metabolites do vary.^{191,194} These proportions are consistent with Genova’s healthy cohort reference ranges.





Urinary Parent Estrogens

Urinary Estrone (E1), Estradiol (E2)



Parent estrogens (E1, E2) are measured in urine. (E3 is discussed with 16 α -hydroxyestrone, since it is a direct metabolite, however, it is also regularly grouped with the parent estrogens.) The clinical utility of urinary parent estrogens is still being elucidated.

High Levels:

Considerations and causes of High Urinary Estrogen:

23,195,196

- Elevated circulating serum estrogens
 - In general, elevated circulating estrogens are associated with multiple diseases and symptoms – see section on [elevated estrogens](#).
 - A larger analysis using pooled data across 4 postmenopausal breast cancer case-control studies showed that higher total estrogen levels (summing all parent estrogens and estrogen metabolites) was strongly associated with breast cancer risk.¹⁸⁷
- Increased estrogen metabolism and excretion
 - Excretion of detoxified estrogen is the goal.
 - According to the US-based Nurses' Health Study II data Increased urinary excretion of parent estrogens can reflect lower overall cancer risk (Premenopausal women – luteal phase).
- Environmental chemicals
 - Higher urinary E1 and E2 may be associated with higher bisphenol-A (BPA) exposure in men and women.
- Alcohol intake
 - Higher urinary E2 was positively associated with premenopausal women who drank alcohol which is consistent with plasma E2 studies.

Therapeutic Options:

- Lifestyle modification to promote healthy detoxification pathways ([see nutrient chart in the high estrogen section](#))
- Optimize the gut microbiome to prevent beta-glucuronidase from recirculating estrogen via enterohepatic circulation and promote excretion of detoxified estrogen in stool.
 - Consider [GI Effects Comprehensive Stool Profile](#)
 - Estrogen metabolism may be improved, and breast cancer risk reduced with high intestinal microbial diversity.¹⁹⁷
- Avoidance of toxins and alcohol
- [Refer to the Estrogen section for strategies to lower overall circulating estrogen](#)

Low Levels:

Considerations and causes of Low Urinary Estrogen:^{121,176,191,193,197-200}

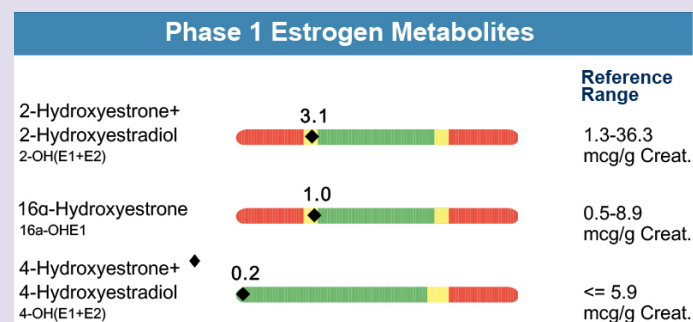
- Low circulating serum estrogen
 - The Shanghai Women's Health Study found that lower urinary parent estrogen levels were associated with decreased breast cancer risk (Postmenopausal women)
- Decreased estrogen metabolism and excretion
- Optimized gut microbiome promoting elimination of detoxified estrogen in stool
- Physical activity
- Green tea
- Soy isoflavones
- Smoking
- Aromatase inhibitors
- Antibiotics

Therapeutic Options:

- Refer to the Estrogen section for considerations regarding [low circulating serum estrogen](#)
- Lifestyle modifications to promote healthy liver detoxification pathways
 - Check glutathione and antioxidant status
- Avoid toxins and smoking

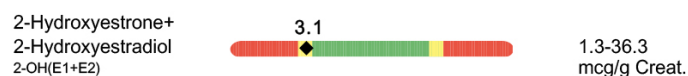
Urinary Estrogen Metabolites

Estrogen Phase 1 CYP Metabolism – Phase 1 Estrogen Metabolites



In phase 1 detoxification, parent estrogens are hydroxylated using CYP enzymes via three major pathways including 2-, 16α- and 4-hydroxylation of estradiol and estrone. These metabolites have earned the names “the good, the bad, and the ugly”, respectively, in reference to their relative risks.

2-hydroxyestrone and 2-hydroxyestradiol (2-OH E1+E2)



During phase 1 liver detoxification, if the hydroxylation of estrogen occurs at carbon position C2, the enzyme CYP1A1 acts and irreversibly yields the metabolites 2-hydroxyestrone and 2-hydroxyestradiol (2-OH-E1+E2). After phase 2 detoxification, these can be excreted in urine and stool.

2-OHE1 may range from only mildly estrogenic to antiestrogenic and is considered the least risky and possibly protective of the three phase 1 metabolites.^{22,116}

- However, both the 2- and 4-OHE can be oxidized to form quinones or semiquinones. The 2-OH quinones form stable DNA adducts, whereas the 4-OH quinones form depurinating adducts and mutations leading to tumorigenesis. These quinones can be safely detoxified by conjugation with glutathione via the enzyme glutathione S-transferase to decrease in DNA damage.^{109,186}

Cancer Risk:

Of the 3 major potential detoxification pathways, 2-hydroxyestrogens made via the CYP1A1 enzyme confer the least amount of cancer risk. Several studies bear this out in postmenopausal women with other studies confirming the same in premenopausal females though with less statistical significance.^{23,187,191} That being said, the next step of phase 2 detoxification and methylation is needed for protection from tumor formation. Methylation via the COMT enzyme converts 2-hydroxyestrogens into a protective, inert form.^{109,201}

As it relates to men, metabolites 2-OHE2 and 4-OHE2 tend to be more potent than E2 in inducing proliferation of a prostate cancer cell line.¹¹⁴ However, a clinical study shows a protective effect of a higher 2OHE1 and higher 2/16 ratio.²⁰²

Osteoporosis Risk:

While higher 2-hydroxyestrogens relative to other metabolic pathways are considered favorable regarding cancer risk, there may be risk associated with osteoporosis if too elevated.²⁰³ Studies suggest that women with predominant metabolism through the 2-hydroxyl pathway have accelerated postmenopausal bone loss and lower bone mineral density compared to those with predominant 16α-hydroxylation who appear to have reduced risk of bone loss. Preventative strategies for osteoporosis may be considered when this metabolite is elevated.

Oxidative Stress and Catecholamines:

Catechol estrogens undergoing phase 1 detoxification are considered active and potentially toxic until stabilized and made inert in phase 2. These catechol estrogens after phase 1, including 2-hydroxy estrogens, can enter into redox cycling and become a source of reactive oxygen species.¹⁰⁹ Specific antioxidants such as N-acetylcysteine and resveratrol can block oxidation of catechol estrogens to their quinones.²⁰⁴ Glutathione status and oxidative stress may be important to assess.

This highly active phase 1 metabolites, 2-hydroxyestrone is a highly efficient competitive inhibitor of COMT, interfering with the degradation of catecholamines, and resulting in prolonged catecholamine activity.²⁰⁵ Therefore, stress management strategies to reduce catecholamine production may be important.



High Levels:

Considerations and causes of high 2-hydroxyestrogens:

23,187,191,203,206-208

- Genetic SNP in the enzyme CYP1A1 results in upregulation and higher levels of 2-hydroxyestrogens
- COMT SNPs result in downregulation of the enzyme and decreased clearance of catechol estrogens and catecholamines.
- Intake of phytonutrients and indole 3-carbinol found in cabbage, cauliflower, Brussels sprouts, broccoli, and kale induces CYP450 2-hydroxylase activity.
- Coffee intake in premenopausal women
- Deemed the most protective of the three phase 1 metabolites- (though phase 2 methylation is still needed to mitigate risk)
- Higher elevations are associated with risk of osteoporosis, oxidative stress, and catecholamine excess

Therapeutic Options:

- Consider SNP testing for CYP1A1 and COMT
 - Genova's [Detoxigenomic Profile](#) or ala carte SNPs add-on to Essential Estrogens
- Assess overall methylation capacity
 - Genova's [Methylation Panel](#)
 - Methylation support may be indicated and includes S-Adenosyl-L-Methionine (SAME), methionine, magnesium, vitamin B12, folate (or folic acid, 5-formyl THF or 5-methyltetrahydrofolate), trimethylglycine (TMG) also known as betaine, and stress management strategies that reduce catecholamine production.^{157,158}
- Evaluate oxidative stress, toxic exposures, as well as antioxidant and glutathione status to prevent quinone formation – (2-hydroxyestrogen quinones are stable and more benign)²⁰⁹
 - Genova's [NutraEval](#) or [Metabolomix+](#) profiles
- Osteoporosis risk assessment
- Stress management

Low Levels:

Considerations and causes of low 2-hydroxyestrogens:

121,176,208

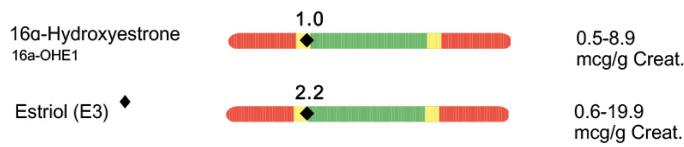
- Low levels of urinary parent hormones
- Decreased metabolism and excretion of circulating parent hormones
- Genetic SNP that upregulates CYP1B1 enzyme resulting in increased estrogen metabolism through the 4OH pathway
- Aromatase inhibitors
- Antibiotic use
- Quinone formation

Therapeutic Options:

- Consider genetic SNP testing for phase 1 CYP450 enzymes
 - Genova's [Detoxigenomic Profile](#)
- Dietary and lifestyle strategies to promote metabolism through this pathway^{137,198,203,210-221}
 - Cruciferous vegetables, soy, flaxseed, green tea catechins, resveratrol, HMR lignan
 - Exercise that increases lean body mass and decreases BMI
 - Supplementation with broccoli derivatives indole-3-carbinol (I3C) or diindolylmethane (DIM)
- Evaluate oxidative stress, toxic exposures, as well as antioxidant and glutathione status in case of quinone formation – (2-hydroxyestrogen quinones are stable and more benign)²⁰⁹
 - Genova's [NutraEval](#) or [Metabolomix+](#) profiles
- If parent hormones are low, refer to section on [low estrogen](#)



16 α -hydroxyestrone (16 α -OHE1) and Estrinol (E3)



During phase 1 liver detoxification, if the hydroxylation of estrogen occurs at carbon position C16, the enzyme CYP3A4 acts and irreversibly yields the metabolite 16 α -hydroxyestrone (16 α -OH) which can then be converted to estrinol (E3). After phase 2 detoxification, these metabolites can be excreted in urine and stool. There is some risk associated with elevations of 16 α -hydroxyestrone.

There are other 16-pathway metabolites including 16-epiestriol, 16-ketoestradiol, and 17-epiestriol. Their clinical relevance is not fully elucidated.^{23,189}

- One study showed urinary 17-epiestriol was positively associated with breast cancer risk in premenopausal women in the luteal phase. However, there is no experimental or epidemiologic evidence for a role of this metabolite in breast carcinogenesis, as there is with 16 α OHE1. These isomers can be reconverted to estrinol and 16 α -OHE1.²³

While 16 α -OH1 has potent estrogenic properties, E3 is the least potent among parent estrogens.^{1,22,100,110} E3 is rapidly excreted in urine, and relatively abundant compared to other urinary metabolites.^{1,23}

Cancer Risk:

As cited previously, population and menopausal status should be considered when interpreting risk from urinary metabolite levels. The US-based Nurses' Health Study II data showed that a higher concentration of urinary 16-pathway estrogens relative to parent estrogens was associated with increased risk of premenopausal breast cancer.²³ A larger analysis using pooled data across 4 postmenopausal breast cancer case-control studies showed a non-statistically significant increase in risk with elevated 16-pathway metabolites.¹⁸⁷

Men with higher 16 α -OHE1 showed a non-significant prostate cancer risk increase, with an increased 2/16 ratio having a protective effect.²⁰²

High Levels:

Considerations and causes of

High 16-hydroxyestrogens:^{222, 223}

- Caffeine intake in premenopausal women
- Association with atypical endometrial hyperplasia
- Obesity

Therapeutic Options:

- Dietary and lifestyle strategies to promote metabolism through the 2-OH safer detoxification pathway^{137,193,198,203,210-221}
 - Cruciferous vegetables, soy, flaxseed, green tea catechins, resveratrol, HMR lignan
 - Exercise that increases lean body mass and decreases BMI
 - Supplementation with broccoli derivatives indole-3-carbinol (I3C) or diindolylmethane (DIM)

Low Levels:

Consideration and causes of Low 16-hydroxestrogens:

^{121,176,199,200}

- Low levels of urinary parent hormones
- Decreased metabolism and excretion of circulating serum parent hormones
- Green tea and smoking in premenopausal women
- Aromatase inhibitors
- Antibiotic use


**In general, lower levels may be considered a favorable finding.*

Therapeutic Options:

- Assess parent hormone levels
- If parent hormone levels are low refer to the Estrogen section for considerations regarding [low circulating serum estrogen](#)



2-OH(E1+E2):16α-OHE1 Ratio

2-OH(E1+E2)/
16α-OHE1 Ratio  0.3-13.7

Genova's 2:16 ratio includes 2-OH (E1+E2) and 16αOHE1. Many studies look at all metabolites within the 2- and 16- pathways, which is different from earlier studies only assessing only 2-OHE1 and 16αOHE1.¹⁸⁷ Assessment of all metabolites in a pathway has received criticism arguing that the 16-hydroxylated pathway metabolites including estriol, 16-epiestriol, and 16-ketoestradiol do not have evidence of tumorigenic or genotoxic properties.¹⁸⁹

- As noted previously, one study showed urinary 17-epiestriol, also a 16-pathway metabolite, was positively associated with breast cancer risk in premenopausal women in the luteal phase. However, there is no experimental or epidemiologic evidence for a role of this metabolite in breast carcinogenesis, as there is with 16αOHE1.²³ Research is ongoing, and Genova continues to monitor the literature.

The 2-OH(E1+E2):16α-OHE1 ratio has been studied as a marker of breast cancer risk, although research is mixed and there has been much debate as to its value.^{22,188,189}

More recent studies show correlation with the urinary 2:16 ratio and breast cancer using LC-MS/MS, which is the same methodology that Genova uses.¹⁸⁷ Translation of the literature is challenging due to different methodologies and sample types, study size, menopausal status, and ethnicity, thus conclusions can be contradictory.

- There is only modest correlation between blood and urine estrogens and metabolites, likely due to differences in metabolism and/or excretion, therefore studies may be mixed.⁴ Having appropriate reference ranges for each sample type is important for analyzing data and assessing risk.
- The 2:16 ratio was similar between breast tissue and urine samples in a small study of 9 women, supporting the use of the ratio in urine as a surrogate marker for breast tissue, however larger studies are needed to confirm this finding.²²⁴
- Studies show that higher ratios in Asian populations who have lower breast cancer risk, decline when these populations migrate West increasing their risk supporting the theory that diet and lifestyle influence estrogen metabolism.¹⁸⁷

High Levels:

Consideration and causes of a High 2OH-(E1+E2):16α-OHE1 Ratio: ^{187,202,208,225}

- Generally considered favorable – inferring metabolism is preferring the safer 2-OH pathway *however phase 2 detoxification is still needed to mitigate risk*
- Higher ratios in males have been associated with reduced risk of prostate cancer.
- Postmenopausal breast cancer case-control studies showed that a higher 2:16 ratio was associated with lower breast cancer risk.
 - A higher ratio is also associated with better long-term mortality among women with breast cancer.

Low Levels:

Consideration and causes of a Low 2OH-(E1+E2):16α-OHE1 Ratio:

- Refer to causes of [elevated 16α-hydroxyestrone](#) and low 2OH-(E1+E2)

Therapeutic Options:

- Diets high in soy isoflavones, DIM, a red clover extract/probiotic formula, and higher levels of physical activity have been shown to increase the 2:16 ratio, although benefits depend on the population being studied.^{193,198,226-230}
- Refer to strategies to [encourage 2OH-\(E1+E2\) metabolism](#)



4-hydroxyestrone and 4-hydroxyestradiol (4-OH E1+E2)



During phase 1 liver detoxification, if the hydroxylation of estrogen occurs at carbon position C4, the enzyme CYP1B1 acts and irreversibly yields the metabolites 4-hydroxyestrone (4-OHE1) and 4-hydroxyestradiol (4OH-E2). After phase 2 detoxification, these metabolites can be excreted in urine and stool. The 4-hydroxyestrogens infer the highest risk. They need to be methylated or conjugated to prevent formation of 3,4 quinones which cause DNA damage.

- Components of the 4-pathway are potent inducers of genotoxic damage.²² Both the 2- and 4-OHE can be oxidized to form quinones or semiquinones. The 2-OH quinones form stable DNA adducts, whereas the 4-OH quinones form depurinating adducts and mutations leading to tumorigenesis. These quinones can be safely detoxified by conjugation with glutathione via the enzyme glutathione S-transferase, resulting in a net decrease in DNA damage.^{109,186}

Cancer Risk:

As cited previously, population and menopausal status should be considered when interpreting risk from urinary metabolite levels. Studies evaluating 4-hydroxyestrogens and breast cancer appear contradictory but are largely the result of menopause status.

Pooled data across 4 postmenopausal breast cancer case-control studies support increased risk with higher 4-OH metabolites.¹⁸⁷ However the US-based Nurses' Health Study II data in premenopausal breast cancer cases found the opposite to be true, though not statistically significant – high urinary 4OH (E1+E2) was less of a risk.²³ This could possibly suggest that the ability to excrete a higher amount of a toxic metabolite may be confer lower risk in premenopausal women. That being said, the need to methylate and/or conjugate this metabolite is needed to prevent quinone formation.^{3,4} Quinone formation can cause DNA damage and thus further increase the risk of breast cancer.^{201,204}

As it relates to males, 4OH-E2 can induce the proliferation of a prostate cancer cell line. However more research is needed to determine the role of catechol estrogens in prostate diseases in males.^{114,231} Again, the need to methylate and/or conjugate 4OH-(E1+E2) is important. A small study showed higher levels of urinary DNA adducts from 4-hydroxylation of estrogens in men with prostate cancer compared to those without.²³²

High Levels:

Considerations and causes of high 4-OH (E1+E2):^{195,200,223}

- Genetic SNP in the CYP1B1 enzyme causing upregulation of the 4-OH pathway
- Bisphenol-A (BPA) exposure
- Smoking
- Diet and lifestyle that lacks strategies to promote healthy metabolism
- Associated with atypical endometrial hyperplasia

Therapeutic Options:

- Dietary and lifestyle strategies to promote metabolism through the 2-OH safer detoxification pathway^{137,198,203,210-221}
 - Cruciferous vegetables, soy, flaxseed, green tea catechins, resveratrol, HMR lignan
 - Exercise that increases lean body mass and decreases BMI
 - Supplementation with broccoli derivatives indole-3-carbinol (I3C) or diindolylmethane (DIM)
- Evaluate antioxidant status and support to prevent quinone formation and oxidative damage
 - Genova's [NutrEval](#) or [Metabolomix+](#) Profiles
 - Cruciferous and allium vegetables, resveratrol, and citrus foods induce glutathione S-transferases.¹⁵⁷ Several natural compounds have exhibited the ability to minimize DNA adduct formation/damage in vitro including N-acetylcysteine, sulforaphane, resveratrol, melatonin, and lipoic acid.^{204,233,234}
- Assess 4-methoxyestrogens and consider methylation support.¹¹⁶
 - Genova's [Methylation Panel](#)
 - Methylation support includes S-Adenosyl-L-Methionine (S-AdoMet), methionine, magnesium, vitamin B12, folate (or folic acid, 5-formyl THF or 5-methyltetrahydrofolate), trimethylglycine (TMG) also known as betaine, and stress management strategies that reduce catecholamine production.^{157,158}
- Genetic SNP testing to evaluate CYP1B1
 - Genova's [Detoxigenomic Profile](#) and add-on ala carte SNPs to Essential Estrogens Profile



Low Levels:

Considerations and causes of Low 4-OH (E1+E2):

121,176,204

- Low levels of urinary parent hormones
- Decreased metabolism and excretion of circulating parent hormones
- Toxic quinones formation
- Aromatase inhibitors
- Antibiotic use

**In general, low 4-OH (E1+E2) levels are considered beneficial due to its association with risk*

Therapeutic Options:

- Assess overall levels of urine estrogen metabolites
 - If overall low, consider supporting estrogen detoxification including sulfation, glucuronidation, methylation and glutathione conjugation, as well as ensuring antioxidant support. Consider also assessing circulating parent estrogen levels for comparison.

Estrogen Phase 2 Methylation –

Phase 2 Estrogen Metabolites

Phase 2 Estrogen Metabolites		
2-Methoxyestrone+ 2-Methoxyestradiol 2-MeO(E1+E2)	1.7	0.2-8.6 mcg/g Creat.
4-Methoxyestrone+ 4-Methoxyestradiol 4-MeO(E1+E2)	<dl	<= 1.0 mcg/g Creat.

After phase I detoxification in the liver, the estrogen catechol molecules are active and potentially toxic. They must go through phase 2 detoxification to be converted to water-soluble forms for safe excretion in both the urine and stool. Both 2- and 4-OH catechol estrogens are methylated and excreted, with the 2-methylethers as the major metabolites of estrone and estradiol in urine. Thus, methylation represents the major detoxification pathway for estrogen.¹⁰⁹

Estrogen methylation is catalyzed by the enzyme catechol-O-methyltransferase (COMT). COMT genetic polymorphisms can impair COMT function, slowing down methylation capability and increasing risk of circulating phase 1 intermediates.¹⁰⁹

2-MeO (E1+E2)

2-Methoxyestrone+ 2-Methoxyestradiol 2-MeO(E1+E2)	1.7	0.2-8.6 mcg/g Creat.
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If the parent estrogen is driven down the 2-OH (E1+E2) pathway, that catechol estrogen intermediate is still active and potentially toxic and requires conjugation. Methylation using the COMT enzyme is the predominant conjugation reaction. In this particular pathway, COMT simultaneously lowers the potential for DNA damage and increases the concentration of 2-methoxyestrogens- now water-soluble and inert.¹⁰⁹

2-MeOE2 has been described to have anti-proliferative, antiangiogenic, and pro-apoptotic activity.¹²² There is evidence that methoxylated estrogens, especially the 2-pathway methoxylated estrogens, are associated with decreased breast cancer risk. One study on postmenopausal breast cancer patients showed elevated 2OHE2 and lower 2-MeOE2 indicating that methylation is an important step in protecting against tumor formation.²⁰¹

High Levels:

Considerations and causes for high 2-MeO (E1+E2):

- High levels of 2-hydroxyestrogens with adequate methylation support

Elevated 2-MeO (E1+E2) relative to 2-OH (E1+E2) is generally thought to be beneficial.

Therapeutic Options:

- Since this is considered beneficial, continued support via diet and lifestyle strategies to promote the 2-OH detoxification pathway with ongoing methylation support

Low Levels:

Considerations and causes for low 2-MEO(E1+E2):

121,176

- Low urinary 2-OH estrogens
- Insufficient methylation nutrients and cofactors
- Genetic SNP of the COMT enzyme which causes downregulation
- Aromatase inhibitors
- Antibiotic use

Therapeutic options:

- Genetic SNP testing to evaluate COMT
 - Genova's [Detoxigenomic Profile](#) and add-on ala carte SNPs to Essential Estrogens Profile
- Assess overall methylation capacity
 - Genova's [Methylation Panel](#)
 - Methylation support may be indicated and includes S-Adenosyl-L-Methionine (SAME), methionine, magnesium, vitamin B12, folate (or folinic acid, 5-formyl THF or 5-methyltetrahydrofolate), trimethylglycine (TMG) also known as betaine, and stress management strategies that reduce catecholamine production.^{157,158}



4-MeO (E1+E2)



If the parent estrogen is driven down the 4-OH (E1+E2) pathway, that catechol estrogen intermediate is still active and potentially toxic and requires conjugation. The 4-hydroxyestrogens infer the highest risk. They need to be methylated or conjugated to prevent formation of 3,4 quinones which cause DNA damage. Methylation using the COMT enzyme is the predominant conjugation reaction. COMT simultaneously lowers the potential for DNA damage and increases the concentration of 4-methoxyestrogens- now water-soluble and inert.¹⁰⁹

Most studies find an increased breast cancer risk associated with the ratio of 4-pathway catechols to 4-pathway methylated catechols. This increased risk has been seen in cases with less extensive methylation of potentially genotoxic 4-hydroxylation pathway catechols; thus, increased relative levels of 4-methoxyestrogens would be considered favorable.^{116,187}

High Levels:

Considerations and causes for high 4-MeO (E1+E2):

- High levels of 4-hydroxyestrogens with adequate methylation support
 - Methylation of the toxic 4-OH metabolite is favorable

Therapeutic Options:

- Continue methylation support
 - S-Adenosyl-L-Methionine (S-AMe), methionine, magnesium, vitamin B12, folate (or folinic acid, 5-formyl THF or 5-methyltetrahydrofolate), trimethylglycine (TMG) also known as betaine.^{157,158}
- Dietary and lifestyle strategies to promote metabolism through the 2-OH safer detoxification pathway
 - Cruciferous vegetables, soy, flaxseed, green tea catechins, resveratrol, HMR lignan
 - Exercise that increases lean body mass and decreases BMI
 - Supplementation with broccoli derivatives indole-3-carbinol (I3C) or diindolylmethane (DIM)^{137,198,203,210-221}

Low Levels:

Considerations and causes of low 4-MeO (E1+E2):^{121,176}

- It is not uncommon to see lower levels of 4-MeO (E1+E2) – healthy populations do not tend to excrete high amounts.²³
- Genetic SNP in COMT
- Low levels of urinary parent hormones and/or 4-OH estrogens
- Aromatase inhibitors
- Antibiotic use

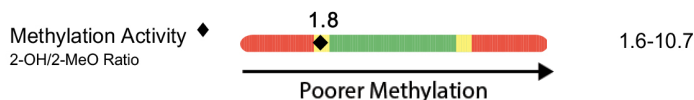
Therapeutic Options:

- Evaluate levels of urinary 4-OH
- Genetic SNP testing to evaluate COMT
 - Genova's [Detoxigenomic Profile](#) and add-on ala carte SNPs to Essential Estrogens Profile
- Assess overall methylation capacity
 - Genova's [Methylation Panel](#)
 - Methylation support may be indicated and includes S-Adenosyl-L-Methionine (S-AMe), methionine, magnesium, vitamin B12, folate (or folinic acid, 5-formyl THF or 5-methyltetrahydrofolate), trimethylglycine (TMG) also known as betaine, and stress management strategies that reduce catecholamine production.^{157,158}



Methylation Activity Ratio

2-OH(E1+E2):2-MeO(E1+E2)



Methylation of the catechol estrogens, 2-OH(E1+E2) and 4-OHE1, is facilitated by the catechol-O-methyltransferase (COMT) enzyme. While both 2- and 4-hydroxy estrogens are methylated, the 2-methylethers are the major metabolites of estrone and estradiol in urine.^{109,235} For this reason, Genova's methylation ratio assesses the 2-hydroxyestrogen pathway in the methylation ratio, versus the 4-hydroxylation pathway.

High Methylation Ratio:

- A high ratio indicates less methylation activity (higher amount of phase 1 metabolites vs. phase 2 methylated)
- Methylation support may be indicated and includes S-Adenosyl-L-Methionine (SAME), methionine, magnesium, vitamin B12, folate (or folinic acid, 5-formyl THF or 5-methyltetrahydrofolate), trimethylglycine (TMG) also known as betaine.^{157,158}
- 2-hydroxyestrone is a highly efficient competitive inhibitor of COMT, interfering with the degradation of catecholamines, and resulting in prolonged catecholamine activity.²⁰⁵ Therefore, it may be prudent to consider stress management strategies that reduce catecholamine production.

Low Methylation Ratio:

- Indicates higher methylation of the catechol estrogens, a favorable finding
- Please note: A low ratio is not necessarily an indication of overmethylation



P/E2 Ratio

The progesterone/estradiol ratio is used clinically to determine dominance of one hormone compared to the other. In a symptomatic patient, the ratio may be important even when the individual levels are within range.

- This ratio is only reported on Genova salivary tests. While progesterone and estradiol are both measured on serum testing, reference ranges for a P/E2 ratio are not included.

In general, a lower ratio (higher relative estrogen) tends to have more clinical implications – estrogen dominance. Progesterone opposes the proliferative effects of estrogen on the endometrium, thus protecting from endometrial cancer, therefore it is important for the P/E2 ratio to be balanced.⁸⁷ This ratio is often used by clinicians to assess balance for a variety of reasons such as monitoring hormone therapy. It should be noted that there is no peer-reviewed literature to support its use as a guide to hormone therapy or to mitigate risk in peri/menopausal women.

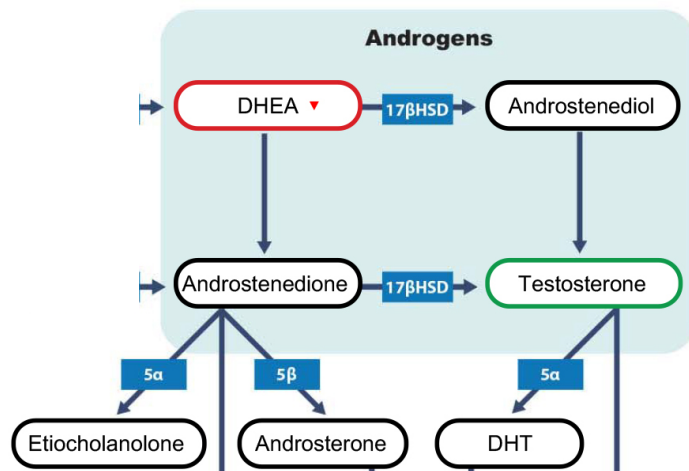
High P/E2 Ratio:

- An elevated ratio with relatively higher progesterone generally does not result in clinical consequence.
- Can be problematic in fertility regimens ²³⁶

Low P/E2 Ratio:

- A low ratio with relatively higher estrogen is known as “estrogen dominance”.
 - This concept was popularized in books written by Dr. John Lee, MD connecting estrogen dominance to a host of symptoms and conditions. Estrogen dominance is widely discussed in the functional medicine space but research using this ratio is still growing.
- A low P/E2 ratio is associated with obesity, endometrial cancer, fibrocystic breast disease, and chronic fatigue syndrome. ^{87,89,237,238}
 - Refer to the [progesterone](#) and [estrogen](#) sections for therapeutic options to optimize balance

Androgens



Androgenic hormones include:

- Testosterone (T)
- Dehydroepiandrosterone (DHEA)
- Dehydroepiandrosterone sulfate (DHEA-S)
- Androstenedione
- Dihydrotestosterone (DHT)

Testosterone and DHT are the most potent.²³⁹

Androstenedione is not biologically active and acts as a precursor for both estrone and testosterone.¹⁴²

Testosterone

While testosterone is thought of as a male hormone and estrogen and progesterone are thought of as female hormones, both males and females synthesize all hormones in varying amounts. Males produce more testosterone as compared to females and thus maintain the masculine phenotype. However, circulating androgens are found in much higher concentrations than estrogens in both sexes.^{239,240}

Testosterone Functions:

Testosterone regulates gonadal function affecting:^{239,241}

- Libido
- Aggressive behavior
- Mood
- Energy
- Psychological well-being
- Muscle mass and strength
- Liver function
- Lipid regulation
- Adipose tissue distribution
- Bone formation
- Erythropoiesis
- Immune function

Although androgens have significant effects on bone, estrogens are more important for bone growth and maintenance.¹¹⁴

The action of androgens and other hormones are mediated via their receptors.^{34,38,242} The androgen receptor (AR) is expressed in the mammary gland, muscle, prostate, skin, vagina, bone marrow, and testes.³⁴

Biosynthesis

Males:

The Hypothalamic-Pituitary-Gonadal Axis (HPG Axis) regulates testosterone production. Luteinizing hormone (LH) is secreted by the anterior pituitary gland in response to hypothalamic signaling. LH acts on the Leydig cells in the interstitium of the testis stimulating testosterone production. In contrast, when follicle-stimulating hormone (FSH) is secreted by the anterior pituitary, this stimulates spermatogenesis and Sertoli cell function.¹⁷³

Over 95% of circulating testosterone is derived from testicular secretion, though testosterone and DHT can also be made in the adrenals from DHEA. The overall contribution of adrenal androgens to circulating testosterone in men is minor (approx 5%), however it contributes to a larger proportion of testosterone in women.²⁴² Testosterone shows a diurnal rhythm in healthy males with levels being highest in the morning and lowest in the evening.^{67,243}

Testosterone and DHEA tend to decline gradually in healthy men after age 40, as sex hormone-binding globulin (SHBG) rises with age.²⁴¹ Circulating testosterone concentrations can decrease by approximately 1-2% annually and DHEA by 2-3% annually.^{241,242}



Females:

Approximately 25% of androgen biosynthesis occurs in the ovaries, 25% in the adrenal gland, and the remainder at tissue sites in the periphery. Androgen secretion by the ovaries and adrenals is stimulated by LH and adrenocorticotrophic hormone (ACTH) respectively.²³⁹

- In healthy females, there is a significant age-related decline in androgens DHEA, total T, free T with the decline being steepest in the early reproductive years and a flattening out in mid-life.
- Natural menopause does not have as large an effect on circulating androgen levels as compared to the decline in estrogens.^{177,244-246}
- Bilateral oophorectomy results in lower total and free T, but not DHEAs or androstenedione among older postmenopausal women. This suggests ongoing ovarian production of androgens many years beyond natural menopause.²⁴⁶
- Androgen levels vary within the menstrual cycle.
 - Testosterone is highest during the middle third of the menstrual cycle.²⁴⁵
 - Women in late reproductive years (ages 43-47) may have a decreased midcycle production of androgens despite normal menstrual cycles.²⁴⁷
 - DHEA-S is not subject to change with the various phases of the menstrual cycle.²⁴⁷

Metabolism

There are different pathways that metabolize testosterone.

1. Testosterone can be converted via the 5 α -reductase enzyme into the more potent DHT, which affects the prostate and hair follicles.²⁴² 5 α -reductase is concentrated in the prostate, reproductive system, and skin.¹⁷³
2. Testosterone can also be converted into estradiol via the CYP19 aromatase enzyme. Aromatase predominates in the liver, adipose, and brain. Obesity and low testosterone levels are involved in a self-perpetuating cycle. Testosterone is aromatized in adipose to estradiol, reducing serum and tissue testosterone levels. The estradiol provides negative feedback to the HPG axis, further reducing testosterone. Low testosterone levels increase fat mass and decrease lean muscle, resulting in increased adipose tissue, and the cycle continues.¹⁷³
3. Testosterone can be oxidized and conjugated in the liver to biologically inactive metabolites for excretion. In phase 2 detoxification, glucuronidation is a major androgen metabolism pathway, though androgens can also be conjugated with glutathione and excreted.^{1,109,242}

Dehydroepiandrosterone (DHEA)

Dehydroepiandrosterone (DHEA) mainly circulates in a conjugated sulfated form (DHEA-S) and is the most abundant steroid in circulation.¹ DHEA-S arises and is secreted primarily from the adrenal cortex zona reticularis and can also be made in the brain as a neurosteroid. DHEA is largely made from the peripheral conversion of DHEA-S and only partially from the adrenal gland.²⁴⁸ DHEA concentrations peak at around 25 years of age and then decline steadily over the following decades.²⁴⁹

Metabolism

DHEA and DHEA-S circulate in blood mostly bound to albumin but some does circulate unbound. Unbound DHEA is rapidly cleared but the DHEA-S metabolism is much slower. DHEA and DHEA-S can interconvert and affect peripheral tissue where they convert to androstenedione, testosterone, and DHT. Both can also be aromatized to estrogen.

DHEA Functions:²⁵⁰

- Neuroprotection
- Neurogenesis
- Apoptosis
- Anti-inflammatory
- Antioxidant
- Catecholamine synthesis and secretion

DHEA -S measured in serum represents a more stable and larger pool of hormone status, however it is a charged, polar molecule and cannot diffuse easily through lipid membranes into saliva. Salivary DHEA is considered an excellent surrogate marker of DHEA status.²⁵¹

DHEA acts as an anabolic hormone, but also appears to function to down-regulate the cellular effects of cortisol. With that, the salivary ratio of DHEA to cortisol represents a measurement of anabolic and catabolic balance and can theoretically enhance the predictive value of HPA axis dysfunction.²⁵¹

DHEA



Measuring Androgens

At Genova Diagnostics, parent androgen hormones can be directly assessed in serum and saliva. There are additional serum measurements to give further insight, as well as considerations to take into account when measuring testosterone in saliva.

Additional Serum Androgen Assessments

Free Androgen Index and Free Testosterone

Sex hormone binding globulin (SHBG) is a high affinity binding protein for testosterone, followed by albumin, corticosteroid binding globulin, and $\alpha 1$ acid glycoprotein, which are low affinity binding proteins. Approximately 44-70% is bound to SHBG with the remainder bound to the other proteins. 1-2% is not protein bound.^{242,252} Since SHBG has the highest affinity, free and albumin-bound testosterone is considered bioavailable testosterone.¹⁷³

There are two common calculations for approximating free, or bioavailable testosterone in serum. The Free Androgen Index and Free Testosterone.

- Genova assesses the Free Androgen Index (FAI) which uses the ratio of total testosterone to SHBG. The FAI assesses bioavailable testosterone and is essentially the measurement of albumin-bound plus unbound testosterone.²⁵³ Testosterone is only weakly bound to albumin and binding sites are shared with other substances; because of more probable spontaneous dissociation from the protein, the albumin-bound testosterone could also have biologically active effects.²⁵²
- The FAI is known to give erroneous results in men, and is commonly used to test for hyperandrogenism in women.²⁵⁴ For this reason, Genova provides FAI results on female, but not male serum studies. However, the FAI may not be reliable when SHBG concentrations fall below 30 nmol/L. Males tend to have lower SHBG compared to females, which contributes to why the FAI is not reliable in males.²⁵⁴
- Another measurement is calculated Free Testosterone which excludes both albumin-bound and SHBG-bound testosterone and only estimates unbound testosterone. Free testosterone can also be measured directly using gold standard equilibrium dialysis, but this method is impractical for routine use.²⁵⁴

Free Testosterone Calculations:

Free Androgen Index (bioavailable testosterone) =
unbound T + albumin-bound T
Free testosterone = unbound T

The idea of free testosterone or FAI adding clinical utility to total testosterone is commonly promoted, however literature is mixed. Several studies support its measurement in male hypogonadism, MAFLD, PCOS, hypertension, male osteoporosis, cognitive issues, and frailty.^{252,255}

PCOS:

Clinical practice guidelines for diagnosis and treatment of PCOS recommend starting with total testosterone. However, some studies show that in women with PCOS, the serum free androgen index and salivary testosterone predicted PCOS better than total testosterone.²⁵⁶

When total T is normal, but the patient presents with hirsutism and clinical evidence of an endocrine disorder, then a free T assessment is recommended.²⁵⁴

Hypogonadism:

In evaluating male hypogonadism, the Endocrine Society recommends measuring total T on two separate mornings, and free T only when men are subject to conditions that alter SHBG or when total T is low or low-normal.²⁵²

Studies show that free testosterone does not add additional information about morbidity or mortality prognosis for older men's health beyond a serum total testosterone.²⁴²

Salivary Testosterone

Salivary testosterone concentrations generally reflect the serum free concentration.^{12,14,67,257,258} Salivary measurements may directly assess 'tissue' concentrations and androgenicity, and therefore bioavailability of testosterone.¹²

Salivary testosterone is unaffected by variations in circulating SHBG and albumin as compared to serum free testosterone.¹²

However, in women with PCOS, salivary T does not correlate with bioavailable testosterone (FAI). Yet both salivary T and the FAI were able to predict PCOS despite not correlating.²⁵⁶

Salivary binding proteins may have more substantial binding effects in women as compared to healthy men, resulting in low salivary testosterone and lesser correlation with serum free testosterone.²⁵⁹



High Levels:

Androgen Excess Signs & Symptoms²⁶⁰⁻²⁶⁶

Menstrual irregularity	Aggression
Hirsutism	Acne
Virilization	Oily skin/seborrhea
Obesity (females)	Androgenic alopecia (temporal hair loss/balding)
Infertility	Voice deepening

Clinical Associations with High Androgens^{242,267,268}

- Polycystic ovary syndrome (PCOS) and anovulation in women
- Infertility
- Idiopathic hyperandrogenism
- Insulin sensitivity
- Elevated serum lipids
- Adrenal gland tumors
- Obesity

Males:

While prostate cancer is androgen dependent, neither circulating testosterone, DHT levels, nor testosterone treatment predicts future prostate cancer.²⁴²

Females:

Androgens are converted to estrogens, therefore estrogenic side effects are possible, which may include increased risk of breast and ovarian cancer.²³⁹

Elevated salivary testosterone is seen in obese women and is thought to be attributed to lower SHBG levels.⁶

Elevated serum estrogens and androgens and low levels of SHBG are associated with higher postmenopausal breast cancer risk.¹⁴⁰

Considerations and causes of high androgens:^{14,67,155,173,242,267,269-271}

- Supplementation
- Low sex hormone binding globulin
 - Due to smoking, thyroid conditions, high insulin etc. ([See section on SHBG](#))
- Hypo- and hyperthyroidism
- PCOS
- Glucocorticoid use
- Progestins
- Adrenal gland or testicular germ cell tumors
- Idiopathic hyperandrogenism
- Blood contamination of salivary sample from gingivitis or bleeding gums
- Up- or downregulation of enzymes within the steroidogenic pathway
 - There are many medications, supplements, nutrients, exposures, and lifestyle factors that can influence hormone levels as well as the enzymes required for steroid production. (Refer to the [steroidogenic pathways chart](#) and other hormone levels to assess whether pathways are up or down regulated.)

Therapeutic Options:

- Lifestyle modification
 - Weight management, calorie reduction, and exercise
- Assess SHBG levels
- Assess medications
- Evaluate thyroid function
 - Genova's Comprehensive Thyroid Assessment or the [Endo+ Profile](#)
- Support detoxification – assess antioxidant status and glutathione levels
 - Genova's [NutraEval](#) or [Metabolomix+](#)



Low Levels:

Androgen Deficiency Signs & Symptoms^{6,38,47,125,173,239,272-275}

Decreased muscle mass/strength	Gynecomastia
Increased adiposity	Decreased body hair
Testicular atrophy	Headaches
Low libido	Hot flashes
Depression	Lack of sense of well-being
Poor cognition	Sleep issues
Fatigue	Erectile dysfunction
Infertility	Bone loss

Clinical Associations with Low Androgens: ^{173,242,276}

- Primary hypogonadism
- Secondary hypogonadism
- Kallmann syndrome
- Obesity
- Diabetes
- Metabolic syndrome
- Hypertension
- Hyperlipidemia
- Osteoporosis
- Obstructive sleep apnea

Males:

Male hypogonadism is a clinical syndrome resulting from failure of the testis to produce enough testosterone and spermatozoa caused by HPG axis dysfunction. Causes of primary hypogonadism (originates in the testes) include normal aging, alcohol abuse, hemochromatosis, cancer treatment, Klinefelter's syndrome, undescended testicles, and mumps orchitis. Elevated serum LH and FSH suggest primary hypogonadism.^{173,276}

Causes of secondary hypogonadism (originates in the hypothalamus or pituitary gland) include obesity, pituitary disorders, stress, glucocorticoid or opiate pain medication use, trauma, surgery, HIV, and Kallmann syndrome. Low or low-normal LH and FSH levels suggest secondary hypogonadism. Prolactin levels can help rule out prolactinoma.^{173,276}

Females:

Androgen insufficiency is also associated with anorexia nervosa, rheumatoid arthritis, systemic lupus erythematosus, Addison's disease, and HIV-AIDS.²³⁹

Symptoms more commonly attributed to estrogen insufficiency may also be seen in androgen insufficiency and include vasomotor instability, vaginal dryness, bone loss, decreased muscle strength, and changes in cognition or memory. It is important to distinguish androgen insufficiency from estrogen insufficiency when determining treatment.²³⁹

Considerations and causes of low androgens:^{1,61,239,244,273,275,277-280}

- Andropause/menopause/advancing age
- Primary or secondary hypogonadism
- Cow's milk
- High endurance exercise
- Hypopituitarism
- Adrenal insufficiency
- Ovarian failure, oophorectomy
- Oral estrogens, oral contraceptives
- Corticosteroids
- Antiandrogenic agents
- Up- or downregulation of enzymes within the steroidogenic pathway
 - There are many medications, supplements, nutrients, exposures, and lifestyle factors that can influence hormone levels as well as the enzymes required for steroid production. (Refer to the [steroidogenic pathways chart](#) and other hormone levels to assess whether pathways are up or down regulated.)

Therapeutic Options:

- Supplementation with testosterone/DHEA
- Resistance training²⁷⁷⁻²⁸⁰
- Korean ginseng was shown to increase testosterone levels in young women and DHEA in older women.²⁸¹
- Ashwagandha and fenugreek seed extracts²⁸² (studies are mixed)

See the following section on "Androgen Supplementation and Testing" for more information.



Androgen Supplementation and Testing

Testosterone and DHEA supplementation is widely prescribed in men and women.

Studies show that supplementation leads to increases in lean muscle and decreases in fat mass. Patients also report an improvement in mood, concentration, motivation, and energy levels when taking testosterone.²⁸³ Androgen-deficient men taking androgen therapy report improvement in one or more symptoms including energy, well-being, psychosocial drive, initiative, assertiveness, libido, ejaculation frequency, increased truncal and facial hair growth and muscular strength and endurance.²⁴²

Literature reflects that there may be no or inconsistent benefits in bone and sexual function, and at times, detrimental effects including cardiovascular disease and polycythemia which makes it important to monitor patients closely. *Androgen therapy is contraindicated in prostate and breast cancer.²⁴²

Supplementing with bioidentical testosterone mimics endogenous testosterone in that it can result in bioactive metabolites DHT and estradiol, whereas synthetic, non-bioidentical testosterone does not.²⁴²

- Oral testosterone supplementation has a short circulating half-life and low oral bioavailability due to rapid hepatic conversion to biologically inactive metabolites that are excreted.
 - Forms such as injectable, implantable, transdermal, sublingual, and buccal are preferred since they bypass this hepatic degradation.²⁴²
- Testosterone pellets provide stable physiologic testosterone levels for up to six months after implantation.²⁴²
- Transdermal delivery via patches, gels, or creams tends to maintain physiologic testosterone levels with daily application.²⁴²
 - Application location is important with thin, highly vascular skin being more permeable than truncal skin.
- Buccal or sublingual delivery of testosterone requires multiple daily dosing to maintain physiologic testosterone levels, and much of this is converted to DHT.²⁴²

After cessation of prolonged high dose androgens, baseline functioning of the HPT axis may be delayed for up to 2 years.²⁴²

Compounded versus commercially produced products:

A study assessing testosterone concentrations in gels and creams that were compounded versus commercially produced showed significant variability among the compounded formulations compared to consistent doses in the commercially prepared products.²⁸⁴

Testosterone Supplementation in Women:

A 2019 global consensus position statement on the use of testosterone therapy for women discusses known benefits and potential risks. The main conclusion was that the only evidence-based indication of T therapy for postmenopausal women is for the treatment of hypoactive sexual desire disorder (HSDD), showing a moderate therapeutic effect.^{285,286}

Testosterone patches and creams tend to be the preferred methods resulting in free testosterone levels within the normal premenopausal range, as compared to subcutaneous implants, IM injections, and oral formulations which often result in supraphysiological concentrations. Guidelines recommend prescribing at 1/10th of a male dose.²⁸⁶

The global consensus is to measure total T, versus free T when monitoring hormone therapy in females.²⁸⁵ A study on postmenopausal women receiving a transdermal T patch showed high correlations between total testosterone, free testosterone, and bioavailable T (free and albumin bound), but no correlation with salivary T. Furthermore, salivary T did not change with treatment potentially making blood testosterone a better sample type for women supplementing with a T patch.²⁸⁷ Guidelines recommend assessing total testosterone and SHBG before initiating therapy, and total testosterone 3-6 weeks after initiating therapy for titrating.

DHEA Supplementation:

Supplementing with DHEA at 50mg/day orally does not translate to adequate blood testosterone for androgen therapy in men, and results in increased circulating estradiol. In women, this dose may result in hyperandrogenism.^{242,288}

Hormone experts promote the use of DHEA's naturally occurring metabolite, 7-keto DHEA as an alternative to DHEA because it is formed through an irreversible reaction and cannot convert to downstream androgens or estrogens. A systematic review showed favorable effects of 7-keto-DHEA on supporting weight loss.²⁸⁹

Androgen Supplementation Side Effects:

The most frequent reported side effects of T treatment in females are hirsutism or acne, with other potential effects including weight gain, permanent lowering of the voice, increased anger and other emotional changes, and adverse lipid changes.^{239,290}

In men, gynecomastia may occur with testosterone supplementation due to systemic aromatization to estrogen.²⁴²

More information can be found in the section entitled ["Hormone Therapy and Testing Impact"](#) at the end of this guide.



Sex Hormone Binding Globulin (SHBG)

Sex hormone binding globulin (SHBG) is a protein produced mainly in the liver that binds and transports testosterone, dihydrotestosterone, and estrogen to tissue receptors for biologic action. It does have a greater affinity for androgens. Not only does it transport hormones, it's been shown that SHBG can regulate hormone bioavailability, affinity, and access to target tissues.²⁷⁰

Because most hormones are bound to SHBG or albumin, changes in SHBG can greatly affect interpretation of measured hormone levels and their clinical effects due to bioavailability. A complete hormonal evaluation should include levels of sex hormones as well as SHBG.

When SHBG is high, there is less bioavailable hormone which can cause symptoms of hormone deficiencies. In the case of testosterone for example, high SHBG causes symptoms of testosterone insufficiency. At the same time, those low testosterone levels stimulate the production of SHBG, further worsening the clinical situation.

Levels of SHBG are influenced by many factors including nutrition, metabolism, hormonal balance, and lifestyle habits.

High Levels:

Because high SHBG can cause decreased testosterone bioavailability, it can present with symptoms of low testosterone (sarcopenia, central obesity, low libido etc.).

Considerations and causes of high SHBG:^{100,270,291-296}

- Aging
- Stress
- Alcohol use
- Smoking
- Cirrhosis/Hepatitis
- HIV
- Hyperthyroidism
- Medications
 - Carbamazepine, phenytoin, thiazolidinediones, oral contraceptives
- Dietary influences
 - Diets low in protein and vegetarian diets are associated with higher SHBG levels.
 - Dietary oleic acid has been shown to increase SHBG production.

- Adiponectin
 - Produced in white adipose tissue (which is inversely correlated to body mass and weight), increases levels of SHBG through molecular signaling
- Menstrual cycle and pregnancy
 - Levels in women can vary within the menstrual cycle and elevate during ovulation and throughout pregnancy.
- Estrogen supplementation
 - Oral E2 and conjugated equine estrogens cause an increase in SHBG, whereas transdermal E2 has no effect.

Therapeutic Options:

- Lifestyle and diet modification
- Assess medications and supplements
- Stress management

Low Levels:

Having low SHBG can potentially present clinically with virilization since it accounts for elevated bioavailable testosterone.

Considerations and causes of low SHBG:^{173,242,270,291,297-299}

- Obesity
- PCOS
- Hypothyroidism
- Systemic inflammation
- Non-alcoholic fatty liver
- Smoking
- Insulin resistance
- Glucocorticoids
- Progestins
- Very high levels of oral testosterone supplementation

Therapeutic Considerations:

- Addressing underlying clinical conditions
- Evaluation for insulin resistance
- Assess medications and supplementation
- Weight loss, smoking cessation, and exercise³⁰⁰
- Resveratrol and DIM may increase SHBG levels in certain populations^{214,229}

Rhythm Profile and the Menstrual Cycle

The Rhythm Profile assesses salivary E2 and progesterone over the course of a 28-day cycle. When it comes to women's hormones throughout a menstrual cycle, there are natural intra- and inter-individual differences in sex hormone levels, which emphasizes the importance of sampling at multiple time points to gather meaningful data.^{7,13}

Menstrual Cycle Dynamics

A series of orchestrated events must happen during a cycle for a fertile window to occur. The menstrual cycle is divided by ovulation into the follicular and luteal phases.

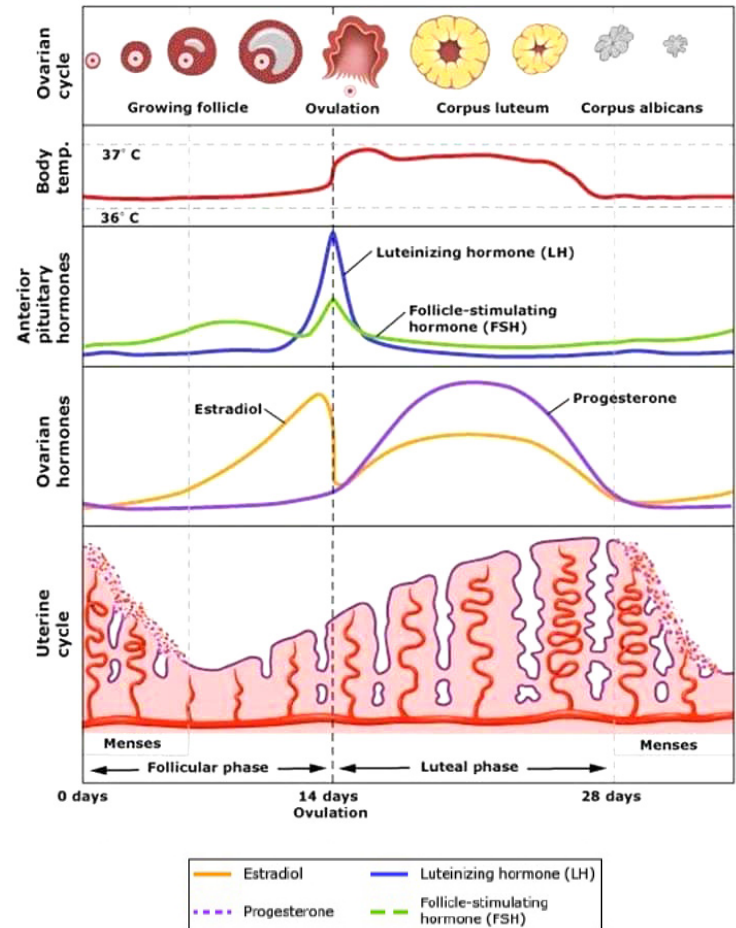
Inside the ovary, multiple follicles grow under the influence of FSH secreted by the pituitary gland. These follicles secrete estrogen, and a dominant follicle emerges. Rising estrogen in the follicular phase indicates that FSH has carried out its function of stimulating the growth of a dominant follicle.

The persistent high level of estrogen causes a release of LH from the pituitary gland, which then triggers ovulation (a release of the egg from the follicle).

The follicle then transforms into a corpus luteum that secretes estrogen and progesterone also known as the luteal phase. Therefore, a rising progesterone would indicate LH has carried out its ovulatory function.³⁰¹⁻³⁰³

The beginning of the rise in progesterone can provide a good reflection of the closure of the fertile window.³⁰⁴ The fertile window begins approximately 3-5 days (sperm lifespan) prior to ovulation and continues to approximately 1-2 days (oocyte lifespan) after ovulation.³⁰²

A healthy, normally cycling woman can have fully fertile cycles interspersed with anovulatory cycles or with a deficient luteal phase.^{78,301,302}



*The menstrual cycle.*³⁰⁵



Menstrual Cycle Length

The standard that has been used to define a normal menstrual cycle is 28 days in length with ovulation occurring on about day 14 – though on average cycles are approximately 25 to 30 days in length.

A cycle shorter than 21 days is termed polymenorrheic, and a cycle longer than 35 days is termed oligomenorrheic.³⁰³

It is widely assumed that ovulation occurs on day 14 of the cycle, but cycle lengths can be variable with the day of ovulation occurring sooner in shorter cycles and later in longer cycles. It is also assumed that the luteal phase is fixed at 14 days, and that it is only the follicular phase that varies. This is untrue as luteal phase lengths are variable, although less variable as compared to the follicular phase. Shortened luteal phases (of approximately less than 10 days) implies inadequate progesterone secretion. Cycle length and follicular phase length tends to decline with age.^{178,306,307}

Testing During the Menstrual Cycle

Hormone testing is often considered for patients planning conception or participating in contraceptive techniques such as natural family planning to determine the infertile period. Since there is inter- and intraindividual variability in cycles, serial collections are preferred to one-time collections making sample types like urine and saliva more practical options as compared to blood.³⁰⁸

Serial collections can also be helpful to assess hormone levels throughout the menstrual cycle when specific symptoms or clinical patterns occur at different points in the cycle, such as migraines or general premenstrual syndrome.

When collected over an entire cycle, the curves for salivary hormones do not differ considerably from those in blood.⁸ Since saliva is a more convenient and less invasive sample type than venipuncture, it is being studied as an alternative to serum hormone monitoring in reproductive medicine.

The Rhythm or Rhythm Plus salivary profiles may provide insight due to its advantageous noninvasive multiple collection data points pertaining to estradiol and progesterone levels and their fluctuations throughout one cycle.

Progesterone: In a normal menstrual cycle, progesterone is lower during the follicular phase, then begins to rise in the days following ovulation.² In a regularly cycling female with a 28-day cycle, progesterone peaks around day 21-23 of the cycle. Progesterone tends to remain elevated days 18-25 in the luteal phase.^{178,304}

During the luteal phase, if there is no fertilized egg, progesterone levels drop, and menstruation begins. If an egg is fertilized, progesterone remains elevated and helps maintain the uterine lining throughout early pregnancy.

Progesterone is thermogenic and basal body temperature can increase by 0.5° C during the luteal phase.³³

Estrogen: In a normal menstrual cycle, E2 is lower during the early follicular phase, then increases in the late follicular phase, peaking during early ovulation, between days 8-12.¹⁷⁸ E2 stimulates endometrial thickening. E2 remains higher during the luteal phase, and when pregnancy does not occur E2 and progesterone levels decrease late in this phase.³³ During pregnancy, E2 levels increase 100-fold.¹⁰⁰

Testosterone: T levels in women are highest between days 8-18 of the menstrual cycle and are lowest in the early follicular and late luteal phase. Midcycle and midluteal levels are similar.¹⁷⁷ On the Rhythm Profile, testosterone is measured using the midluteal day 23 sample.

Ovulation: Ovulation occurs between the estrogen peak day and the rise of progesterone approximately 10-12 hours following the LH surge.³⁰¹ The beginning of the LH surge occurs approximately 34 to 36 hours prior to ovulation and is a relatively precise predictor for timing ovulation.³⁰³ It is possible to ovulate without seeing a LH peak and not ovulate despite seeing a LH peak, bringing into question the reliability of LH as the gold standard indicator of ovulation.³⁰¹

While FSH and LH play important roles in ovulation, neither guarantees that the expected result occurs. Instead, measuring markers that directly relate to the actual physiologic events of follicle growth, ovulation, and corpus luteum formation may be more important.³⁰¹



Genova’s salivary testing may be suggestive of but cannot confirm ovulation. There are several methods used to measure or confirm ovulation:

- Transvaginal ultrasound (TVUS): TVUS is considered the gold standard. It provides a detailed view of the ovaries and is often used as a reference examination to compare hormone levels. However, this may not be the most convenient method for routine use.
- Urinary LH Tests: A popular and convenient over-the-counter test for predicting ovulation is the at-home urinary LH test that identifies the mid-cycle surge of LH that precedes ovulation by 1-2 days.^{78,304,309}
- Blood LH levels: Blood LH levels are also sensitive and specific for ovulation, however without knowing the exact date of ovulation, multiple samples over several days may be invasive and inconvenient.³⁰²
- Estrogen and Progesterone Levels: While FSH and LH play important roles in ovulation, measuring hormones directly may be informative. Studies show good correlation between urine and serum hormones which can be used interchangeably for ovulation confirmation.³¹⁰ One single blood hormone measurement can confirm ovulation, but only if collected on the right day; a clinician or patient may not necessarily know the exact day of their cycle that this happens. The clinician would have to order several blood tests over a span of several days to have comparative reference points for that individual patient.
 - After ovulation has occurred, a serum progesterone or the urinary metabolite of progesterone, pregnanediol glucuronide can confirm ovulation.³⁰² Genova assesses serum progesterone, but not pregnanediol glucuronide. Over-the-counter urinary pregnanediol glucuronide kits have become recently available for confirming ovulation.^{311,312} The literature cites a cutpoint of ≥ 3 -5 ng/mL of a single serum progesterone as an indicator confirming ovulation.^{78,313}
 - Another marker measured prior to ovulation is estradiol in serum or the urinary metabolite estrone glucuronide. Genova assesses serum estradiol, but not estrone glucuronide in urine. Over-the-counter fertility monitor testing can assess both LH and estrone-3-glucuronide.⁷⁸

**Genova has not validated any of the hormone profile reference ranges to specifically predict or confirm ovulation.*

Rhythm Test Interpretation

When interpreting the Rhythm Profile, it is important to assess patterns as well as absolute values of hormones. For symptom information and causes of high/low levels of hormones, visit earlier parts of the guide for information on estradiol, progesterone, and testosterone.

While there is no standard criteria for salivary menstrual cycle assessments, one research group created specific criteria to classify cycles as normal, likely normal, or not normal upon salivary testing (see table 1).⁷ These can be used as a guideline when interpreting the Rhythm Profile.

TABLE 1
Criteria for menstrual cycle assessment
Normal Cycle:
1. Two estradiol peaks are present.
2. Primary (earlier) estradiol peak precedes the second estradiol peak by 5 days or more.
3. Primary peak is higher than the second peak.
4. Progesterone rising over the course of the cycle (a little variation/a few small dips are acceptable).
5. Progesterone peaks within 2 days (+ or -) of the second estradiol peak: (e.g., if estradiol peaks on day 27, progesterone peaks on day 25, 26, 27, 28, or 29)
Likely normal cycle:
One or more of the criteria cannot be judged on account of missing data, but the criteria that can be judged are fulfilled.
Clearly abnormal cycle:
Sufficient data are available and one or more of the criteria for normal cycle are not fulfilled.



Hormone Therapy and Testing Impact

Hormone therapy (HT) or hormone replacement therapy (HRT) prescribing is a widespread practice. Not all hormone therapies are equivalent – clinicians must consider multiple variables including bioidentical versus non-bioidentical prescribing, route of delivery, dose, and testing impact. For more information on hormone supplementation, please see the [“Suggested Reading”](#) section at the end of this document. For information about specific hormones, visit each section: [Progesterone Supplementation and Testing](#), [Estrogen Supplementation and Testing](#), and [Androgen Supplementation and Testing](#).

- Genova Diagnostics’ Assays:** Genova’s hormone assays detect endogenous or bioidentical hormones but do not detect non-bioidentical hormones. Genova does not recommend hormone testing in females taking hormonal contraception, which is generally non-bioidentical. If a patient is taking non-bioidentical hormones, the negative feedback loop may result in lower levels of endogenous hormones.²⁴²
- Baseline Testing Prior to HRT:** Clinicians often utilize testing to establish a baseline to determine if hormones are indicated. They will often follow up with testing after beginning a treatment regimen to monitor the need for titrating the dose. Any sample type is appropriate for establishing baseline levels – serum, saliva, or urine. It is recommended that if a certain sample type is chosen for baseline measurement, that the same sample type be utilized for monitoring. There are multiple caveats to consider with each sample type and hormone regimen before choosing the ideal sample type for testing.
- Compounded versus Conventional HRT:** Customized compounding presents unique challenges for laboratories that test hormones. Healthcare practitioners and pharmacists are generally not aware of safety and efficacy of bioidentical hormone therapy and compounding as compared to conventional preparations.³¹⁴ Since the Women’s Health Initiative (WHI) landmark study published in 2002 that ended early due to risks with hormone therapy, clinicians and patients have been skeptical of conventional preparations and thus turned to the practice of prescribing compounded bioidentical formulations. It was assumed that risk was higher with oral hormones, so transdermal preparations have become popular. With the onset of more custom compounding came the desire for patient monitoring through testing and hence the need for reliable and convenient testing methods.
- Salivary Testing with HRT:** There is literature using salivary hormone testing for HRT monitoring. However, studies are lacking that provide guidance around when to test with regards to route of administration and timing of dose. The clinical application of some of these studies as it relates to increasing or decreasing dosages is not yet fully elucidated.
- Urinary Testing with HRT:** If clinicians want to see absolute urinary estrogen concentrations as well as estrogen metabolism in supplemented patients, a 24-hour urine collection can account for peak and trough levels. 24-hour testing removes the problem of variable daily fluctuations in hormone levels seen with hormone supplementation. If the clinician is only interested in how a supplemented patient is metabolizing estrogens, an FMV collection may be acceptable. Please note, Endo+ includes FMV only. Clinicians wanting to see 24-hour urine results can order Essential Estrogens as a stand-alone profile.
 - Many clinicians assess absolute estrogen values with saliva or serum and use urine strictly for assessing estrogen metabolism. It is unclear how hormone therapies influence estrogen metabolism pathways. (Note: These are general guidelines. Literature to guide best practices with regards to urine collection and interpretation in supplemented patients is lacking.)
- Testing vs. Symptom Monitoring:** Expert organizations generally do not recommend monitoring hormones with testing, but rather to adjust dose based on symptoms only, regardless of hormone levels. This is mainly due to lack of high-powered studies demonstrating optimal hormone levels with hormone therapy.³¹⁵ Still, some clinicians prefer to have some level of guidance offered by testing to determine if therapies are significantly off base, or if there is a true deficiency or excess prior to treatment. Functional medicine organizations encourage clinicians to test regularly to ensure levels are not too elevated, and to combine laboratory and clinical information to make decisions about HT.
- Lasting Impacts:** Persistent elevations may be seen on salivary testing even after discontinuation of hormone creams. It is unclear as to why salivary levels remain elevated for sometimes months after discontinuation, especially progesterone, and other hormones to a lesser degree. Progesterone is very lipophilic and along with lipophilic ingredients of creams, may saturate the fatty layer below the dermis.¹⁰⁴ Age, hydration and skin condition are among many factors that affect absorption of drugs across the skin, leading to wide interindividual variability.¹⁵
- Unintentional Exposure:** Unintentional exposure can occur and should be taken into account with unexpected elevations in hormone levels and/or symptoms of hormone excess.³¹⁶⁻³²⁰ Exposure can be from close contact with others using prescription or over the counter transdermals including family members or occupational (i.e., massage therapy, pharmacies, health care facilities), and exposure to items that a person using transdermals touched including gym equipment, towels, doorknobs, sheets, etc.. Additionally, over-the-counter products can contain hormones including anti-aging and other creams and lotions, cosmetics, and body care products.



- *The degree of impact that therapies (whether intentional or unintentional) may have on an individual's hormone levels is highly individual and is determined by many variables: dose, route of administration, site of administration, timing of administration, acute versus chronic absorption (steady state), formulation and pharmacokinetics of the preparation, other medications, alcohol ingestion, patient compliance, adiposity, and patient metabolic individuality.*^{15,103,321-323}

Common Hormone Therapy Misconceptions

There are several common misconceptions with hormone prescribing that must be considered, especially when hormone testing is involved.

Misconception: Bioidentical hormones must be compounded.

- A bioidentical hormone simply refers to the chemical structure of the hormone as matching that of an endogenous-produced human hormone. In fact, there are several commercial products that have been FDA-approved that are bioidentical.¹⁰¹
- Conservative indications for compounding include allergy to a component of the FDA-approved preparation, or if a desired dose or an FDA-approved preparation is unavailable despite being scientifically acceptable.³²⁴ Still, many clinicians choose compounded bioidentical hormones due to the benefits observed in clinical practice, ability to create customized formulations, and more natural or hypoallergenic additives and/or inactive ingredients.
- **Impact on testing:** *Genova's hormone assays detect bioidentical hormones, versus non-bioidentical or unique equine estrogens. Both compounded and commercially available FDA-approved bioidentical hormones can be detected using Genova's hormone assays.*

Misconception: Bioidentical hormones are natural, not synthetic.

- All hormones are synthetic with the exception of one preparation: conjugated equine estrogens (CEE). This commercial product, widely known as Premarin, is truly a natural hormone because it contains estrogen from horse urine, a natural product. However, even though this product is technically "natural", some argue that there are estrogens unique to horses that a human body may not recognize or metabolize in a healthy manner. All other products have some level of synthesis in the manufacturing process, including plant sources used as precursors such as yams or soybeans.¹⁰¹

- **Impact on testing:** *Genova's hormone assays detect bioidentical hormones, versus non-bioidentical or unique equine estrogens. Genova does not recommend hormone testing in females taking hormonal contraception, which is generally non-bioidentical. If a patient is taking non-bioidentical hormones, the negative feedback loop may result in lower levels of endogenous hormones.*²⁴²

Misconception: Bioidentical hormones are safer.

- The WHI showed risk with oral CEE and medroxyprogesterone, but not oral CEE alone. Subsequent studies using FDA-cleared bioidentical hormone preparations such as topical estradiol with oral micronized progesterone have shown a better risk profile. Still, all hormone therapy has risk and there are no randomized trials demonstrating superior safety of bioidentical over non-bioidentical.³²⁵⁻³²⁸
- **Impact on testing:** *There are no standard reference ranges that would be considered "safe" reference ranges for any hormone regimen. A "supplemented healthy" reference range determined by individual laboratories simply shows a range for patients taking hormones that are currently asymptomatic. Each laboratory is responsible for setting reference ranges and there are numerous ways of doing so. Genova has "supplemented healthy" ranges for saliva testing. Reference ranges do not guarantee long-term safety.*

Misconception: Compounded hormones are safer.

- There are no long-term safety studies on compounded hormone formulations. A few studies assessing short term safety and efficacy of compounded formulations show favorable results.³²⁹⁻³³¹ However, it is known that more serious diseases such as cancer or heart disease require longer-term studies. FDA-cleared preparations are required to include black-box warnings related to conditions such as cancer or cardiovascular disease risk. Compounded products are not required to have warning labels, which may add to the misconception that compounded bioidentical products are safer.
- **Impact on testing:** *Being that there are no long-term safety studies on compounded hormones, there are no standard reference ranges that would be considered "safe" reference ranges. A "supplemented healthy" reference range determined by individual laboratories simply shows a range for patients taking hormones that are currently asymptomatic. Each laboratory is responsible for setting reference ranges and there are numerous ways of doing so. Genova has "supplemented healthy" ranges for saliva testing. Reference ranges do not guarantee long-term safety.*

**Misconception: Compounded products are equivalent to commercially produced, FDA-approved products.**

- Studies show that FDA-approved bioidentical hormone doses are consistent from batch to batch as compared to compounded products which can vary significantly. However, high-volume compounding pharmacies seem to show better consistency and accuracy.^{332,333} A study assessing testosterone concentrations in gels and creams that were compounded versus commercially produced showed significant variability among the compounded formulations compared to consistent doses in the commercially prepared products.²⁸⁴
- Furthermore, the inactive substances that serve as the vehicle or medium of hormone delivery or other ingredients mixed with the hormone(s) may affect the pharmacokinetics of the product, thus affecting the dose delivered to tissues.¹⁰³ For example, a study comparing transdermal carrier Vanicream with VersaBase cream showed that VersaBase delivers more than four times as much progesterone to the dermis.³³⁴
- The Women's Health Initiative (WHI) is an ongoing study that assesses the safety of FDA-approved hormones, including FDA-approved bioidentical hormones. However, the data from this study cannot be extrapolated to compounded formulations since they are not standardized and contain other ingredients that can affect the pharmacokinetics of the product.
- In addition to compounded products, it is important for practitioners to be aware that hormone creams and supplements are readily available over the counter, particularly those containing pregnenolone, progesterone, estriol, and DHEA.¹⁰⁶ Patients may experiment with hormones to alleviate symptoms and it is important to query patients on their use of these unregulated supplements.
- **Impact on testing:** *The possibility of variability in compounded formulations or over the counter supplements must be considered when interpreting test results. It is unknown how each hormone preparation may impact test results. For example, a compounded estradiol cream may have different ingredients as compared to a conventional topical estradiol preparation which may change the pharmacokinetics of the product. Pharmacokinetic studies on a commercially produced product cannot necessarily be extrapolated to a similar compounded or over-the-counter product.*

Suggested Reading

- Reed BG, Carr BR. [The Normal Menstrual Cycle and the Control of Ovulation](#) Endotext. Updated 2018. South Dartmouth (MA): MDText.com, Inc.; 2000.
- Schiffer L, et. al. [Hormone biosynthesis and metabolism: a comprehensive review](#) J Steroid Biochem Mol Biol. 2019 Nov;194:105439.
- Hodges RE, Minich DM. [Modulation of Metabolic Detoxification Pathways Using Foods and Food-Derived Components: A Scientific Review with Clinical Application](#) J Nutr Metab. 2015;2015:760689.
- Dandona P, Rosenberg MT. [A practical guide to male hypogonadism in the primary care setting](#) Int J Clin Pract. 2010 May;64(6):682-96.
- Handelsman DJ. [Androgen Physiology, Pharmacology, Use and Misuse](#) Endotext. Updated 2020. South Dartmouth (MA): MDText.com, Inc.; 2000.
- Davis SR, et. al. [Global Consensus Position Statement on the Use of Testosterone Therapy for Women](#) J Clin Endocrinol Metab. 2019 Oct;104(10):4660-4666.
- Parish SJ, et. al. [International Society for the Study of Women's Sexual Health Clinical Practice Guidelines for the Use of Systemic Testosterone for Hypoactive Sexual Desire Disorder in Women](#) J Womens Health (Larchmt). 2021 Apr;30(4):474-491.
- Kuhl H. [Pharmacology of estrogens and progestens: influence of different routes of administration](#) Climacteric. 2005;8 Suppl 1:3-63.
- Stanczyk FZ, et. al. [Progestogens Used in Postmenopausal Hormone Therapy: Differences in Their Pharmacological Properties, Intracellular Actions, and Clinical Effects](#) Endocr Rev. 2013 Apr;34(2):171-208.
- Stanczyk FZ, Matharu H, Winer SA. [Bioidentical Hormones](#) Climacteric. 2021 Feb;24(1):38-45.



References

- Schiffer L, Barnard L, Baranowski ES, et al. Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: A comprehensive review. *J Steroid Biochem Mol Biol*. 2019;194:105439.
- Taraborrelli S. Physiology, production and action of progesterone. *Acta Obstetrica Gynecol Scand*. 2015;94 Suppl 161:8-16.
- Konishi S, Brindle E, Guyton A, O'Connor KA. Salivary concentration of progesterone and cortisol significantly differs across individuals after correcting for blood hormone values. *Am J Phys Anthropol*. 2012;149(2):231-241.
- Coburn SB, Stanczyk FZ, Falk RT, et al. Comparability of serum, plasma, and urinary estrogen and estrogen metabolite measurements by sex and menopausal status. *Cancer Causes Control* 2019;30(1):75-86.
- Rahman SA, Grant LK, Gooley JJ, Rajaratnam SMW, Czeisler CA, Lockley SW. Endogenous Circadian Regulation of Female Reproductive Hormones. *J Clin Endocrinol Metab*. 2019;104(12):6049-6059.
- Gavrilova N, Lindau ST. Salivary sex hormone measurement in a national, population-based study of older adults. *J Gerontol Series B, Psychol Sci Soc Sci*. 2009;64 Suppl 1(Suppl 1):i94-105.
- Gandara BK, Leresche L, Mancl L. Patterns of salivary estradiol and progesterone across the menstrual cycle. *Ann NY Acad Sci*. 2007;1098:446-450.
- Celec P, Ostaniková D, Skoknová M, Hodosy J, Putz Z, Kúdela M. Salivary sex hormones during the menstrual cycle. *Endocrine J*. 2009;56(3):521-523.
- Huang T, Howse FM, Stachenfeld NS, Usselman CW. Correlations between salivary- and blood-derived gonadal hormone assessments and implications for inclusion of female participants in research studies. *Am J Phys Heart Circ Physiol*. 2023;324(1):H33-h46.
- Thomasson R, Baillot A, Jollin L, et al. Correlation between plasma and saliva adrenocortical hormones in response to submaximal exercise. *J Physiol Sci*. 2010;60(6):435-439.
- Dielen C, Fiers T, Somers S, Deschepper E, Gerris J. Correlation between saliva and serum concentrations of estradiol in women undergoing ovarian hyperstimulation with gonadotropins for IVF/ICSI. *Facts, Views Vision ObGyn*. 2017;9(2):85-91.
- Keevil BG, MacDonald P, Macdonald W, Lee DM, Wu FC. Salivary testosterone measurement by liquid chromatography tandem mass spectrometry in adult males and females. *Ann Clin Biochem*. 2014;51(Pt 3):368-378.
- Chatterton RT, Jr., Mateo ET, Hou N, et al. Characteristics of salivary profiles of oestradiol and progesterone in premenopausal women. *J Endocrinol*. 2005;186(1):77-84.
- Higashi T. Salivary hormone measurement using LC/MS/MS: specific and patient-friendly tool for assessment of endocrine function. *Bio Pharmaceut Bull*. 2012;35(9):1401-1408.
- Zargar-Shoshtari S, Wahhabaghei H, Mehraei A, Wen J, Alany R. Transdermal delivery of bioidentical progesterone using dutasteride (A 5 α -reductase inhibitor): a pilot study. *J Pharm Pharmaceut Sci*. 2010;13(4):626-636.
- Rottiers AS, Dalewyn L, Somers S, Alper MM, Sakkas D, Gerris J. Correlation between sonographic follow-up of follicular growth, serum and salivary estradiol in women undergoing controlled ovarian stimulation (IVF/ICSI). *Facts Views Vision ObGyn*. 2018;10(4):173-179.
- Hofman LF. Human saliva as a diagnostic specimen. *J Nutrition*. 2001;131(5):1621s-1625s.
- Granger DA, Taylor MK. *Saliv Biosci*. Springer Nature; 2020.
- Behr GA, Patel JP, Coote M, et al. A statistical method to calculate blood contamination in the measurement of salivary hormones in healthy women. *Clin Biochem*. 2017;50(7-8):436-439.
- Pedersen AML, Sørensen CE, Proctor GB, Carpenter GH, Ekström J. Salivary secretion in health and disease. *J Oral Rehab*. 2018;45(9):730-746.
- Fuhrman BJ, Xu X, Falk RT, et al. Assay reproducibility and interindividual variation for 15 serum estrogens and estrogen metabolites measured by liquid chromatography-tandem mass spectrometry. *Cancer Epidemiol Biomarkers Prevent*. 2014;23(12):2649-2657.
- Faupel-Badger JM, Fuhrman BJ, Xu X, et al. Comparison of liquid chromatography-tandem mass spectrometry, RIA, and ELISA methods for measurement of urinary estrogens. *Cancer Epidemiol Biomarkers Prevent*. 2010;19(1):292-300.
- Eliassen AH, Spiegelman D, Xu X, et al. Urinary estrogens and estrogen metabolites and subsequent risk of breast cancer among premenopausal women. *Cancer Res*. 2012;72(3):696-706.
- Maskarinec G, Beckford F, Morimoto Y, Franke AA, Stanczyk FZ. Association of estrogen measurements in serum and urine of premenopausal women. *Biomarkers Med*. 2015;9(5):417-424.
- Speight AC, Hancock KW, Oakey RE. Non-protein-bound oestrogens in plasma and urinary excretion of unconjugated oestrogens in non-pregnant women. *J Endocrinol*. 1979;83(3):385-391.
- Speight AC, Hancock KW, Oakey RE. Non-protein-bound oestrogens in plasma and urinary excretion of unconjugated oestrogens in men. *Clin Endocrinol*. 1979;10(4):329-341.
- Munro CJ, Stabenfeldt GH, Cragun JR, Addiego LA, Overstreet JW, Lasley BL. Relationship of serum estradiol and progesterone concentrations to the excretion profiles of their major urinary metabolites as measured by enzyme immunoassay and radioimmunoassay. *Clin Chem*. 1991;37(6):838-844.
- Baker TS, Jennison KM, Kellie AE. The direct radioimmunoassay of oestrogen glucuronides in human female urine. *Biochem J*. 1979;177(2):729-738.
- Stanczyk FZ, Miyakawa I, Goebelsmann U. Direct radioimmunoassay of urinary estrogen and pregnenediol glucuronides during the menstrual cycle. *Am J Obst Gynecol*. 1980;137(4):443-450.
- Biezanski JJ, Millner SN. Correlation between morning urine estriol concentration and 24-hour estriol excretion. *Obstetrics and gynecology*. 1976;48(6):678-681.
- Baker TS, Jennison KM, Kellie AE. The direct radioimmunoassay of oestrogen glucuronides in human female urine. *Biochem J*. 1979;177(2):729-738.
- Medscape. progesterone micronized (Rx). *Drugs Dis* 2023; 2023.
- Knudtson. *Female Reproductive Endocrinology*. In: Merck Manual.2019:.
- Stanczyk FZ, Hapgood JP, Winer S, Mishell DR, Jr. Progestogens used in postmenopausal hormone therapy: differences in their pharmacological properties, intracellular actions, and clinical effects. *Endo Rev*. 2013;34(2):171-208.
- Regidor PA. Progesterone in Peri- and Postmenopause: A Review. *Geburtshilfe und Frauenheilkunde*. 2014;74(11):995-1002.
- Modi DN, Shah C, Puri CP. Non-genomic membrane progesterone receptors on human spermatozoa. *Soc Reproduct Fertility Suppl*. 2007;63:515-529.
- Oettel M, Mukhopadhyay AK. Progesterone: the forgotten hormone in men? *Aging Male*. 2004;7(3):236-257.
- De Groot LJ. *JL Endocrinol Adult Ped*. 6th ed. Philadelphia, PA: Saunders Elsevier; 2010.
- Kimball A, Dichtel LE, Nyer MB, et al. The allopregnanolone to progesterone ratio across the menstrual cycle and in menopause. *Psychoneuroendocrinology*. 2020;112:104512.



40. Sundström-Poromaa I, Comasco E, Sumner R, Luders E. Progesterone - Friend or foe? *Front Neuroendocrinol.* 2020;59:100856.
41. Martinez PE, Rubinow DR, Nieman LK, et al. 5 α -Reductase Inhibition Prevents the Luteal Phase Increase in Plasma Allopregnanolone Levels and Mitigates Symptoms in Women with Premenstrual Dysphoric Disorder. *Neuropsychopharmacology.* 2016;41(4):1093-1102.
42. Apgar BS, Greenberg G. Using progestins in clinical practice. *Am Family Phys.* 2000;62(8):1839-1846, 1849-1850.
43. Cappelletti M, Wallen K. Increasing women's sexual desire: The comparative effectiveness of estrogens and androgens. *Hormones Behav.* 2016;78:178-193.
44. Herrera AY, Nielsen SE, Mather M. Stress-induced increases in progesterone and cortisol in naturally cycling women. *Neurobiol Stress.* 2016;3:96-104.
45. Glick ID, Bennett SE. Psychiatric complications of progesterone and oral contraceptives. *J Clin Psychopharmacol.* 1981;1(6):350-367.
46. Standeven LR, McEvoy KO, Osborne LM. Progesterone, reproduction, and psychiatric illness. *Best practice & research Clin Obst Gynaecol.* 2020;69:108-126.
47. Morssinkhof MWL, van Wylick DW, Priester-Vink S, et al. Associations between sex hormones, sleep problems and depression: A systematic review. *Neurosci Biobehav Rev.* 2020;118:669-680.
48. Charkoudian N, Stachenfeld N. Sex hormone effects on autonomic mechanisms of thermoregulation in humans. *Autonomic neurosci.* 2016;196:75-80.
49. Oztekin D, Senkaya AR, Gunes ME, Keskin O, Dogdu IA. Early Initiation and Long-Term Use of Vaginal Progesterone may Cause Gestational Diabetes Mellitus. *Zeitschrift fur Geburtshilfe und Neonatologie.* 2022;226(3):173-177.
50. Calabria A. Congenital Adrenal Hyperplasia Caused by 21-Hydroxylase Deficiency. *Merck Manual* 2020.
51. Kawai Y, Ishikawa H, Segawa T, Teramoto S, Tanaka T, Shozu M. High serum progesterone associated with infertility in a woman with nonclassic congenital adrenal hyperplasia. *J Obst Gynaecol Res.* 2017;43(5):946-950.
52. Wingfield M, O'Herlihy C, Finn MM, Tallon DF, Fottrell PF. Follicular and luteal phase salivary progesterone profiles in women with endometriosis and infertility. *Gynecol Endocrinol.* 1994;8(1):21-25.
53. Vuorento T, Hovatta O, Kurunmäki H, Ratsula K, Huhtaniemi I. Measurements of salivary progesterone throughout the menstrual cycle in women suffering from unexplained infertility reveal high frequency of luteal phase defects. *Fertility Sterility.* 1990;54(2):211-216.
54. Ku CW, Allen JC, Jr., Lek SM, Chia ML, Tan NS, Tan TC. Serum progesterone distribution in normal pregnancies compared to pregnancies complicated by threatened miscarriage from 5 to 13 weeks gestation: a prospective cohort study. *BMC Preg Childbirth.* 2018;18(1):360.
55. Matsuoka A, Tate S, Nishikimi K, Ishikawa H, Shozu M. Elevated serum progesterone levels in postmenopausal women with mucinous ovarian tumors. *Menopause.* 2016;23(5):544-549.
56. Heinonen PK, Koivula T, Pystynen P. Elevated progesterone levels in serum and ovarian venous blood in patients with ovarian tumors. *Acta Obst Gynecol Scand.* 1985;64(8):649-652.
57. Oliver R, Pillarisetty LS. Anatomy, Abdomen and Pelvis, Ovary Corpus Luteum. In: *StatPearls.* Treasure Island (FL): StatPearls Publishing LLC.; 2022.
58. Kotsopoulos J, Eliassen AH, Missmer SA, Hankinson SE, Tworoger SS. Relationship between caffeine intake and plasma sex hormone concentrations in premenopausal and postmenopausal women. *Cancer.* 2009;115(12):2765-2774.
59. Zumoff B, Miller L, Levit CD, et al. The effect of smoking on serum progesterone, estradiol, and luteinizing hormone levels over a menstrual cycle in normal women. *Steroids.* 1990;55(11):507-511.
60. 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. *Fertility Sterility.* 2003;80(2):459-461.
61. Maruyama K, Oshima T, Ohyama K. Exposure to exogenous estrogen through intake of commercial milk produced from pregnant cows. *Ped Int.* 2010;52(1):33-38.
62. Wirth MM. Beyond the HPA Axis: Progesterone-Derived Neuroactive Steroids in Human Stress and Emotion. *Front Endocrinol.* 2011;2:19.
63. Wirth MM, Meier EA, Fredrickson BL, Schultheiss OC. Relationship between salivary cortisol and progesterone levels in humans. *Biol Psychol.* 2007;74(1):104-107.
64. Wirth MM, Schultheiss OC. Effects of affiliation arousal (hope of closeness) and affiliation stress (fear of rejection) on progesterone and cortisol. *Hormones Behavior.* 2006;50(5):786-795.
65. Lewis JG, McGill H, Patton VM, Elder PA. Caution on the use of saliva measurements to monitor absorption of progesterone from transdermal creams in postmenopausal women. *Maturitas.* 2002;41(1):1-6.
66. Stanczyk FZ. Treatment of postmenopausal women with topical progesterone creams and gels: are they effective? *Climacteric.* 2014;17 Suppl 2:8-11.
67. Matsui F, Koh E, Yamamoto K, et al. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay for simultaneous measurement of salivary testosterone and cortisol in healthy men for utilization in the diagnosis of late-onset hypogonadism in males. *Endo J.* 2009;56(9):1083-1093.
68. Gaskins AJ, Mumford SL, Wactawski-Wende J, Schisterman EF. Effect of daily fiber intake on luteinizing hormone levels in reproductive-aged women. *Eur J Nutr.* 2012;51(2):249-253.
69. Gaskins AJ, Mumford SL, Zhang C, et al. Effect of daily fiber intake on reproductive function: the BioCycle Study. *Am J Clin Nutr.* 2009;90(4):1061-1069.
70. Kossman DA, Williams NI, Domchek SM, Kurzer MS, Stopfer JE, Schmitz KH. Exercise lowers estrogen and progesterone levels in premenopausal women at high risk of breast cancer. *J Applied Physiol.* 2011;111(6):1687-1693.
71. Williams NI, Reed JL, Leidy HJ, Legro RS, De Souza MJ. Estrogen and progesterone exposure is reduced in response to energy deficiency in women aged 25-40 years. *Human Reproduct.* 2010;25(9):2328-2339.
72. Knight JA, Wong J, Blackmore KM, Raboud JM, Vieth R. Vitamin D association with estradiol and progesterone in young women. *Cancer Causes Control* 2010;21(3):479-483.
73. Sanchez EG, Giviziez CR, Sanchez HM, Agostinho PL, Barros PS, Approbato MS. Low progesterone levels and ovulation by ultrasound assessment in infertile patients. *JBRA Assisted Reproduct.* 2016;20(1):13-16.
74. Ziolkiewicz A, Pawlowski B, Ellison PT, Lipson SF, Thune I, Jasienska G. Higher luteal progesterone is associated with low levels of premenstrual aggressive behavior and fatigue. *Biol Psychol.* 2012;91(3):376-382.
75. Vorherr H. Fibrocystic breast disease: pathophysiology, pathomorphology, clinical picture, and management. *Am J Obst Gynecol.* 1986;154(1):161-179.
76. Eren T, Aslan A, Ozemir IA, et al. Factors Effecting Mastalgia. *Breast Care.* 2016;11(3):188-193.
77. Macgregor EA. Menstrual migraine: therapeutic approaches. *Thera Adv Neurol Dis.* 2009;2(5):327-336.



78. Schliep KC, Mumford SL, Hammoud AO, et al. Luteal phase deficiency in regularly menstruating women: prevalence and overlap in identification based on clinical and biochemical diagnostic criteria. *J Clin Endocrinol Metab*. 2014;99(6):E1007-1014.
79. Andrews MA, Schliep KC, Wactawski-Wende J, et al. Dietary factors and luteal phase deficiency in healthy eumenorrheic women. *Human Reproduct*. 2015;30(8):1942-1951.
80. Rochester D, Jain A, Polotsky AJ, et al. Partial recovery of luteal function after bariatric surgery in obese women. *Fertility Sterility*. 2009;92(4):1410-1415.
81. Gude D. Thyroid and its indispensability in fertility. *J Human Reproduct Sci*. 2011;4(1):59-60.
82. Diagnosis and treatment of luteal phase deficiency: a committee opinion. *Fertility Sterility*. 2021;115(6):1416-1423.
83. Tremellen K, Syedi N, Tan S, Pearce K. Metabolic endotoxaemia—a potential novel link between ovarian inflammation and impaired progesterone production. *Gynecol Endocrinol*. 2015;31(4):309-312.
84. Maguire MJ, Nevitt SJ. Treatments for seizures in catamenial (menstrual-related) epilepsy. *Cochrane Database Systematic Rev*. 2021;9(9):Cd013225.
85. Goh JY, He S, Allen JC, Malhotra R, Tan TC. Maternal obesity is associated with a low serum progesterone level in early pregnancy. *Hormone Mol Biol Clin Invest*. 2016;27(3):97-100.
86. Blanchette S, Marceau P, Biron S, Brochu G, Tchernof A. Circulating progesterone and obesity in men. *Hormone Metab Res*. 2006;38(5):330-335.
87. Carlson MJ, Thiel KW, Yang S, Leslie KK. Catch it before it kills: progesterone, obesity, and the prevention of endometrial cancer. *Discovery Med*. 2012;14(76):215-222.
88. Lukanova A, Kaaks R. Endogenous hormones and ovarian cancer: epidemiology and current hypotheses. *Cancer Epidemiol Biomarkers Prevent*. 2005;14(1):98-107.
89. Boneva RS, Maloney EM, Lin JM, et al. Gynecological history in chronic fatigue syndrome: a population-based case-control study. *J Womens Health (2002)*. 2011;20(1):21-28.
90. Xiao E, Xia-Zhang L, Ferin M. Inadequate luteal function is the initial clinical cyclic defect in a 12-day stress model that includes a psychogenic component in the Rhesus monkey. *J Clin Endocrinol Metab*. 2002;87(5):2232-2237.
91. Xiao E, Xia-Zhang L, Barth A, Zhu J, Ferin M. Stress and the menstrual cycle: relevance of cycle quality in the short- and long-term response to a 5-day endotoxin challenge during the follicular phase in the rhesus monkey. *J Clin Endocrinol Metab*. 1998;83(7):2454-2460.
92. Sarkola T, Mäkilä H, Fukunaga T, Eriksson CJ. Acute effect of alcohol on estradiol, estrone, progesterone, prolactin, cortisol, and luteinizing hormone in premenopausal women. *Alcoholism, clinical and experimental research*. 1999;23(6):976-982.
93. Rachdaoui N, Sarkar DK. Effects of alcohol on the endocrine system. *Endocrinol Metab Clin North Am*. 2013;42(3):593-615.
94. Meenakumari KJ, Agarwal S, Krishna A, Pandey LK. Effects of metformin treatment on luteal phase progesterone concentration in polycystic ovary syndrome. *Brazil J Med Biol Res*. 2004;37(11):1637-1644.
95. Hernández García IA, Gutiérrez Gutiérrez AM, Gallardo Lozano E. [Effect of weight reduction on the clinical and hormonal condition of obese anovulatory women]. *Ginecol Obstetr Mexico*. 1999;67:433-437.
96. Korevaar TIM, Derakhshan A, Taylor PN, et al. Association of Thyroid Function Test Abnormalities and Thyroid Autoimmunity With Preterm Birth: A Systematic Review and Meta-analysis. *JAMA*. 2019;322(7):632-641.
97. Datta M, Roy P, Banerjee J, Bhattacharya S. Thyroid hormone stimulates progesterone release from human luteal cells by generating a proteinaceous factor. *J Endocrinol*. 1998;158(3):319-325.
98. Westphal LM, Polan ML, Trant AS. Double-blind, placebo-controlled study of Fertilityblend: a nutritional supplement for improving fertility in women. *Clin Exp Obstet Gynecol*. 2006;33(4):205-208.
99. van Die MD, Burger HG, Teede HJ, Bone KM. Vitex agnus-castus extracts for female reproductive disorders: a systematic review of clinical trials. *Planta medica*. 2013;79(7):562-575.
100. Kuhl H. Pharmacology of estrogens and progestogens: influence of different routes of administration. *Climacteric*. 2005;8 Suppl 1:3-63.
101. Stanczyk FZ, Matharu H, Winer SA. Bioidentical hormones. *Climacteric*. 2021;24(1):38-45.
102. Bolaji, II, Tallon DF, O'Dwyer E, Fottrell PF. Assessment of bioavailability of oral micronized progesterone using a salivary progesterone enzymeimmunoassay. *Gynecol Endocrinol*. 1993;7(2):101-110.
103. 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.
104. Stanczyk FZ, Paulson RJ, Roy S. Percutaneous administration of progesterone: blood levels and endometrial protection. *Menopause*. 2005;12(2):232-237.
105. Constantine GD, Kessler G, Graham S, Goldstein SR. Increased Incidence of Endometrial Cancer Following the Women's Health Initiative: An Assessment of Risk Factors. *J Womens Health (2002)*. 2019;28(2):237-243.
106. Hermann AC, Nafziger AN, Victory J, Kulawy R, Rocci ML, Jr., Bertino JS, Jr. Over-the-counter progesterone cream produces significant drug exposure compared to a food and drug administration-approved oral progesterone product. *J Clin Pharmacol*. 2005;45(6):614-619.
107. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and actions of estrogens. *NEJM*. 2002;346(5):340-352.
108. Qureshi R, Picon-Ruiz M, Aurrekoetxea-Rodriguez I, et al. The Major Pre- and Postmenopausal Estrogens Play Opposing Roles in Obesity-Driven Mammary Inflammation and Breast Cancer Development. *Cell Metab*. 2020;31(6):1154-1172.e1159.
109. Raftogianis R, Creveling C, Weinshilboum R, Weisz J. Estrogen metabolism by conjugation. *J Nat Cancer Inst Monographs*. 2000(27):113-124.
110. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. *Trends Mol Med*. 2013;19(3):197-209.
111. Simpson ER, Merrill JC, Hollub AJ, Graham-Lorence S, Mendelson CR. Regulation of estrogen biosynthesis by human adipose cells. *Endocrine Rev*. 1989;10(2):136-148.
112. Rocha-Rodrigues S, Sousa M, Lourenço Reis P, et al. Bidirectional Interactions between the Menstrual Cycle, Exercise Training, and Macronutrient Intake in Women: A Review. *Nutrients*. 2021;13(2).
113. Patel S, Homaei A, Raju AB, Meher BR. Estrogen: The necessary evil for human health, and ways to tame it. *Biomed Pharmacotherap*. 2018;102:403-411.
114. Cooke PS, Nanjappa MK, Ko C, Prins GS, Hess RA. Estrogens in Male Physiology. *Physiol Rev*. 2017;97(3):995-1043.
115. Cohen PG. Aromatase, adiposity, aging and disease. The hypogonadal-metabolic-atherogenic-disease and aging connection. *Med Hypoth*. 2001;56(6):702-708.
116. Ziegler RG, Fuhrman BJ, Moore SC, Matthews CE. Epidemiologic studies of estrogen metabolism and breast cancer. *Steroids*. 2015;99(Pt A):67-75.
117. Song WC. Biochemistry and reproductive endocrinology of estrogen sulfotransferase. *Ann NY Acad Sci*. 2001;948:43-50.
118. Ervin SM, Redinbo MR. The Gut Microbiota Impact Cancer Etiology through "Phase IV Metabolism" of Xenobiotics and Endobiotics. *Cancer Prevent Res*. 2020;13(8):635-642.



119. Huang H, Lan BD, Zhang YJ, et al. Inhibition of Human Sulfotransferases by Phthalate Monoesters. *Front Endocrinol.* 2022;13:868105.
120. Prusakiewicz JJ, Harville HM, Zhang Y, Ackermann C, Voorman RL. Parabens inhibit human skin estrogen sulfotransferase activity: possible link to paraben estrogenic effects. *Toxicology.* 2007;232(3):248-256.
121. Kwa M, Plottel CS, Blaser MJ, Adams S. The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer. *J Nat Cancer Instit.* 2016;108(8).
122. Starek-Świechowicz B, Budziszewska B, Starek A. Endogenous estrogens-breast cancer and chemoprevention. *Pharmacol Rep.* 2021;73(6):1497-1512.
123. Sacco S, Ricci S, Degan D, Carolei A. Migraine in women: the role of hormones and their impact on vascular diseases. *J Headache Pain.* 2012;13(3):177-189.
124. Brandes JL. The influence of estrogen on migraine: a systematic review. *JAMA.* 2006;295(15):1824-1830.
125. Delaruelle Z, Ivanova TA, Khan S, et al. Male and female sex hormones in primary headaches. *J Headache Pain.* 2018;19(1):117.
126. Cheng G, Yeater KM, Hoyer LL. Cellular and molecular biology of *Candida albicans* estrogen response. *Eukaryotic Cell.* 2006;5(1):180-191.
127. Seippel L, Bäckström T. Luteal-phase estradiol relates to symptom severity in patients with premenstrual syndrome. *J Clinical Endocrinol Metab.* 1998;83(6):1988-1992.
128. Stachenfeld NS. Sex hormone effects on body fluid regulation. *Exercise Sport Sci Rev.* 2008;36(3):152-159.
129. Stachenfeld NS. Hormonal changes during menopause and the impact on fluid regulation. *Reproduct Dci.* 2014;21(5):555-561.
130. Oriel KA, Schrager S. Abnormal uterine bleeding. *Am Family Phys.* 1999;60(5):1371-1380; discussion 1381-1372.
131. van den Beld AW, de Jong FH, Grobbee DE, Pols HA, Lamberts SW. Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density, and body composition in elderly men. *J Clinical Endocrinol Metab.* 2000;85(9):3276-3282.
132. Huang Z, Willett WC, Colditz GA, et al. Waist circumference, waist:hip ratio, and risk of breast cancer in the Nurses' Health Study. *Am J Epidemiol.* 1999;150(12):1316-1324.
133. Mancini A, Milardi D, Bianchi A, Summaria V, De Marinis L. Increased estradiol levels in venous occlusive disorder: a possible functional mechanism of venous leakage. *Int J Impotence Res.* 2005;17(3):239-242.
134. Suzuki K, Ito K, Ichinose Y, et al. Endocrine environment of benign prostatic hyperplasia: prostate size and volume are correlated with serum estrogen concentration. *Scand J Urol Nephrol.* 1995;29(1):65-68.
135. Jasonni VM, Bulletti C, Bolelli GF, et al. Estrone sulfate, estrone and estradiol concentrations in normal and cirrhotic postmenopausal women. *Steroids.* 1983;41(5):569-573.
136. Olivo J, Gordon GG, Rafii F, Southren AL. Estrogen metabolism in hyperthyroidism and in cirrhosis of the liver. *Steroids.* 1975;26(1):47-56.
137. Wiggs AG, Chandler JK, Aktas A, Sumner SJ, Stewart DA. The Effects of Diet and Exercise on Endogenous Estrogens and Subsequent Breast Cancer Risk in Postmenopausal Women. *Front Endocrinol.* 2021;12:732255.
138. Trabert B, Coburn SB, Falk RT, et al. Circulating estrogens and postmenopausal ovarian and endometrial cancer risk among current hormone users in the Women's Health Initiative Observational Study. *Cancer Causes Control.* 2019;30(11):1201-1211.
139. Brown SB, Hankinson SE. Endogenous estrogens and the risk of breast, endometrial, and ovarian cancers. *Steroids.* 2015;99(Pt A):8-10.
140. de Roon M, May AM, McTiernan A, et al. Effect of exercise and/or reduced calorie dietary interventions on breast cancer-related endogenous sex hormones in healthy postmenopausal women. *Breast Cancer Res.* 2018;20(1):81.
141. Walker K, Bratton DJ, Frost C. Premenopausal endogenous oestrogen levels and breast cancer risk: a meta-analysis. *Br J Cancer.* 2011;105(9):1451-1457.
142. Samavat H, Kurzer MS. Estrogen metabolism and breast cancer. *Cancer Lett.* 2015;356(2 Pt A):231-243.
143. Xie Y, Xie W. The Role of Sulfotransferases in Liver Diseases. *Drug Metab Disposition.* 2020;48(9):742-749.
144. Zu Y, Yang J, Zhang C, Liu D. The Pathological Mechanisms of Estrogen-Induced Cholestasis: Current Perspectives. *Front Pharmacol.* 2021;12:761255.
145. Mori T, Ito F, Koshiba A, et al. Local estrogen formation and its regulation in endometriosis. *Reproduct Med Biol.* 2019;18(4):305-311.
146. Ginsburg ES, Mello NK, Mendelson JH, et al. Effects of alcohol ingestion on estrogens in postmenopausal women. *JAMA.* 1996;276(21):1747-1751.
147. Sriprasert I, Kono N, Karim R, et al. Factors Associated With Serum Estradiol Levels Among Postmenopausal Women Using Hormone Therapy. *Obstet Gynecol.* 2020;136(4):675-684.
148. Muti P, Trevisan M, Micheli A, et al. Alcohol consumption and total estradiol in premenopausal women. *Cancer Epidemiol Biomarkers Prevent.* 1998;7(3):189-193.
149. McTiernan A, Wu L, Chen C, et al. Relation of BMI and physical activity to sex hormones in postmenopausal women. *Obesity.* 2006;14(9):1662-1677.
150. Galbraith RA, Michnovicz JJ. The effects of cimetidine on the oxidative metabolism of estradiol. *NEJM.* 1989;321(5):269-274.
151. Thompson III GR, Surampudi PN, Odermatt A. Gynecomastia and hypertension in a patient treated with posaconazole. *Clinical Case Rep.* 2020;8(12):3158.
152. Parida S, Sharma D. The Microbiome-Estrogen Connection and Breast Cancer Risk. *Cells.* 2019;8(12).
153. Ruo SW, Alkayyali T, Win M, et al. Role of Gut Microbiota Dysbiosis in Breast Cancer and Novel Approaches in Prevention, Diagnosis, and Treatment. *Cureus.* 2021;13(8):e17472.
154. McTiernan A, Tworoger SS, Ulrich CM, et al. Effect of exercise on serum estrogens in postmenopausal women: a 12-month randomized clinical trial. *Cancer Res.* 2004;64(8):2923-2928.
155. Silva JF, Ocarino NM, Serakides R. Thyroid hormones and female reproduction. *Biol Reproduct.* 2018;99(5):907-921.
156. Worda C, Sator MO, Schneeberger C, Jantschew T, Ferlitsch K, Huber JC. Influence of the catechol-O-methyltransferase (COMT) codon 158 polymorphism on estrogen levels in women. *Human Reproduct.* 2003;18(2):262-266.
157. Hodges RE, Minich DM. Modulation of Metabolic Detoxification Pathways Using Foods and Food-Derived Components: A Scientific Review with Clinical Application. *J Nutr Metab.* 2015;2015:760689.
158. Goodman JE, Lavigne JA, Wu K, et al. COMT genotype, micronutrients in the folate metabolic pathway and breast cancer risk. *Carcinogenesis.* 2001;22(10):1661-1665.
159. Institute M. Estrogen Metabolism Science Review. In:2019.
160. Marsden J, Marsh M, Rigg A. British Menopause Society consensus statement on the management of estrogen deficiency symptoms, arthralgia and menopause diagnosis in women treated for early breast cancer. *Post Reproduct Health.* 2019;25(1):21-32.



161. Mirkin S, Ryan KA, Chandran AB, Komm BS. Bazedoxifene/conjugated estrogens for managing the burden of estrogen deficiency symptoms. *Maturitas*. 2014;77(1):24-31.
162. Lambert MNT, Langkjer ST, Jeppesen PB. [Isoflavones have therapeutic efficacy in estrogen deficiency]. *Ugeskrift for læger*. 2020;182(21).
163. Gambrell RD, Jr. The menopause. *Investigative radiology*. 1986;21(4):369-378.
164. Franić D, Fističić I. Laser Therapy in the Treatment of Female Urinary Incontinence and Genitourinary Syndrome of Menopause: An Update. *BioMed Res Int*. 2019;2019:1576359.
165. Robinson D, Toozs-Hobson P, Cardozo L. The effect of hormones on the lower urinary tract. *Menopause international*. 2013;19(4):155-162.
166. Dalal PK, Agarwal M. Postmenopausal syndrome. *Indian J Psych*. 2015;57(Suppl 2):S222-232.
167. Rzepecki AK, Murase JE, Juran R, Fabi SG, McLellan BN. Estrogen-deficient skin: The role of topical therapy. *Int J Womens Dermatol*. 2019;5(2):85-90.
168. Mirmirani P. Hormonal changes in menopause: do they contribute to a 'midlife hair crisis' in women? *Br J Dermatol*. 2011;165 Suppl 3:7-11.
169. Nelson LM. Clinical practice. Primary ovarian insufficiency. *NEJM*. 2009;360(6):606-614.
170. Santoro N. Update in hyper- and hypogonadotropic amenorrhea. *J Clin Endocrinol Metab*. 2011;96(11):3281-3288.
171. Silveira LF, Latronico AC. Approach to the patient with hypogonadotropic hypogonadism. *J Clin Endocrinol Metab*. 2013;98(5):1781-1788.
172. Biegon A, Alia-Klein N, Fowler JS. Potential contribution of aromatase inhibition to the effects of nicotine and related compounds on the brain. *Front Pharmacol*. 2012;3:185.
173. Dandona P, Rosenberg MT. A practical guide to male hypogonadism in the primary care setting. *Int J Clin Pract*. 2010;64(6):682-696.
174. Gates MA, Tworoger SS, Eliassen AH, Missmer SA, Hankinson SE. Analgesic use and sex steroid hormone concentrations in postmenopausal women. *Cancer Epidemiol Biomarkers Prevent*. 2010;19(4):1033-1041.
175. Hudson AG, Gierach GL, Modugno F, et al. Nonsteroidal anti-inflammatory drug use and serum total estradiol in postmenopausal women. *Cancer Epidemiol Biomarkers Prevent*. 2008;17(3):680-687.
176. Lønning PE. Estradiol measurement in translational studies of breast cancer. *Steroids*. 2015;99(Pt A):26-31.
177. Rothman MS, Carlson NE, Xu M, et al. Reexamination of testosterone, dihydrotestosterone, estradiol and estrone levels across the menstrual cycle and in postmenopausal women measured by liquid chromatography-tandem mass spectrometry. *Steroids*. 2011;76(1-2):177-182.
178. Allen AM, McRae-Clark AL, Carlson S, et al. Determining menstrual phase in human biobehavioral research: A review with recommendations. *Exp Clin Psychopharmacol*. 2016;24(1):1-11.
179. Gorbach SL, Goldin BR. Diet and the excretion and enterohepatic cycling of estrogens. *Prev Med*. 1987;16(4):525-531.
180. Geller SE, Studee L. Botanical and dietary supplements for menopausal symptoms: what works, what does not. *J Womens Health* (2002). 2005;14(7):634-649.
181. Bower-Cargill C, Yarandi N, Petróczi A. A systematic review of the versatile effects of the Peruvian Maca Root (*Lepidium meyenii*) on sexual dysfunction, menopausal symptoms and related conditions. *Phytomedicine Plus*. 2022:100326.
182. Schindler AE. [Transdermal and oral hormone substitution with estrogens: a comparison]. *Zentralblatt Gynakol*. 1995;117(10):504-507.
183. Slater CC, Hodis HN, Mack WJ, Shoupe D, Paulson RJ, Stanczyk FZ. Markedly elevated levels of estrone sulfate after long-term oral, but not transdermal, administration of estradiol in postmenopausal women. *Menopause*. 2001;8(3):200-203.
184. Ginsburg ES, Walsh BW, Shea BF, Gao X, Gleason RE, Barbieri RL. The effects of ethanol on the clearance of estradiol in postmenopausal women. *Fertility Sterility*. 1995;63(6):1227-1230.
185. Tahboub R, Arafah BM. Sex steroids and the thyroid. *Best Pract Res Clin Endocrinol Metab*. 2009;23(6):769-780.
186. Fuhrman BJ, Schairer C, Gail MH, et al. Estrogen metabolism and risk of breast cancer in postmenopausal women. *J Nat Cancer Inst*. 2012;104(4):326-339.
187. Sampson JN, Falk RT, Schairer C, et al. Association of Estrogen Metabolism with Breast Cancer Risk in Different Cohorts of Postmenopausal Women. *Cancer Res*. 2017;77(4):918-925.
188. Jacob Schor N. Estrogen Metabolite Ratios: Time for Us to Let Go.
189. Stanczyk FZ. The 2-/16 α -Hydroxylated Estrogen Ratio-Breast Cancer Risk Hypothesis: Insufficient Evidence for its Support. *J Steroid Biochem Mol Biol*. 2020;201:105685.
190. Moore SC, Matthews CE, Ou Shu X, et al. Endogenous Estrogens, Estrogen Metabolites, and Breast Cancer Risk in Postmenopausal Chinese Women. *JNCI*. 2016;108(10).
191. Moore SC, Matthews CE, Ou Shu X, et al. Endogenous Estrogens, Estrogen Metabolites, and Breast Cancer Risk in Postmenopausal Chinese Women. *JNCI*. 2016;108(10).
192. Goldin BR, Adlercreutz H, Gorbach SL, et al. The relationship between estrogen levels and diets of Caucasian American and Oriental immigrant women. *Am J Clin Nutr*. 1986;44(6):945-953.
193. Xu X, Duncan AM, Merz BE, Kurzer MS. Effects of soy isoflavones on estrogen and phytoestrogen metabolism in premenopausal women. *Cancer Epidemiol Biomarkers Prevent*. 1998;7(12):1101-1108.
194. Fuhrman BJ, Brinton LA, Pfeiffer RM, et al. Estrogen metabolism and mammographic density in postmenopausal women: a cross-sectional study. *Cancer Epidemiol Biomarkers Prevent*. 2012;21(9):1582-1591.
195. Kim EJ, Lee D, Chung BC, Pyo H, Lee J. Association between urinary levels of bisphenol-A and estrogen metabolism in Korean adults. *Sci Total Environ*. 2014;470-471:1401-1407.
196. Hartman TJ, Sisti JS, Hankinson SE, Xu X, Eliassen AH, Ziegler R. Alcohol Consumption and Urinary Estrogens and Estrogen Metabolites in Premenopausal Women. *Hormones Cancer*. 2016;7(1):65-74.
197. Fuhrman BJ, Feigelson HS, Flores R, et al. Associations of the fecal microbiome with urinary estrogens and estrogen metabolites in postmenopausal women. *J Clin Endocrinol Metab*. 2014;99(12):4632-4640.
198. Matthews CE, Fortner RT, Xu X, Hankinson SE, Eliassen AH, Ziegler RG. Association between physical activity and urinary estrogens and estrogen metabolites in premenopausal women. *J Clin Endocrinol Metab*. 2012;97(10):3724-3733.
199. Fuhrman BJ, Pfeiffer RM, Wu AH, et al. Green tea intake is associated with urinary estrogen profiles in Japanese-American women. *Nutrition J*. 2013;12:25.
200. Gu F, Caporaso NE, Schairer C, et al. Urinary concentrations of estrogens and estrogen metabolites and smoking in caucasian women. *Cancer Epidemiol Biomarkers Prevent*. 2013;22(1):58-68.
201. Huang J, Sun J, Chen Y, et al. Analysis of multiplex endogenous estrogen metabolites in human urine using ultra-fast liquid chromatography-tandem mass spectrometry: a case study for breast cancer. *Analyt chim Acta*. 2012;711:60-68.
202. Barba M, Yang L, Schünemann HJ, et al. Urinary estrogen metabolites and prostate cancer: a case-control study and meta-analysis. *J Exp Clin Cancer Res*. 2009;28(1):135.



203. Napoli N, Armamento-Villareal R. Estrogen Hydroxylation in Osteoporosis. In: *Adv Clin Chem*. Vol 43. Elsevier; 2007:211-227.
204. Cavalieri EL, Rogan EG. Unbalanced metabolism of endogenous estrogens in the etiology and prevention of human cancer. *J Steroid Biochem Mol Biol*. 2011;125(3-5):169-180.
205. BALL P, KNUPPEN R, HAUPT M, BREUER H. Interactions between estrogens and catechol amines III. Studies on the methylation of catechol estrogens, catechol amines and other catechols by the catechol-O-methyltransferase of human liver. *J Clin Endocrinol Metab*. 1972;34(4):736-746.
206. Napoli N, Donepudi S, Sheikh S, Rini GB, Armamento-Villareal R. Increased 2-hydroxylation of estrogen in women with a family history of osteoporosis. *J Clin Endocrinol Metab*. 2005;90(4):2035-2041.
207. Napoli N, Armamento-Villareal R. Estrogen hydroxylation in osteoporosis. *Adv Clin Chem*. 2007;43:211-227.
208. Greenlee H, Chen Y, Kabat GC, et al. Variants in estrogen metabolism and biosynthesis genes and urinary estrogen metabolites in women with a family history of breast cancer. *Breast Cancer research and treatment*. 2007;102(1):111-117.
209. Bolton JL, Thatcher GR. Potential mechanisms of estrogen quinone carcinogenesis. *Chem Res Toxicol*. 2008;21(1):93-101.
210. Michnovicz JJ, Adlercreutz H, Bradlow HL. Changes in levels of urinary estrogen metabolites after oral indole-3-carbinol treatment in humans. *JNCI*. 1997;89(10):718-723.
211. Michnovicz JJ. Increased estrogen 2-hydroxylation in obese women using oral indole-3-carbinol. *Int J Obesity*. 1998;22(3):227-229.
212. Green T, See J, Schauch M, et al. A randomized, double-blind, placebo-controlled, cross-over trial to evaluate the effect of EstroSense(®) on 2-hydroxyestrone:16α-hydroxyestrone ratio in premenopausal women. *J Complement Integ Med*. 2023;20(1):199-206.
213. Campbell KL, Westerlind KC, Harber VJ, Bell GJ, Mackey JR, Courneya KS. Effects of aerobic exercise training on estrogen metabolism in premenopausal women: a randomized controlled trial. *Cancer Epidemiol Biomarkers Prevent*. 2007;16(4):731-739.
214. Chow HH, Garland LL, Heckman-Stoddard BM, et al. A pilot clinical study of resveratrol in postmenopausal women with high body mass index: effects on systemic sex steroid hormones. *J Transl Med*. 2014;12:223.
215. Laidlaw M, Cockerline CA, Sepkovic DW. Effects of a breast-health herbal formula supplement on estrogen metabolism in pre- and post-menopausal women not taking hormonal contraceptives or supplements: a randomized controlled trial. *Breast Cancer*. 2010;4:85-95.
216. Higdon JV, Delage B, Williams DE, Dashwood RH. Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmacol Res*. 2007;55(3):224-236.
217. Rajoria S, Suriano R, Parmar PS, et al. 3,3'-diindolylmethane modulates estrogen metabolism in patients with thyroid proliferative disease: a pilot study. *Thyroid*. 2011;21(3):299-304.
218. Xu X, Duncan AM, Wangen KE, Kurzer MS. Soy consumption alters endogenous estrogen metabolism in postmenopausal women. *Cancer Epidemiol Biomarkers Prevent*. 2000;9(8):781-786.
219. Brooks JD, Ward WE, Lewis JE, et al. Supplementation with flaxseed alters estrogen metabolism in postmenopausal women to a greater extent than does supplementation with an equal amount of soy. *Am J Clin Nutr*. 2004;79(2):318-325.
220. Fuhrman BJ, Pfeiffer R, Xu X, et al. Soy intake is associated with increased 2-hydroxylation and decreased 16α-hydroxylation of estrogens in Asian-American women. *Cancer Epidemiol Biomarkers Prevent*. 2009;18(10):2751-2760.
221. Davis SJ, Arscott SA, Goltz S, Muir C, Binkley N, Tanumihardjo SA. Urinary 2- to 16α-hydroxyestrone ratio did not change with cruciferous vegetable intake in premenopausal women. *Int J Vitamin Nutr Res*. 2023.
222. Sisti JS, Hankinson SE, Caporaso NE, et al. Caffeine, coffee, and tea intake and urinary estrogens and estrogen metabolites in premenopausal women. *Cancer Epidemiol Biomarkers Prevent*. 2015;24(8):1174-1183.
223. Zhao H, Yang H, Li J, et al. Association of Urinary Levels of Estrogens and Estrogen Metabolites with the Occurrence and Development of Endometrial Hyperplasia Among Premenopausal Women. *Reproduct Sci*. 2023.
224. Taioli E, Im A, Xu X, Veenstra TD, Ahrendt G, Garte S. Comparison of estrogens and estrogen metabolites in human breast tissue and urine. *Reproduct Biol Endocrinol*. 2010;8:93.
225. Wang T, Nichols HB, Nyante SJ, et al. Urinary Estrogen Metabolites and Long-Term Mortality Following Breast Cancer. *JNCI Cancer Spectrum*. 2020;4(3):pkaa014.
226. Lu LJ, Cree M, Josyula S, Nagamani M, Grady JJ, Anderson KE. Increased urinary excretion of 2-hydroxyestrone but not 16α-hydroxyestrone in premenopausal women during a soya diet containing isoflavones. *Cancer Res*. 2000;60(5):1299-1305.
227. Martini MC, Dancisak BB, Haggans CJ, Thomas W, Slavin JL. Effects of soy intake on sex hormone metabolism in premenopausal women. *Nutrition Cancer*. 1999;34(2):133-139.
228. Lambert MNT, Thybo CB, Lykkeboe S, et al. Combined bioavailable isoflavones and probiotics improve bone status and estrogen metabolism in postmenopausal osteopenic women: a randomized controlled trial. *Am J Clin Nutr*. 2017;106(3):909-920.
229. Thomson CA, Chow HHS, Wertheim BC, et al. A randomized, placebo-controlled trial of diindolylmethane for breast cancer biomarker modulation in patients taking tamoxifen. *Breast Cancer Res Treat*. 2017;165(1):97-107.
230. Gee JR, Saltzstein DR, Messing E, et al. Phase Ib placebo-controlled, tissue biomarker trial of diindolylmethane (BR-DIMNG) in patients with prostate cancer who are undergoing prostatectomy. *Eur J Cancer Prev*. 2016;25(4):312-320.
231. Mosli HA, Tolba MF, Al-Abd AM, Abdel-Naim AB. Catechol estrogens induce proliferation and malignant transformation in prostate epithelial cells. *Toxicology Lett*. 2013;220(3):247-258.
232. Markushin Y, Gaikwad N, Zhang H, et al. Potential biomarker for early risk assessment of prostate cancer. *Prostate*. 2006;66(14):1565-1571.
233. Yager JD. Mechanisms of estrogen carcinogenesis: The role of E2/E1-quinone metabolites suggests new approaches to preventive intervention--A review. *Steroids*. 2015;99(Pt A):56-60.
234. Zahid M, Gaikwad NW, Rogan EG, Cavalieri EL. Inhibition of depurinating estrogen-DNA adduct formation by natural compounds. *Chem Res Toxicol*. 2007;20(12):1947-1953.
235. Hirose Y, Tsutsui TW, Ohno M, Barrett JC, Tsutsui T. Effects of a catechol-O-methyltransferase inhibitor on catechol estrogen-induced cellular transformation, chromosome aberrations and apoptosis in Syrian hamster embryo cells. *Int J Cancer*. 2007;120(8):1627-1633.
236. Arora R, Chan C, Ye XY, Greenblatt EM. Progesterone, progesterone/estradiol and ART outcomes in day-5 transfer cycles. *Gynecol Endocrinol*. 2018;34(1):59-63.
237. Rizner TL, Smuc T, Ruprecht R, Sinkovec J, Penning TM. AKR1C1 and AKR1C3 may determine progesterone and estrogen ratios in endometrial cancer. *Mol Cellular Endocrinol*. 2006;248(1-2):126-135.
238. 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.
239. Bachmann G, Bancroft J, Braunstein G, et al. Female androgen insufficiency: the Princeton consensus statement on definition, classification, and assessment. *Fertility Sterility*. 2002;77(4):660-665.
240. Glaser R, Dimitrakakis C. Testosterone therapy in women: myths and misconceptions. *Maturitas*. 2013;74(3):230-234.



241. Feldman HA, Longcope C, Derby CA, et al. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts male aging study. *J Clin Endocrinol Metab.* 2002;87(2):589-598.
242. Handelsman DJ. Androgen Physiology, Pharmacology, Use and Misuse. In: Feingold KR, Anawalt B, Boyce A, et al., eds. *Endotext*. MDText.com, Inc.; 2000.
243. Barberia JM, Giner J, Cortes-Gallegos V. Diurnal variations of plasma testosterone in men. *Steroids.* 1973;22(5):615-626.
244. Haring R, Hannemann A, John U, et al. Age-specific reference ranges for serum testosterone and androstenedione concentrations in women measured by liquid chromatography-tandem mass spectrometry. *J Clin Endocrinol Metab.* 2012;97(2):408-415.
245. Guay A, Munarriz R, Jacobson J, et al. Serum androgen levels in healthy premenopausal women with and without sexual dysfunction: Part A. Serum androgen levels in women aged 20-49 years with no complaints of sexual dysfunction. *Int J Impotence Res.* 2004;16(2):112-120.
246. Davison SL, Bell R, Donath S, Montalto JG, Davis SR. Androgen levels in adult females: changes with age, menopause, and oophorectomy. *J Clin Endocrinol Metab.* 2005;90(7):3847-3853.
247. Guay A, Jacobson J, Munarriz R, et al. Serum androgen levels in healthy premenopausal women with and without sexual dysfunction: Part B: Reduced serum androgen levels in healthy premenopausal women with complaints of sexual dysfunction. *Int J Impotence Res.* 2004;16(2):121-129.
248. Longcope C. The metabolism of dehydroepiandrosterone sulfate and dehydroepiandrosterone. *Aging Male.* 1998;1(1):51-55.
249. Maggio M, De Vita F, Fisichella A, et al. DHEA and cognitive function in the elderly. *J Steroid Biochem Mol Biol.* 2015;145:281-292.
250. Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). *Front Neuroendocrinol.* 2009;30(1):65-91.
251. Guillems TG, Edwards L. Chronic stress and the HPA axis. *The Standard.* 2010;9(2):1-12.
252. Narinx N, David K, Walravens J, et al. Role of sex hormone-binding globulin in the free hormone hypothesis and the relevance of free testosterone in androgen physiology. *Cell Mol Life Sci : CMLS.* 2022;79(11):543.
253. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab.* 1999;84(10):3666-3672.
254. Keevil BG, Adaway J, Fiers T, Moghetti P, Kaufman JM. The free androgen index is inaccurate in women when the SHBG concentration is low. *Clinical Endocrinol.* 2018;88(5):706-710.
255. Chen J, Wang Q, Pei Y, Li N, Han J, Yu J. Effect of free androgen index on blood pressure variability and target organ damage in postmenopausal hypertensive women: findings from a cross-sectional study. *Menopause.* 2021;28(11):1264-1270.
256. Sathyapalan T, Al-Qaissi A, Kilpatrick ES, et al. Salivary testosterone measurement in women with and without polycystic ovary syndrome. *Sci Rep.* 2017;7(1):3589.
257. Morley JE, Perry HM, 3rd, Patrick P, Dollbaum CM, Kells JM. Validation of salivary testosterone as a screening test for male hypogonadism. *Aging Male.* 2006;9(3):165-169.
258. Goncharov N, Katsya G, Dobracheva A, et al. Diagnostic significance of free salivary testosterone measurement using a direct luminescence immunoassay in healthy men and in patients with disorders of androgenic status. *Aging Male.* 2006;9(2):111-122.
259. Fiers T, Delanghe J, T'Sjoen G, Van Caenegem E, Wierckx K, Kaufman JM. A critical evaluation of salivary testosterone as a method for the assessment of serum testosterone. *Steroids.* 2014;86:5-9.
260. Yildiz BO. Diagnosis of hyperandrogenism: clinical criteria. *Best Pract Res Clin Endocrinol Metab.* 2006;20(2):167-176.
261. Li C, Xing C, Zhang J, Zhao H, Shi W, He B. Eight-hour time-restricted feeding improves endocrine and metabolic profiles in women with anovulatory polycystic ovary syndrome. *J Transl Med.* 2021;19(1):148.
262. Sutton-Tyrrell K, Zhao X, Santoro N, et al. Reproductive hormones and obesity: 9 years of observation from the Study of Women's Health Across the Nation. *Am J Epidemiol.* 2010;171(11):1203-1213.
263. Cussen L, McDonnell T, Bennett G, Thompson CJ, Sherlock M, O'Reilly MW. Approach to androgen excess in women: Clinical and biochemical insights. *Clin Endocrinol.* 2022;97(2):174-186.
264. Tchernof A, Brochu D, Maltais-Payette I, et al. Androgens and the Regulation of Adiposity and Body Fat Distribution in Humans. *Comprehensive Physiol.* 2018;8(4):1253-1290.
265. Olweus D, Mattsson A, Schalling D, Löw H. Testosterone, aggression, physical, and personality dimensions in normal adolescent males. *Psychosomatic Med.* 1980;42(2):253-269.
266. Hsieh TC, Pastuszak AW, Hwang K, Lipshultz LI. Concomitant intramuscular human chorionic gonadotropin preserves spermatogenesis in men undergoing testosterone replacement therapy. *J Urol.* 2013;189(2):647-650.
267. Carmina E. The spectrum of androgen excess disorders. In: Vol 85: Elsevier; 2006:1582-1585.
268. Witchel SF, Oberfield SE, Peña AS. Polycystic Ovary Syndrome: Pathophysiology, Presentation, and Treatment With Emphasis on Adolescent Girls. *J Endocrine Soc.* 2019;3(8):1545-1573.
269. Tawfeek MA, Alfarhli EM, Alayoubi AM, El-Beshbishy HA, Habib FA. Sex hormone binding globulin as a valuable biochemical marker in predicting gestational diabetes mellitus. *BMC Womens Health.* 2017;17(1):18.
270. Basulto-Alarcón C, Llanos P, García-Rivas G, et al. Classic and Novel Sex Hormone Binding Globulin Effects on the Cardiovascular System in Men. *Int J Endocrinol.* 2021;2021:5527973.
271. Kooij CD, Mavinkurve-Groothuis AMC, Kremer Hovinga ICL, et al. Familial Male-limited Precocious Puberty (FMPP) and Testicular Germ Cell Tumors. *J Clin Endocrinol Metab.* 2022;107(11):3035-3044.
272. Sansone A, Romanelli F, Sansone M, Lenzi A, Di Luigi L. Gynecomastia and hormones. *Endocrine.* 2017;55(1):37-44.
273. Bolour S, Braunstein G. Testosterone therapy in women: a review. *Int J Impotence Res.* 2005;17(5):399-408.
274. Mohr BA, Bhasin S, Link CL, O'Donnell AB, McKinlay JB. The effect of changes in adiposity on testosterone levels in older men: longitudinal results from the Massachusetts Male Aging Study. *Eur J Endocrinol.* 2006;155(3):443-452.
275. Cohen J, Nassau DE, Patel P, Ramasamy R. Low Testosterone in Adolescents & Young Adults. *Front Endocrinol.* 2019;10:916.
276. Sizar O, Schwartz J. Hypogonadism. In: StatPearls. StatPearls Publishing LLC.; 2023.
277. Hooper DR, Tenforde AS, Hackney AC. Treating exercise-associated low testosterone and its related symptoms. *Phys Sports Med.* 2018;46(4):427-434.
278. Hackney AC, Zieff GH, Lane AR, Register-Mihalik JK. Marathon Running and Sexual Libido in Adult Men: Exercise Training and Racing Effects. *J Endocrinol Sci.* 2022;4(1):10-12.
279. Ntovas P, Loumprin N, Maniatakis P, Margaritidis L, Rahiotis C. The Effects of Physical Exercise on Saliva Composition: A Comprehensive Review. *Dentistry J.* 2022;10(1).
280. Geisler S, Aussieker T, Paldauf S, et al. Salivary testosterone and cortisol concentrations after two different resistance training exercises. *J Sports Med Phys Fitness.* 2019;59(6):1030-1035.



281. Al-Dujaili EAS, Abu Hajleh MN, Chalmers R. Effects of Ginseng Ingestion on Salivary Testosterone and DHEA Levels in Healthy Females: An Exploratory Study. *Nutrients*. 2020;12(6).
282. Smith SJ, Lopresti AL, Teo SY, Fairchild TJ. Examining the effects of herbs on testosterone concentrations in men: A systematic review. *Adv Nutr*. 2021;12(3):744-765.
283. Scott A, Newson L. Should we be prescribing testosterone to perimenopausal and menopausal women? A guide to prescribing testosterone for women in primary care. *Br J Gen Pract*. 2020;70(693):203-204.
284. Grober ED, Garbens A, Božović A, Kulasingam V, Fanipour M, Diamandis EP. Accuracy of testosterone concentrations in compounded testosterone products. *J Sex Med*. 2015;12(6):1381-1388.
285. Davis SR, Baber R, Panay N, et al. Global Consensus Position Statement on the Use of Testosterone Therapy for Women. *K Clin Endocrinol Metab*. 2019;104(10):4660-4666.
286. Parish SJ, Simon JA, Davis SR, et al. International Society for the Study of Women's Sexual Health Clinical Practice Guideline for the Use of Systemic Testosterone for Hypoactive Sexual Desire Disorder in Women. *J Womens Health* (2002). 2021;30(4):474-491.
287. Flyckt RL, Liu J, Frasure H, Wekselman K, Buch A, Kingsberg SA. Comparison of salivary versus serum testosterone levels in postmenopausal women receiving transdermal testosterone supplementation versus placebo. *Menopause*. 2009;16(4):680-688.
288. Young J, Couzinet B, Nahoul K, et al. Panhypopituitarism as a model to study the metabolism of dehydroepiandrosterone (DHEA) in humans. *J Clin Endocrinol Metab* 1997;82(8):2578-2585.
289. Jeyaprakash N, Maeder S, Janka H, Stute P. A systematic review of the impact of 7-keto-DHEA on body weight. *Arch Gynecol Obst*. 2022.
290. Schwenkhagen A, Studd J. Role of testosterone in the treatment of hypoactive sexual desire disorder. *Maturitas*. 2009;63(2):152-159.
291. Cooper LA, Page ST, Amory JK, Anawalt BD, Matsumoto AM. The association of obesity with sex hormone-binding globulin is stronger than the association with ageing--implications for the interpretation of total testosterone measurements. *Clin Endocrinol*. 2015;83(6):828-833.
292. English KM, Pugh PJ, Parry H, Scutt NE, Channer KS, Jones TH. Effect of cigarette smoking on levels of bioavailable testosterone in healthy men. *Clin Sci*. 2001;100(6):661-665.
293. Lennartsson AK, Kushnir MM, Bergquist J, Billig H, Jonsdottir IH. Sex steroid levels temporarily increase in response to acute psychosocial stress in healthy men and women. *Int J Psychophysiol*. 2012;84(3):246-253.
294. Hirko KA, Spiegelman D, Willett WC, Hankinson SE, Eliassen AH. Alcohol consumption in relation to plasma sex hormones, prolactin, and sex hormone-binding globulin in premenopausal women. *Cancer epidemiol Biomarkers Prevent*. 2014;23(12):2943-2953.
295. Karelis AD, Fex A, Filion M-E, Adlercreutz H, Aubertin-Leheudre M. Comparison of sex hormonal and metabolic profiles between omnivores and vegetarians in pre- and post-menopausal women. *Br J Nutr*. 2010;104(2):222-226.
296. Sáez-López C, Soriguer F, Hernandez C, et al. Oleic acid increases hepatic sex hormone binding globulin production in men. *Mol Nutr Food Res*. 2014;58(4):760-767.
297. Shin JY, Kim SK, Lee MY, et al. Serum sex hormone-binding globulin levels are independently associated with nonalcoholic fatty liver disease in people with type 2 diabetes. *Diabetes Res Clin Pract*. 2011;94(1):156-162.
298. Liao CH, Li HY, Yu HJ, et al. Low serum sex hormone-binding globulin: marker of inflammation? *C Int J Clin Chem*. 2012;413(7-8):803-807.
299. Wang Y. Definition, Prevalence, and Risk Factors of Low Sex Hormone-Binding Globulin in US Adults. *J Clin Endocrinol Metab*. 2021;106(10):e3946-e3956.
300. Hawkins VN, Foster-Schubert K, Chubak J, et al. Effect of exercise on serum sex hormones in men: a 12-month randomized clinical trial. *Med Sci Sports Exercise*. 2008;40(2):223-233.
301. Blackwell L, Cooke D, Brown S. Self-Monitoring of Fertility Hormones: A New Era for Natural Family Planning? *Linacre Quarterly*. 2018;85(1):26-34.
302. Su HW, Yi YC, Wei TY, Chang TC, Cheng CM. Detection of ovulation, a review of currently available methods. *Bioeng Transl Med*. 2017;2(3):238-246.
303. Reed BG, Carr BR. The Normal Menstrual Cycle and the Control of Ovulation. In: Feingold KR, Anawalt B, Boyce A, et al., eds. *Endotext*. MDText.com, Inc.; 2000.
304. Johnson S, Weddell S, Godbert S, Freundl G, Roos J, Gnath C. Development of the first urinary reproductive hormone ranges referenced to independently determined ovulation day. *CCLM / FESCC*. 2015;53(7):1099-1108.
305. Buchheit KB, J. Progestogen hypersensitivity. 2020.UptoDate.
306. Bull JR, Rowland SP, Scherwitzl EB, Scherwitzl R, Danielsson KG, Harper J. Real-world menstrual cycle characteristics of more than 600,000 menstrual cycles. *NPJ Digit Med*. 2019;2:83.
307. Najmabadi S, Schliep KC, Simonsen SE, Porucznik CA, Egger MJ, Stanford JB. Menstrual bleeding, cycle length, and follicular and luteal phase lengths in women without known subfertility: A pooled analysis of three cohorts. *Paed Perinatal Epidemiol*. 2020;34(3):318-327.
308. Finn MM, Gosling JP, Tallon DF, Baynes S, Meehan FP, Fottrell PF. The frequency of salivary progesterone sampling and the diagnosis of luteal phase insufficiency. *Gynecol Endocrinol*. 1992;6(2):127-134.
309. Roos J, Johnson S, Weddell S, et al. Monitoring the menstrual cycle: Comparison of urinary and serum reproductive hormones referenced to true ovulation. *Eur J Contracept Reproduct Health Care*. 2015;20(6):438-450.
310. Metcalf MG, Evans JJ, Mackenzie JA. Indices of ovulation: comparison of plasma and salivary levels of progesterone with urinary pregnanediol. *J Endocrinol*. 1984;100(1):75-80.
311. Leiva R, McNamara-Kilian M, Niezgoda H, Ecochard R, Bouchard T. Pilot observational prospective cohort study on the use of a novel home-based urinary pregnanediol 3-glucuronide (PDG) test to confirm ovulation when used as adjunct to fertility awareness methods (FAMs) stage 1. *BMJ Open*. 2019;9(5):e028496.
312. Handelsman DJ, Nimmagadda R, Desai R, et al. Direct measurement of pregnanediol 3-glucuronide (PDG) in dried urine spots by liquid chromatography-mass spectrometry to detect ovulation. *J Steroid Biochem Mol Biol*. 2021;211:105900.
313. Usala SJ, Allende ME, Trindade AA. The Fertility Indicator Equation Using Serum Progesterone and Urinary Pregnanediol-3-Glucuronide for Assessment of Ovulatory to Luteal Phase Transition. *Medicina*. 2021;57(2).
314. Whelan AM, Thebeau JP, Jurgens TM, Hurst E. Bioidentical hormone therapy: Nova Scotia pharmacists' knowledge and beliefs. *Pharm Pract*. 2012;10(3):159-167.
315. Constantine GD, Archer DF, Graham S, Bernick BA, Mirkin S. Prescribing of FDA-approved and compounded hormone therapy differs by specialty. *Menopause*. 2016;23(10):1075-1082.
316. Franklin SL. Effects of unintentional exposure of children to compounded transdermal sex hormone therapy. *Ped Endocrinol Rev*. 2011;8(3):208-212.
317. Busse KL, Maibach HI. Transdermal estradiol and testosterone transfer in man: existence, models, and strategies for prevention. *Skin Pharmacol Physiol*. 2011;24(2):57-66.
318. Hadgraft J, Lane ME. Transdermal delivery of testosterone. *Eur J Pharm Biopharmaceut*. 2015;92:42-48.



319. Stahlman J, Britto M, Fitzpatrick S, et al. Serum testosterone levels in non-dosed females after secondary exposure to 1.62% testosterone gel: effects of clothing barrier on testosterone absorption. *Curr Med Res Opin*. 2012;28(2):291-301.
320. Taylor MB, Gutierrez MJ. Absorption, bioavailability, and partner transfer of estradiol from a topical emulsion. *Pharmacotherapy*. 2008;28(6):712-718.
321. Burry KA, Patton PE, Hermsmeyer K. Percutaneous absorption of progesterone in postmenopausal women treated with transdermal estrogen. *Am J Obst Gynecol*. 1999;180(6 Pt 1):1504-1511.
322. Santen RJ. Vaginal administration of estradiol: effects of dose, preparation and timing on plasma estradiol levels. *Climacteric*. 2015;18(2):121-134.
323. Ginsburg ES, Gao X, Shea BF, Barbieri RL. Half-life of estradiol in postmenopausal women. *Gynecol Obst Invest*. 1998;45(1):45-48.
324. L'Hermite M. Custom-compounded bioidentical hormone therapy: why so popular despite potential harm? The case against routine use. *Climacteric*. 2017;20(3):205-211.
325. L'Hermite M. Bioidentical menopausal hormone therapy: registered hormones (non-oral estradiol ± progesterone) are optimal. *Climacteric*. 2017;20(4):331-338.
326. Newson LR, Lass A. Effectiveness of transdermal oestradiol and natural micronised progesterone for menopausal symptoms. *Br J Gen Pract*. 2018;68(675):499-500.
327. Stute P, Wildt L, Neulen J. The impact of micronized progesterone on breast cancer risk: a systematic review. *Climacteric*. 2018;21(2):111-122.
328. Conaway E. Bioidentical hormones: an evidence-based review for primary care providers. *JAOA*. 2011;111(3):153-164.
329. Stephenson K, Neuenschwander PF, Kurdowska AK. The effects of compounded bioidentical transdermal hormone therapy on hemostatic, inflammatory, immune factors; cardiovascular biomarkers; quality-of-life measures; and health outcomes in perimenopausal and postmenopausal women. *Int J Pharmaceut Compound*. 2013;17(1):74-85.
330. Ruiz AD, Daniels KR, Barner JC, Carson JJ, Frei CR. Effectiveness of compounded bioidentical hormone replacement therapy: an observational cohort study. *BMC Womens Health*. 2011;11:27.
331. Holtorf K. The bioidentical hormone debate: are bioidentical hormones (estradiol, estriol, and progesterone) safer or more efficacious than commonly used synthetic versions in hormone replacement therapy? *Postgrad Med*. 2009;121(1):73-85.
332. Wiley TS, Odegard RD, Raden J, Haraldsen JT. The standardization of nonsterile compounding: a study in quality control and assessment for hormone compounding. *Int J Pharmaceut Compound*. 2014;18(2):162-168.
333. Stanczyk FZ, Niu C, Azen C, Mirkin S, Amadio JM. Determination of estradiol and progesterone content in capsules and creams from compounding pharmacies. *Menopause*. 2019;26(9):966-971.
334. Allen Jr LV. Progesterone 50 mg/g in VersaBase Cream. *US Pharm*. 2017;42(9):47-48.

Update Coming Soon



 Measurable in Urine  Measurable in Blood  Measurable in Saliva

REFERENCES

3 β HSD (3beta-hydroxysteroid dehydrogenase)

Lower activity:

- Alcohol: Chiao YB, et al. Alcohol Clin Exp Res. 1981 Spring;5(2):230-6.
- PCBs: Andric SA, et al. Environ Health Perspect. 2000 Oct;108(10):955-9.
- Progestins: Lee TC, et al. J Clin Endocrinol Metab. 1999 Jun;84(6):2104-10.
- Isoflavonoids: Deluca D, et al. J Steroid Biochem Mol Biol. 2005 Feb;93(2-5):285-92.

Higher activity:

- Hyperadrenalism: Simonian MH. J Steroid Biochem. 1986 Dec;25(6):1001-6.
- Hyperinsulinemia: DeClue TJ, et al. J Clin Endocrinol Metab. 1991 Jun;72(6):1308-11.
- PCOS: Strauss JF 3rd. Ann N Y Acad Sci. 2003 Nov;997:42-8.
- Forskolin: Bird IM, et al. J Endocrinol. 1996 Sep;150 Suppl:S165-73.

5 α (5alpha-reductase)

Lower activity:

- Flaxseed, isoflavones: Evans BA, et al. J Endocrinol. 1995 Nov;147(2):295-302.
- EGCG: Hiipakkka RA, et al. Biochem Pharmacol. 2002 Mar 15;63(6):1165-76.
- Progesterone: Cassidenti DL, et al. Obstet Gynecol. 1991 Jul;78(1):103-7.
- Saw palmetto: Drsata J, et al. Cas Lek Cesk. 2002 Oct 11;141(20):630-5.

Higher activity:

- Insulin resistance, obesity: Westerbacka J, et al. J Clin Endocrinol Metab. 2003 Oct;88(10):4924-31.
- Essential HT: Soro A, et al. Hypertension. 1995 Jan;25(1):67-70.
- DHEA: Stomati M, et al. Gynecol Endocrinol. 2000 Oct;14(5):342-63.

5 β (5beta-reductase)

Lower activity:

- Licorice: Tamura Y, et al. Arzneimittelforschung. 1979;29(4):647-9.

Higher activity:

- Insulin resistance, fatty liver: Westerbacka J, et al. J Clin Endocrinol Metab. 2003 Oct;88(10):4924-31.

CYP11 β 1 (11beta-hydroxylase)

Lower activity:

- Azole antifungals: Ayub M, et al. J Steroid Biochem. 1989 Apr;32(4):515-24.
- DHEA: Stomati M, et al. Gynecol Endocrinol. 2000 Oct;14(5):342-63.

11HSD (11beta-hydroxysteroid dehydrogenase)

More cortisol:

- Metabolic syndrome: Seckle JR, et al. Recent Prog Horm Res. 2004;59:359-93.
- Inflammation: Cai TQ, et al. J Steroid Biochem Mol Biol. 2001 May;77(2-3):117-22.

- Hypothyroid: Hoshiro M, et al. Clin Endocrinol (Oxf). 2006 Jan;64(1):37-45.
- Licorice: Ferrari P, et al. Hypertension. 2001 Dec 1;38(6):1330-6.

Less cortisol:

- Hyperthyroidism: Taniyama M, et al. Thyroid. 1993 Fall;3(3):229-33.
- Coffee: Atanasov AG, et al. FEBS Lett. 2006 Jul 24;580(17):4081-5. Epub 2006 Jun 27.
- Ketoconazole: Stiefel P, et al. Endocrine. 2002 Aug;18(3):279-84.
- Rosiglitazone: Berthiaume M, et al. Am J Physiol Regul Integr Comp Physiol. 2004 Nov;287(5):R1116-23.

17 α -hydroxylase

Lower activity:

- Smoking: Yeh J, et al. J Steroid Biochem. 1989 Oct;33(4A):627-30.
- Antifungals: Weber MM, et al. Clin Investig. 1993 Nov;71(11):933-8.
- Spironolactone: Kossor DC, et al. Mol Pharmacol. 1991 Aug;40(2):321-5.

Higher activity:

- Hyperglycemia: Ueshiba H, et al. Eur J Endocrinol. 2002 Mar;146(3):375-80.
- Hyperinsulinemia: Nestler JE, et al. N Engl J Med. 1996 Aug 29;335(9):617-23.
- Stress: Sirianni R, et al. J Clin Endocrinol Metab. 2005 Jan;90(1):279-85. Epub 2004 Oct 19.
- Alcohol: Chiao YB, et al. Alcohol Clin Exp Res. 1981 Spring;5(2):230-6.

17,20 lyase

Lower activity:

- Hyperglycemia: Ueshiba H, et al. Eur J Endocrinol. 2002 Mar;146(3):375-80.
- Azole antifungals: Ayub M, Levell MJ. J Steroid Biochem. 1989 Apr;32(4):515-24.
- Dioxins: Moran FM, et al. Biol Reprod. 2003 Jan;68(1):244-51.
- Licorice: Deluca D, et al. J Steroid Biochem Mol Biol. 2005 Feb;93(2-5):285-92.

Higher activity:

- PCBs: Andric SA, et al. Environ Health Perspect. 2000 Oct;108(10):955-9.
- DHEA: Stomati M, et al. Gynecol Endocrinol. 2000 Oct;14(5):342-63.
- Anti-epileptics (valproate): Nelson-DeGrave VL, et al. Endocrinology. 2004 Feb;145(2):799-808. Epub 2003 Oct 23.

17 β -HSD (17beta-hydroxysteroid dehydrogenase)

Lower activity of 17 β HSD type 1 (e.g., resulting in less E2 or testosterone)

- Phytoestrogens: Krazeisen A, et al. Adv Exp Med Biol. 2002;505:151-61.
- Licorice: Armanini D, et al. N Engl J Med. 1999 Oct 7;341(15):1158.
- Pasqualini JR, Ebert C. Gynecol Endocrinol. 1999 Jun;13 Suppl 4:11-9.
- Tamoxifen: Speirs V, et al. J Steroid Biochem Mol Biol. 1993 Nov;46(5):605-11.

Higher activity of 17 β HSD type 1 (e.g., resulting in more E2 or testosterone)

- Alcohol: Sarkola T, et al. Alcohol Alcohol. 2000 Jan;35(1):84-90.
- Abdominal obesity: Corbould AM, et al. Int J Obes Relat Metab Disord. 2002 Feb;26(2):165-75.
- DHEA: Bonney RC, et al. J Steroid Biochem. 1983 Jan;18(1):59-64.

CYP19 (Aromatase)

Lower activity:

- Dioxins: Drenth HJ, et al. Toxicol Appl Pharmacol. 1998 Jan;148(1):50-5.
- Phytoestrogens: Krazeisen A, et al. Mol Cell Endocrinol. 2001 Jan 22;171(1-2):151-62.
- EGCG, green tea catechins: Kapiszewska M, et al. Br J Nutr. 2006 May;95(5):989-95.
- Chrysin: Edmunds KM, et al. Reprod Nutr Dev. 2005 Nov-Dec;45(6):709-20.
- Inflammation: Cutolo M, et al. Ann N Y Acad Sci. 2006 Nov;1089:538-47.
- Licorice: Takeuchi T, et al. Am J Chin Med. 1991;19(1):73-8.
- Vitamin D3: Lou YR, et al. J Steroid Biochem Mol Biol. 2005 Feb;94(1-3):151-7. Epub 2005 Feb 17.

CYP21 (21alpha-hydroxylase)

Lower activity:

- Late-onset adrenal hyperplasia: Carmina E, et al. J Endocrinol Invest. 1984 Apr;7(2):89-92.
- Primary adrenal insufficiency: Nikfarjam L, et al. Eur J Endocrinol. 2005 Jan;152(1):95-101.
- Resveratrol: Supornsilchai V, et al. Horm Res. 2005;64(6):280-6. Epub 2005 Nov 1.
- DHEA: Stomati M, et al. Gynecol Endocrinol. 2000 Oct;14(5):342-63.
- Sodium depletion: Tremblay A, et al. J Biol Chem. 1991 Feb 5;266(4):2245-51.
- High prolactin: Kau MM, et al. J Cell Biochem. 1999 Feb 1;72(2):286-93.

CYP1A1

Lower activity:

- Excess sugar: Peters LP, Teel RW. Anticancer Res. 2003 Jan-Feb;23(1A):399-403.
- Excess omega 6 fats: Lord RS, et al. Altern Med Rev. 2002 Apr;7(2):112-29.
- Oral contraceptives: Jernstrom H, et al. Carcinogenesis. 2003 May;24(5):991-1005.
- Cimetidine: Galbraith RA, Michnovicz JJ. N Engl J Med. 1989 Aug 3;321(5):269-74.
- Strawberries, blackberries, raspberries: Sowers MR, et al. J Nutr. 2006 Jun;136(6):1588-95.
- Soy isoflavones: Lu LJ. Cancer Res. 2000 Mar 1;60(5):1299-305.
- Caffeine: Sowers MR, et al. J Nutr. 2006 Jun;136(6):1588-95.
- Thyroxine: Michnovicz JJ, Galbraith RA. Steroids. 1990 Jan;55(1):22-6.

CYP1B1

Lower activity:

- Hops: Henderson MC, et al. Xenobiotica. 2000 Mar;30(3):235-51.
- Bioflavonoids: Doostdar H, et al. Toxicology. 2000 Apr 3;144(1-3):31-8.
- Grapefruit: Girennavar B, et al. Bioorg Med Chem. 2006 Apr 15;14(8):2606-12.

Higher activity:

- PAHs, PCBs: Carpenter DO, et al. Environ Health Perspect. 1998 Dec;106 Suppl 6:1263-70.

CYP3A4

Lower activity:

- Grapefruit: Fukuda K, et al. Pharmacogenetics. 1997 Oct;7(5):391-6.
- Rosemary: Zhu BT, et al. Carcinogenesis. 1998 Oct;19(10):1821-7.
- Wild yam: Wu WH, et al. J Am Coll Nutr. 2005 Aug;24(4):235-43.
- Peppermint oil: Dresser GK, et al. Clin Pharmacol Ther. 2002 Sep;72(3):247-55.
- Hypothyroidism: Liddle C, et al. J Clin Endocrinol Metab. 1998 Jul;83(7):2411-6.
- Pesticides: Bradlow HL, et al. Environ Health Perspect. 1995 Oct;103 Suppl 7:147-50.
- Smoking or caffeine: Sowers MR, et al. J Nutr. 2006 Jun;136(6):1588-95.

COMT—Methylation support

- Rule out Hg toxicity: Houston MC. Altern Ther Health Med. 2007 Mar-Apr;13(2):S128-33.
- Rule out oxidative stress: James SJ, et al. Am J Clin Nutr. 2004 Dec;80(6):1611-7.
- Reduce stress: Dubey RK, et al. J Clin Endocrinol Metab. 2004 Aug;89(8):3922-31.



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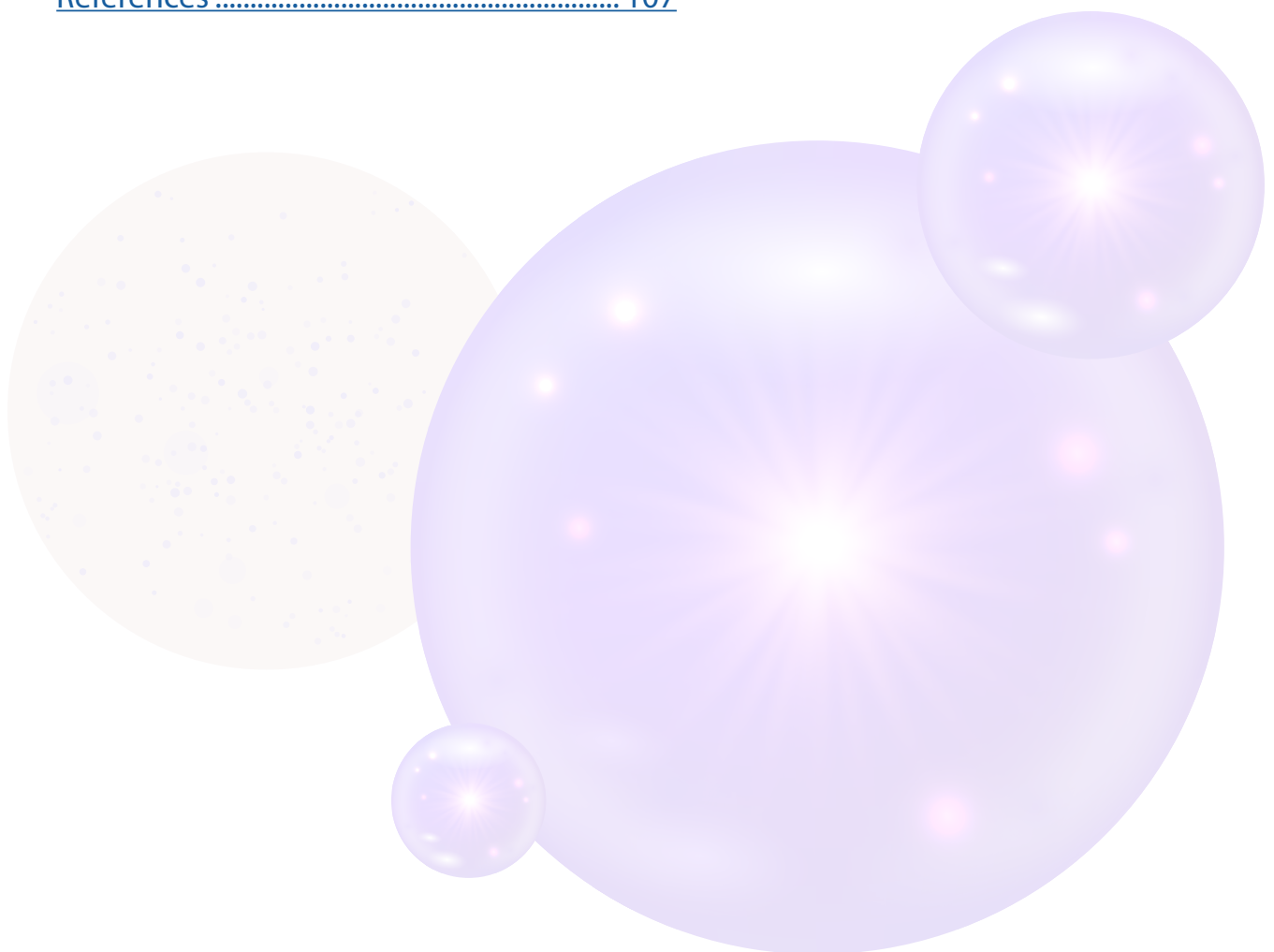
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Introduction:

The **Comprehensive Melatonin Profile** is a salivary assessment using three samples collected over 24 hours to capture the levels and circadian rhythm of melatonin. Melatonin is a hormone that affects many bodily functions, regulates the sleep-wake cycle, and is an important systemic antioxidant. Measuring the circadian rhythm of melatonin might be helpful in patients with:

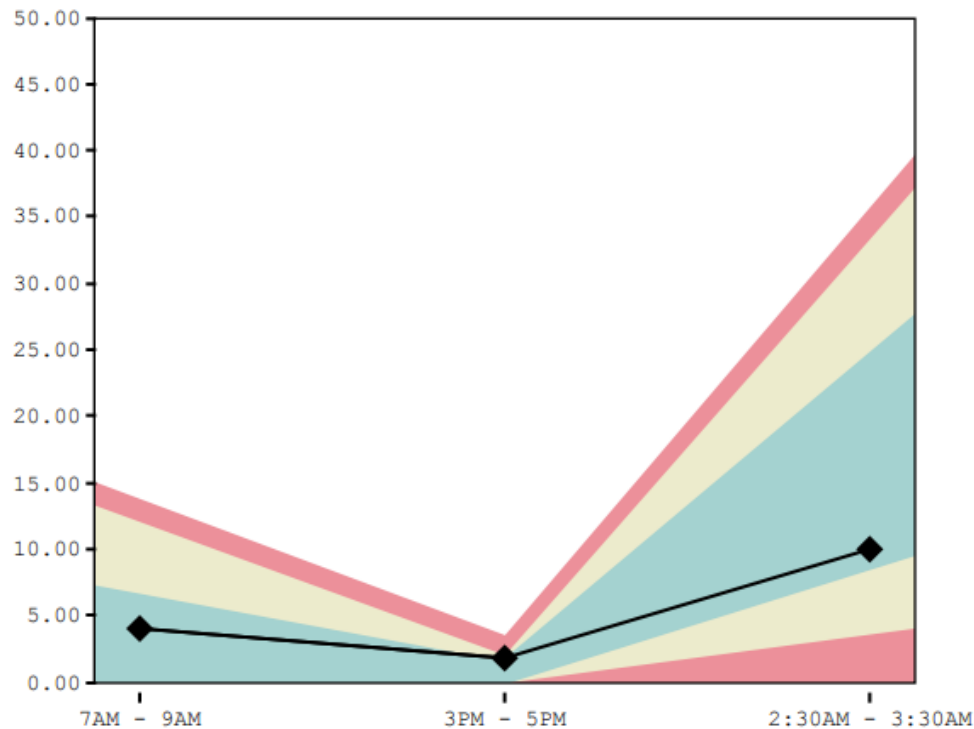
- Sleep disturbances
 - Jet lag
 - Chronic fatigue
 - Altered sleep patterns (i.e., shift- workers)
- Mood disorders
 - Neurodegenerative disorders
 - Hormonal imbalances
 - Cardiovascular risk
- Advanced aging
 - Cancer
 - Metabolic dysfunction



4108 Comprehensive Melatonin Profile

Methodology: EIA

Salivary Melatonin



Results

	7AM-9AM*	3PM-5PM*	2:30AM - 3:30AM*
Patient Results (pg/mL) >>	4.00	1.80	10.00
Reference Range (pg/mL)	<=12.12	<=1.97	3.71-33.38
*Based on Collection Times			



Melatonin Synthesis

We often think of melatonin as a hormone produced in the pineal gland, but our body's melatonin reserves come from many sources:¹⁻⁴

- pineal gland via visible light signaling
- extra-pineal cells/tissues/organs such as the retina, bone marrow, airway epithelium, and skin
- the microbiome of the GI tract, skin, mouth, and vagina
- dietary sources
- mitochondrial production via near-infrared light regulation
- activated macrophages and local immune-competent cells

Most circulating melatonin comes from the pineal gland. The pineal gland produces an average between 0.1 – 0.9 mg of melatonin per day.⁵ Extra-pineal production contributes very little to circulating concentrations in mammals. In fact, after pinealectomy, melatonin levels are mostly undetectable.⁶⁻⁸ It should be noted that although these other locations do not significantly contribute to systemic levels, melatonin exerts important local effects in these extra-pineal areas.

In the brain, melatonin is synthesized in the pineal gland from tryptophan. Tryptophan is a dietary essential amino acid. After ingestion and absorption, it binds to albumin for transport systemically and eventually across the blood-brain-barrier. Tryptophan is taken up by the pineal gland and converted to serotonin then to melatonin via methylation reactions.⁹

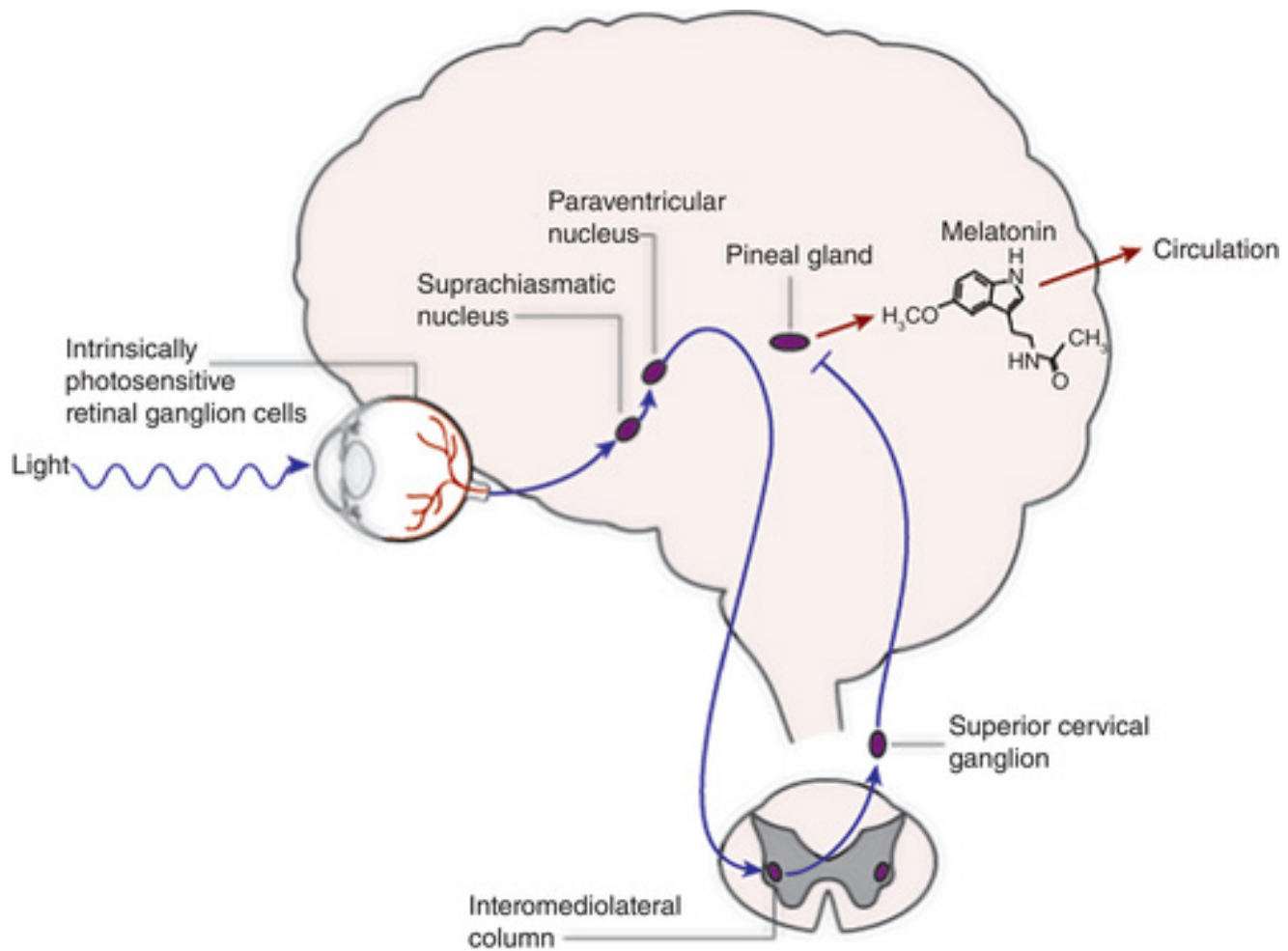
At birth, levels are almost undetectable, the only fetal source being via the placenta. A rhythm appears at around 2-3 months increasing to a lifetime peak in prepubertal children. In fact, melatonin levels may play a role in pubertal Tanner stages of development. Mean adult concentrations in the late teen years remain stable until 35-40, then decline. Patients >90 years old have levels that are less than 20% of young adult levels.⁹

Melatonin Metabolism

Once melatonin is synthesized, it acts in the brain as a day/night signal but is also carried in circulation, metabolized, and excreted. No pineal storage is available; therefore, plasma levels reflect pineal activity fairly well.

Blood melatonin is mainly bound to albumin (70%) and 30% is free and excreted into saliva through passive diffusion. Circulating melatonin can reach all body tissues, including the ability to cross the blood-brain barrier to modulate brain activity.⁷ After its secretion, it immediately diffuses through cell membranes and can be detected in blood, CSF, saliva, breast milk, semen, amniotic fluid, or as its urine metabolite 6-sulfatoxymelatonin.¹⁰ The half-life of melatonin ranges from 20-40 minutes.

The liver clears more than 90% of circulating melatonin. In the liver it is hydroxylated then excreted in the urine as sulfate and glucuronide conjugates. Approximately 50-80% is converted to sulfate derivatives (i.e., 6-sulfatoxymelatonin) and 5-30% is converted to glucuronide derivatives (i.e., 6-hydroxymelatonin glucuronide). It can be found unchanged approximately 1% of the time in the urine.¹¹



Light activation pathway to pineal gland melatonin suppression.¹³

Physiology of the Day/Night Cycle

Light signals from the retina are sent to the suprachiasmatic nucleus (SCN), the major rhythm-generating system or “clock” in mammals, and from there to other hypothalamic areas.

- When light hits the SCN, it secretes gamma-amino butyric acid (GABA), which inhibits the neurons in the paraventricular nucleus (PVN) of the hypothalamus. GABA causes the signal to the pineal gland to be interrupted and melatonin is not made.^{10,12}
- Alternately, when there is no light (darkness), the SCN secretes glutamate. This PVN signal communicates with various neuronal pathways including the superior cervical ganglion to secrete norepinephrine (NE) which triggers the pineal gland to produce melatonin.⁷ The highest secretion of melatonin happens between 2-4am.¹⁰

Blindness or lack of light perception: It is important to note that in blindness or lack of light perception, the SCN continues to generate rhythmic output regardless of light suppression since it functions as an endogenous oscillator (master pacemaker or clock). The rhythm is different from the usual 24h cycle and ‘free-runs’ in the absence of that important light time cue. Light-dark cycles that serve to synchronize the melatonin rhythm to a 24h cycle are absent in blindness or lack of light perception. There is however a small subset of blind patients who retain an intact tract between the retina and hypothalamus resulting in a normal response in spite of no light perception.⁹



Sleep and Circadian Rhythm Disorders

Air travelers know that after crossing several time zones during transcontinental flights they can experience many jet-lag symptoms, including fatigue, sleepiness, irritability, apathy, digestive upset, memory lapses, lack of concentration, impaired judgment and decision making, and headache. The majority of studies (both controlled and uncontrolled) indicate that melatonin administration is useful for improving jetlag symptoms. Interestingly, the improvement is greater with the number of time zones, and in an eastward direction compared to westward.¹⁴

In many blind people, especially in those with no conscious light perception and free running (non-24-h) rhythms, disrupted rhythms of sleep-wake cycle, core body temperature, cortisol, and melatonin are very common. Many blind subjects have unusual melatonin circadian profiles with the range of the endogenous rhythm varying from 23h50min to 25h00min rather than a typical 24-hour cycle. Melatonin supplementation has been shown to shift and reset the circadian clock in blind people. It may stabilize sleep onset and sometimes improve quality and duration of sleep.^{14,15}

Circadian rhythms are also disturbed in shift workers (especially permanent night shift workers) who often complain of fatigue, sleep disturbances, and gastrointestinal problems. In night-shift workers, there is great variability in circadian melatonin profiles. Melatonin, when administered at the desired bedtime in spite of a night shift, may improve sleep and increase daytime alertness, and may prove to be a useful strategy for helping night workers adapt to working night shifts.¹⁴

Melatonin and Cortisol

There is some debate regarding the relationship between melatonin and cortisol.

Cortisol and melatonin typically have an inverse relationship. Cortisol helps regulate the body's response to stress and is involved in the body's wakefulness and alertness, while melatonin regulates the sleep-wake cycle and promotes sleep. When cortisol levels are high, melatonin levels are likely low.

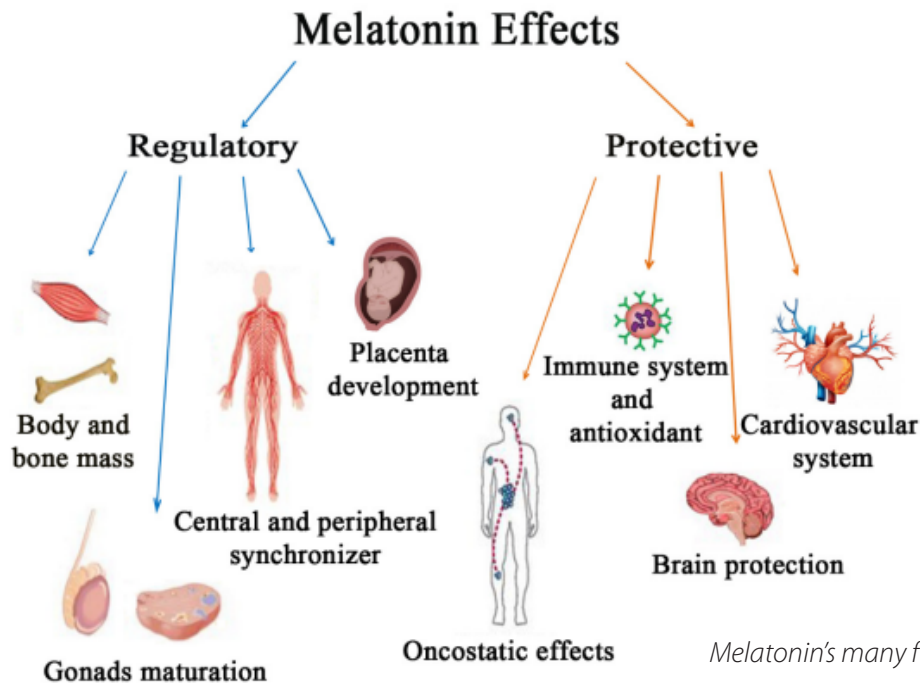
Melatonin is an antioxidant and exerts important local effects, therefore many believe levels rise to compensate for chronically high cortisol levels. That being said, literature to validate this is limited at present, and mainly consists of animal studies – though studies are ongoing.¹⁶⁻¹⁸ Most literature tells us that melatonin will follow its natural circadian rhythm with a few nuances. The interplay between the two hormones is important since they regulate alertness and sleep.

High cortisol levels, especially if they persist over an extended period, can affect, and suppress melatonin production and delay the timing of release.¹⁹⁻²²



Systemic Role of Melatonin

As noted, there is no melatonin storage in the pineal gland. It is secreted into systemic circulation. Melatonin is both hydrophilic and lipophilic. It can cross cell membranes and affect various systemic receptors found in the heart, arteries, kidney, liver, gall bladder, GI tract, adipocytes, ovaries, breast, prostate, and skin. It works to regulate various cycles and its non-receptor-mediated actions are mainly that of acting as an antioxidant and free radical scavenger.



Melatonin Has Many Roles:

1. **Antioxidant:** Not only does melatonin act as a free radical scavenger, but it also stimulates antioxidant enzymes. It increases the levels of several antioxidative enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase. On the other hand, melatonin inhibits the pro-oxidative enzyme nitric oxide synthase.⁷ Compared to such well-known scavengers like glutathione and mannitol, melatonin is 4X and 14X more effective respectively.²³ It is also twice as active as Vitamin E.¹⁰
2. **GI Tract Protection:** Melatonin can be made in the enterochromaffin cells (EC) of the GI tract. Within these EC cells, tryptophan is taken up and converted to serotonin. That serotonin can also be converted to melatonin. This is independent from pineal synthesis. Levels of gut melatonin exceed the amount present in the pineal gland by about 400-500 times – though they do not contribute much at all to systemic levels and rather exert more local effects.
 - a. Locally, melatonin provides protection against intestinal damage. It has been shown that impairment of aged GI-mucosa can be restored by melatonin supplementation.²⁴
 - b. Melatonin is also effective against damage induced by reflux esophagitis, pancreatitis, ulcers and is protective of the liver and biliary tract.
3. **Immune Regulation - The Immune-Pineal Axis:** Pineal melatonin contributes to surveillance and defense responses of the immune system. In normal states, elevated nocturnal melatonin keeps adhesion molecules and leukocytes from adhering to the endothelium. In the face of acute inflammation, proinflammatory cytokines and inflammatory mediators block nocturnal pineal melatonin synthesis. With acute inflammatory insults, local melatonin production in various organs as well as local macrophages produce melatonin 'on demand' at very high levels to help promote local healing and avoid overactivation of the immune response. Restoration of the nocturnal melatonin surge happens via signaling by pro-inflammatory mediators and circulating glucocorticoids from the inflammatory response.^{3,25}

Some research shows that melatonin may have a dual role in immunomodulation. It may activate the immune system after a pathogenic challenge by stimulating and enhancing Th1 cell activity, macrophage function, natural killer (NK) cells, and



cytokine production. Additionally, it is thought that the long-term circadian effects of altered melatonin production can affect hematopoiesis and spleen and thymus function.^{26,27}

These same mechanisms can affect the inflammatory cascade as well. Melatonin has been shown to reduce neutrophil recruitment, regulate nitric oxide synthesis in vasodilation/vasoconstriction, and reduce adhesion of white blood cells to the endothelium.²⁷

4. Hormonal Balance:

- a. The circadian annual rhythm of prolactin secretion depends on circadian melatonin signals. Melatonin regulates and coordinates with prolactin-secreting cells which function together as an intrapituitary pacemaker.^{28,29}
 - b. Melatonin can act on the adrenal glands as an endogenous pacemaker. Glucocorticoid levels are low after onset of darkness and rise through the night concomitantly with a decrease in melatonin levels. There is an inhibitory effect of melatonin on glucocorticoid production.⁹
 - c. Melatonin plays an important role as a regulator of ovulation and positively influences embryonic implant. It is also involved in placenta homeostasis.^{30,31}
 - d. Melatonin has positive effects in gynecological disorders, such as polycystic ovary syndrome (PCOS), premature ovarian failure, and oophoritis, by reducing follicular cell death due to its anti-apoptotic activity. Higher levels of melatonin have been found in women affected by PCOS, likely as a compensative response resulting from body attempts to neutralize high reactive oxygen species (ROS).³²
5. **Skin:** Melatonin is made in the skin. Melatonin and its metabolites regulate and preserve the physical and functional integrity of the skin. Because of its antioxidant and radical scavenger properties, melatonin is protective against UV-mediated skin damage, aging, and may promote wound healing.³³⁻³⁵
6. **Bone Health:** Melatonin may play an important role in age-, menopausal-, and immobility-related diseases, including osteoporosis. Especially around menopause, there is a marked decrease in the secretion of melatonin. Immobility further decreases melatonin, while some studies reveal that levels are increased in response to activity in women.³⁶ Studies indicate that bone turnover also exhibits a circadian rhythm with an increase in bone resorption during the night. The age-related decrease in melatonin levels may interact with

this mechanism and cause accelerated nighttime bone resorption and bone loss in the elderly.

A number of in vitro studies have suggested beneficial effects of melatonin on bone metabolism including bone anabolic effects as well as antiresorptive effects. The effects of melatonin on bone cells may, at least in part, be mediated through reduced oxidative stress.³⁶⁻³⁸

7. **Brain and Neurodegenerative disease:** Nocturnal secretion of melatonin decreases with age. Since it is important for regulating inflammation and is an antioxidant, there is a relationship between decreased melatonin and brain inflammaging. Melatonin rhythms are blunted in neurodegenerative diseases (i.e., Alzheimer's and Parkinson's disease, Huntington's disease, autism, etc.), and melatonin therapy helps to manage insomnia in these patients and can help with cognitive function, memory, and anxiety. Given the wide distribution of melatonin receptors in the CNS, melatonin seems to be one of the promising neuroprotective agents to be tested in humans. However, it should be noted that some studies have found deleterious effects of melatonin in elderly demented patients.^{9,39}
8. **Cancer:** Melatonin's benefits in cancer include its antiproliferative actions, immunostimulatory effects on host anticancer defenses and antioxidant activity. Interestingly, there appears to be some circadian rhythm component to antitumor action and the timing of melatonin supplementation is important. Morning administration of melatonin has shown some stimulation of tumor growth in isolated reports. There are also studies using melatonin as an adjunct to chemotherapy with improvement of survival and quality of life.⁷
9. **Metabolic Dysfunction:** Melatonin can regulate adipose tissue and adipokines, lipolysis of fat deposition, brown adipose tissue growth, and white adipose tissue browning – thereby affecting energy expenditure. There is no consensus on melatonin supplementation in treating metabolic diseases, though given it's varied physiologic effects, there is potential and research is ongoing.⁴⁰
10. **Cardiovascular Disease:** Due to its antioxidant nature, having low melatonin levels is a risk factor for cardiovascular diseases such as ischemic myocardial injury, hypertension, atherosclerosis, and heart failure. Administration of melatonin promotes angiogenesis and blood vessel formation to damaged cardiac tissue to reduce cellular death and cardiac insufficiency.^{10,41}



Laboratory Assessment

Salivary melatonin measurement is well-represented in literature. Salivary levels are significantly lower than serum levels, estimated to be approximately 30% of serum. Saliva measures unbound, bioavailable melatonin, while serum reflects free melatonin and melatonin bound to carrier proteins. The patterns of salivary melatonin secretion can

help to assess the circadian rhythm and are easily sampled at home. Melatonin levels peak between 2:00-5:00 am then begin to fall with low levels expected throughout the day.^{42,43} It should be noted that when sampling salivary melatonin in the middle of the night, samples should be obtained without turning on a bright light which will cause levels to drop.

Quick Melatonin Interpretation

Result	Potential Contributing Factors	Potential Treatment Strategies
High	<ul style="list-style-type: none"> An extended dark phase of the day Supplementation: melatonin (especially sublingual), tryptophan, 5HTP, cannabis, St. John's Wort^{44,45} Medications: SSRIs, SNRIs, MAO inhibitors⁴⁶⁻⁴⁸ Foods high in melatonin: oats, meat, milk, sweet corn, rice, ginger, tomatoes, cherries, berries, bananas, barley, Japanese radish⁴⁹ Foods high in tryptophan: spirulina, seaweed, soybean, cottage cheese, chicken liver, pumpkin seeds, turkey, chicken, watermelon seeds, almonds, peanuts, brewer's yeast, malted milk, milk, ice cream, yogurt Slow metabolism of melatonin by the liver (hydroxylation, then conjugation with sulphate and glucuronide)⁵⁰ SNP of the MTNR1B gene (melatonin receptor), which may extend duration of melatonin production⁵¹ Compensatory response to chronically elevated cortisol (empirical evidence) Pineal gland tumor (excess secretion) <ul style="list-style-type: none"> - Rare: 0.4-1.0% of brain tumors in adults - Usually leads to headache, vision changes, nausea, vomiting, problems with balance/coordination Caffeine (both stimulatory and inhibitory – delays clearance of melatonin)⁵² Exercise Some studies indicate exercise can increase melatonin in women – though studies are mixed³⁶ 	<ul style="list-style-type: none"> Consider increasing morning exposure to bright light - natural or full-spectrum light source Check for presence of medications, supplements, or foods containing melatonin or tryptophan and consider adjusting, if medically appropriate Consider checking liver function Consider nutrients that support liver detoxification pathways: <ul style="list-style-type: none"> • Sulfation: allium vegetables, methionine, taurine, cysteine • Glucuronidation: cruciferous vegetables (or DIM or I3C) Consider adrenal adaptogens if elevated cortisol levels Consider referral for appropriate pineal gland imaging study, especially if chronic headache, vision changes, nausea, vomiting, problems with balance/coordination Monitor caffeine intake

(continued on following page)



Result	Potential Contributing Factors	Potential Treatment Strategies
Low	<ul style="list-style-type: none"> An extended light phase of the day Medications: NSAIDs, SSRIs, benzodiazepines, beta-blockers, adrenergics, calcium channel blockers, and steroids^{48,53} Caffeine, tobacco, alcohol, high dose vitamin B12^{52,54} Exercise may suppress melatonin levels for up to 3 hours post-workout and cause sleep phase delays – though studies are mixed^{55,56} Exposure to electromagnetic fields⁵⁷ Decreased production of melatonin by the pineal gland (ex: calcification or damage secondary to head trauma)⁵⁸ Advanced age Tryptophan deficiency and lack of cofactors to make melatonin such as vitamin B6 and folate⁷ 	<ul style="list-style-type: none"> Consider minimizing bright light exposure at night Consider reducing exposure to electromagnetic fields Consider adjusting the timing of medications, if medically appropriate Consider exercising earlier in the day to prevent nighttime suppression Consider single or divided low dose melatonin supplementation, if appropriate Consider increasing dietary intake of melatonin and tryptophan containing foods (mentioned above) Consider nutrients required for melatonin production: vitamin B3, vitamin B6, calcium, and magnesium Consider adjusting timing of vitamin B12 supplementation (if high dose)

Melatonin as a Therapeutic

There are many animal and plant dietary sources of melatonin, such as tomatoes, spinach, nuts, grains, berries and cherries, legumes, eggs, beef, pork, salmon, milk, and much more.⁴⁹ Although the amounts of melatonin in most foods seem relatively low (nanograms per gram) compared to physiologic levels, consuming foods rich in melatonin may increase overall systemic antioxidant status.⁵

Physiologic ranges can be reached by oral melatonin supplementation of 0.1-0.3mg. Higher doses can cause supraphysiologic levels, though there are no reported overt toxic effects. 1-10mg can raise plasma melatonin levels from 3-60 fold its normal peak¹⁰ Overall, it appears safe for adults without serious side effects (mild effects include dizziness, headache, nausea, morning drowsiness, decreased body temperature).⁴⁰ When supplemented during the daytime, these symptoms can interfere with day-to-day activities.⁹

Plant-based melatonin supplementation (phytomelatonin) is being widely studied and is shown to be more effective than synthetic melatonin. Phytomelatonin has been shown to have stronger COX-2 inhibition and stronger free-radical scavenging ability as compared to synthetic melatonin supplementation. This is likely due to other plant constituents found in the supplement such as chlorophyll, beta-carotene, lutein, and other antioxidant nutrients.⁵

In general, there is no general consensus regarding dosage of melatonin supplementation. A wide range of dose formulations are available, and the usage varies depending on the clinical application. The usual advice is to start with the lowest dose available. Low doses 0.1 to 0.3 mg/d that produce near physiological melatonin concentrations can be used for central clock synchronization, doses ranging from 0.6 to 5 mg/d for sleep disorders, or doses as high as 300 mg/d for neurodegenerative disorders. Whether dosage should be changed in chronic melatonin treatment according to the annual season requires further investigation; further studies are needed on the undesirable consequences of melatonin suppression in the long term (for example by beta blocking drugs or shiftwork).⁹



References

1. Tan D-X, Reiter RJ, Zimmerman S, Hardeland R. Melatonin: Both a Messenger of Darkness and a Participant in the Cellular Actions of Non-Visible Solar Radiation of Near Infrared Light. *Biology*. 2023;12(1):89.
2. Meng X, Li Y, Li S, et al. Dietary Sources and Bioactivities of Melatonin. *Nutrients*. 2017;9(4).
3. Markus RP, Sousa KS, da Silveira Cruz-Machado S, Fernandes PA, Ferreira ZS. Possible Role of Pineal and Extra-Pineal Melatonin in Surveillance, Immunity, and First-Line Defense. *Int J Mol Sci*. 2021;22(22):12143.
4. Zefferino R, Di Gioia S, Conese M. Molecular links between endocrine, nervous and immune system during chronic stress. *Brain Behavior*. 2021;11(2):e01960.
5. Minich DM, Henning M, Darley C, Fahoum M, Schuler CB, Frame J. Is melatonin the "Next Vitamin D"? A review of emerging science, clinical uses, safety, and dietary supplements. *Nutrients*. 2022;14(19):3934.
6. Lewy AJ, Tetsuo M, Markey SP, Goodwin FK, Kopin IJ. Pinelectomy abolishes plasma melatonin in the rat. *J Clin Endocrinol Metab*. 1980;50(1):204-205.
7. Claustrat B, Brun J, Chazot G. The basic physiology and pathophysiology of melatonin. *Sleep Med Rev*. 2005;9(1):11-24.
8. Hardeland R. Melatonin—more than just a pineal hormone. *Biomed J Sci Tech Res*. 2017;1(4):1-4.
9. Arendt J, Aulinas A. Physiology of the Pineal Gland and Melatonin. In: Feingold KR, Anawalt B, Blackman MR, et al., eds. *Endotext*. South Dartmouth (MA): MDText.com, Inc. 2000.
10. Ferlazzo N, Andolina G, Cannata A, et al. Is melatonin the cornucopia of the 21st century? *Antioxidants*. 2020;9(11):1088.
11. Gunata M, Parlakpinar H, Acet H. Melatonin: A review of its potential functions and effects on neurological diseases. *Revue Neurolog*. 2020;176(3):148-165.
12. Repova K, Baka T, Krajcovicova K, et al. Melatonin as a Potential Approach to Anxiety Treatment. *Int J Mol Sci*. 2022;23(24):16187.
13. Ostrin LA. Ocular and systemic melatonin and the influence of light exposure. *Clin Exp Optometr*. 2019;102(2):99-108.
14. Karasek M, Winczyk K. Melatonin in humans. *J Physiol Pharmacol*. 2006;57:19.
15. Lockley SW, Skene DJ, Arendt J, Tabbandeh H, Bird AC, DeFrance R. Relationship between melatonin rhythms and visual loss in the blind. *J Clin Endocrinol Metab*. 1997;82(11):3763-3770.
16. Dagnino-Subiabre A, Orellana JA, Carmona-Fontaine C, et al. Chronic stress decreases the expression of sympathetic markers in the pineal gland and increases plasma melatonin concentration in rats. *J Neurochem*. 2006;97(5):1279-1287.
17. Fernandes PA, Tamura EK, D'Argenio-Garcia L, et al. Dual Effect of Catecholamines and Corticosterone Crosstalk on Pineal Gland Melatonin Synthesis. *Neuroendocrinology*. 2017;104(2):126-134.
18. Ito Y, Iida T, Yamamura Y, et al. Relationships between Salivary Melatonin Levels, Quality of Sleep, and Stress in Young Japanese Females. *Int J Tryptoph Res*. 2013;6s1:1JTR. S11760.
19. Bob P, Fedor-Freybergh P. Melatonin, consciousness, and traumatic stress. *J Pineal Res*. 2008;44(4):341-347.
20. Hirotsu C, Tufik S, Andersen ML. Interactions between sleep, stress, and metabolism: From physiological to pathological conditions. *Sleep Sci*. 2015;8(3):143-152.
21. Riemann D, Klein T, Rodenbeck A, et al. Nocturnal cortisol and melatonin secretion in primary insomnia. *Psych Res*. 2002;113(1-2):17-27.
22. Ackermann K, Plomp R, Lao O, et al. Effect of sleep deprivation on rhythms of clock gene expression and melatonin in humans. *Chronobiol Int*. 2013;30(7):901-909.
23. Tan D-X. Melatonin: a potent, endogenous hydroxyl radical scavenger. *Endocr J*. 1993;1:57-60.
24. Brzozowski T, Jaworek J. Editorial (Thematic issues: Basic and clinical aspects of melatonin in the gastrointestinal tract. new advancements and future perspectives). *Curr Pharmaceut Design*. 2014;20(30):4785-4787.
25. Markus RP, Ferreira ZS. The immune-pineal axis: the role of pineal and extra-pineal melatonin in modulating inflammation. *Adv Neuroimm Biol*. 2011;1(1):95-104.
26. Macchi MM, Bruce JN. Human pineal physiology and functional significance of melatonin. *Front Neuroendocrinol*. 2004;25(3-4):177-195.
27. Szczepanik M. Melatonin and its influence on immune system. *J Physiol Pharmacol*. 2007;58(6):115-124.
28. Webley GE, Lenton EA. The temporal relationship between melatonin and prolactin in women. *Fertility Sterility*. 1987;48(2):218-222.
29. Arendt J, Aulinas A. *Physiol Pineal Gland Melatonin*. MDText.com, Inc., South Dartmouth (MA); 2000.
30. Carlomagno G, Minini M, Tilotta M, Unfer V. From implantation to birth: insight into molecular melatonin functions. *Int J Mol Sci*. 2018;19(9):2802.
31. Rai S, Gosh H. Modulation of human ovarian function by melatonin. *Front Bioscience-Elite*. 2020;13(1):140-157.
32. Jamilian M, Foroozanfar F, Mirhosseini N, et al. Effects of Melatonin Supplementation on Hormonal, Inflammatory, Genetic, and Oxidative Stress Parameters in Women With Polycystic Ovary Syndrome. *Front Endocrinol*. 2019;10:273.
33. Kleszczynski K, Fischer TW. Melatonin and human skin aging. *Dermato-endocrinology*. 2012;4(3):245-252.
34. Rusanova I, Martinez-Ruiz L, Florido J, et al. Protective effects of melatonin on the skin: Future perspectives. *Int J Mol Sci*. 2019;20(19):4948.
35. Slominski A, Tobin DJ, Zmijewski MA, Wortsman J, Paus R. Melatonin in the skin: synthesis, metabolism and functions. *Trends Endocrinol Metab*. 2008;19(1):17-24.
36. Amstrup A, Sikjaer T, Mosekilde L, Rejnmark L. Melatonin and the skeleton. *Osteoporosis Int*. 2013;24:2919-2927.
37. Amstrup AK, Sikjaer T, Heickendorff L, Mosekilde L, Rejnmark L. Melatonin improves bone mineral density at the femoral neck in postmenopausal women with osteopenia: a randomized controlled trial. *J Pineal Res*. 2015;59(2):221-229.
38. Kotlarczyk MP, Lassila HC, O'Neil CK, et al. Melatonin osteoporosis prevention study (MOPS): a randomized, double-blind, placebo-controlled study examining the effects of melatonin on bone health and quality of life in perimenopausal women. *J Pineal Res*. 2012;52(4):414-426.
39. Hardeland R, Cardinali DP, Brown GM, Pandi-Perumal SR. Melatonin and brain inflammation. *Progress Neurobiol*. 2015;127:46-63.
40. Guan Q, Wang Z, Cao J, Dong Y, Chen Y. Mechanisms of Melatonin in Obesity: A Review. *Int J Mol Sci*. 2021;23(1).
41. Rahbarghazi A, Siahkhouhian M, Rahbarghazi R, et al. Role of melatonin in the angiogenesis potential; highlights on the cardiovascular disease. *J Inflamm*. 2021;18(1):4.
42. Arendt J, Skene DJ. Melatonin as a chronobiotic. *Sleep Med Rev*. 2005;9(1):25-39.
43. Brzezinski A. Melatonin in humans. *NEJM*. 1997;336(3):186-195.
44. Murch SJ, Saxena PK. A melatonin-rich germplasm line of St John's wort (*Hypericum perforatum* L.). *J Pineal Res*. 2006;41(3):284-287.



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