# Endo+

## SUPPORT GUIDE

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HPA axis
CORTISOL AWAKENING RESPONSE
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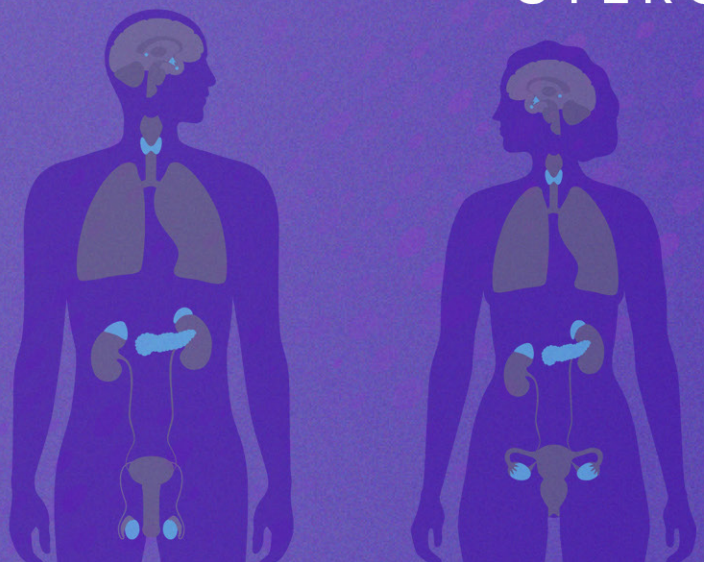

GENOVA DIAGNOSTICS®

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# Endo+ Support Guide

## 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.

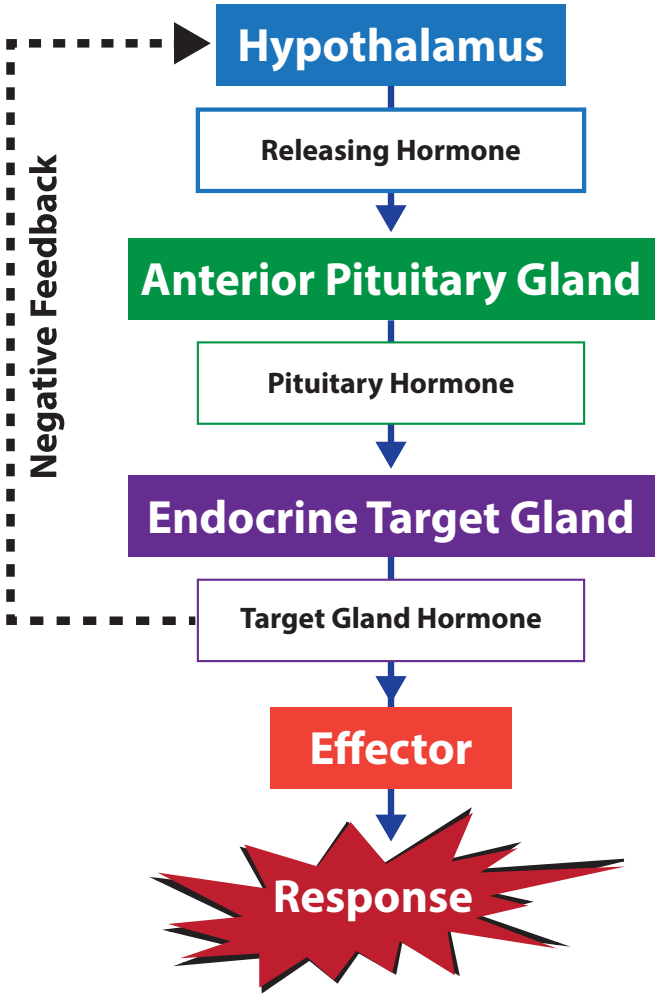
Endo+ is a unique profile that combines multiple hormonal assessments and synthesizes the information so the clinician can quickly identify imbalances. Clinicians can build an individualized hormone assessment for each patient choosing from six component profiles.

## 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-methyoxyestrone, 4-methyoxyestradiol, 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

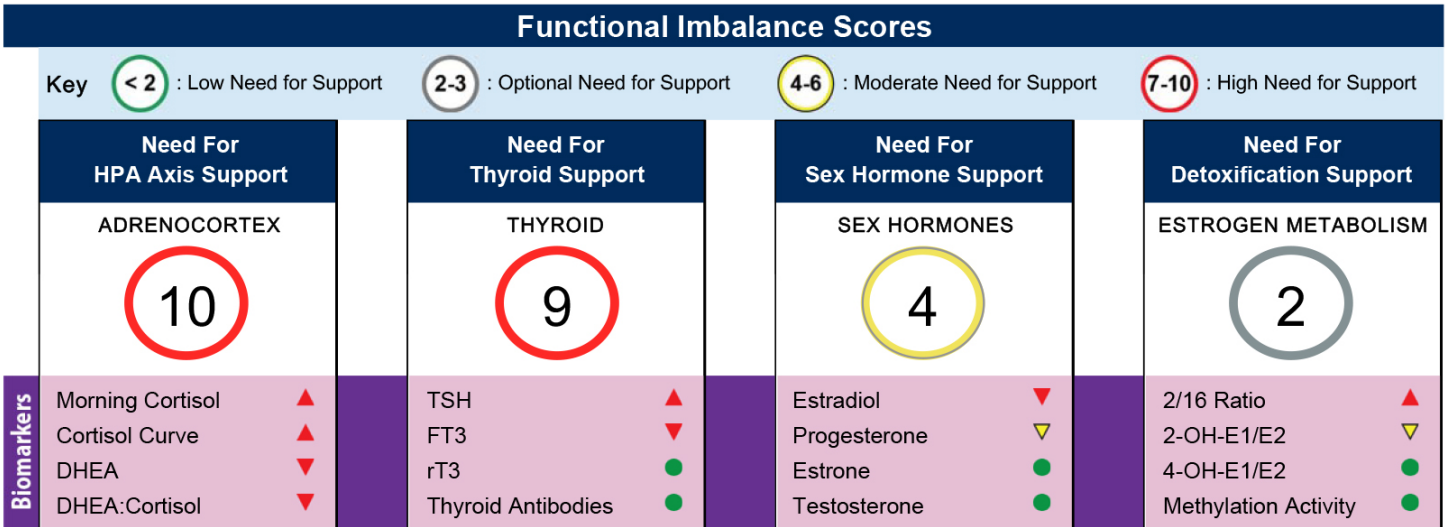
Hormone imbalance symptoms can be associated with multiple hormones.
**E** Estrogen; **P** Progesterone; **T** Testosterone; **TH** Thyroid; **M** Melatonin; **C** Cortisol

| | | | | | | |
|---|---|---|---|---|---|---|
| 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




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.

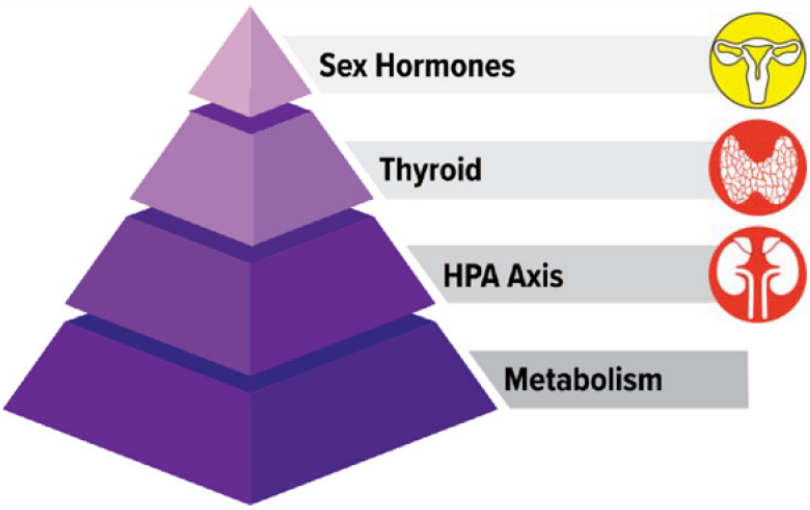
## Therapeutic Support Options

| Therapeutic Support Options | | | |
|---|---|---|---|
| • Stress Reduction<br>• Mindfulness Training<br>• HRV Biofeedback<br>• Breathwork Training<br>• Exercise/Yoga/Tai Chi<br>• Herbal Adaptogens<br>• Lower Blood Sugar<br>• B-Vitamins<br>• Phosphoserine<br>• Glandulars | • Minerals: Se, Zn, Iron<br>• Tyrosine<br>• Adequate Iodine<br>• Stress Reduction<br>• Reduce Environmental Exposures<br>• Antioxidant Support<br>• Assess Medications<br>• Smoking Cessation<br>• Consider Thyroid HRT | • Address Thyroid Imbalance<br>• Address HPA Axis<br>• Phytoestrogens (for estrogen support)<br>• Phytoandrogens (for testosterone support)<br>• Consider Glandulars<br>• Consider Hormone Precursors<br>• Consider BHRT | • Methylation Support: B-Vitamins, Mg, Betaine<br>• Cruciferous vegetables, berries, rosemary<br>• Soy isoflavones, DIM, I3C<br>• Bioflavanoids, Glutathione<br>• Reduce sugar, stress, environmental toxicity<br>• Reduce adiposity<br>• Physical activity |

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.

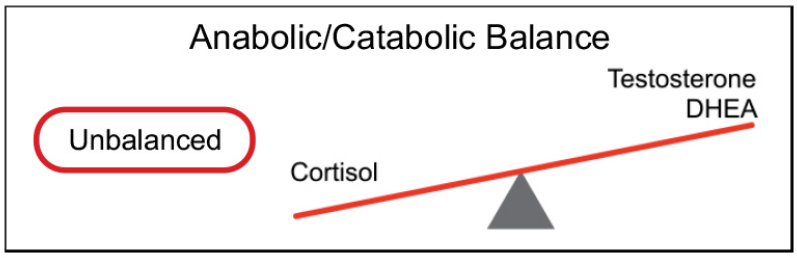



The colored circles to the right of the pyramid reflect the functional imbalance scores discussed above and show the relative need for therapeutic support in that category. Green represents a low need for support, grey represents an optional need, yellow indicates moderate, and red indicates a high need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

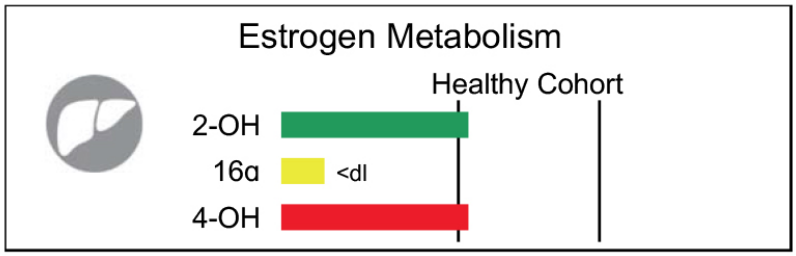



The Anabolic/Catabolic Balance graphic shows the ratio of total cortisol to testosterone and DHEA based on the percentiles of where they fall within their respective reference ranges. The analysis is similar to comparing the relative abundances of testosterone plus DHEA, and total cortisol. When you 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.


# A·S·P

ADRENOCORTEX STRESS PROFILE

# SUPPORT GUIDE

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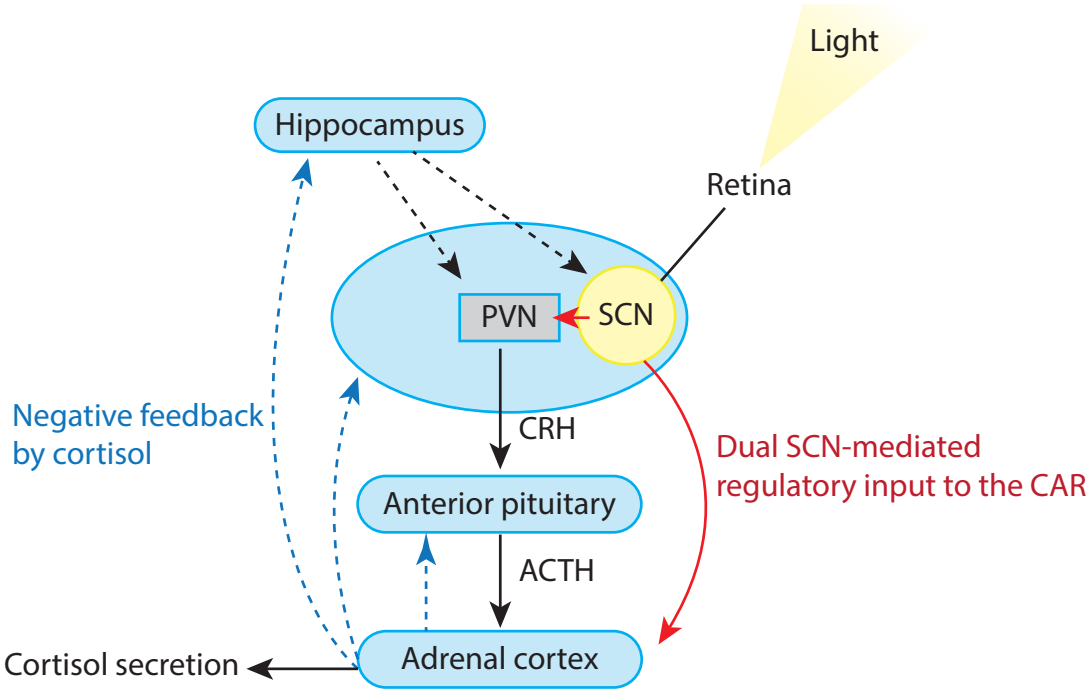
## Hypothalamic-Pituitary-Adrenal (HPA) Axis and the Stress Response:

In response to any stressor, the hypothalamus produces corticotropin releasing hormone (CRH), which stimulates the anterior pituitary to secrete adrenocorticotropic 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-chiasmic 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 triiodthryonine (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-1β, 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 reponse, 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:

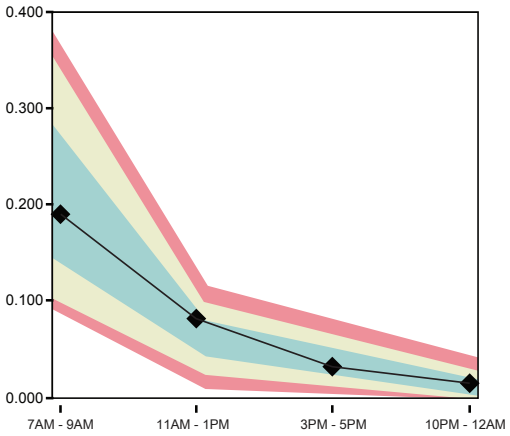
Specific cortisol findings throughout a diurnal rhythm may be affected by any number of acute mental, emotional, and physical daily stressors. Abnormal results should be correlated with each patient's clinical presentation and daily routine.[2,16]

| TIMING | SIGNIFICANCE | CONSIDERATIONS WHEN HIGH | CONSIDERATIONS WHEN LOW |
|---|---|---|---|
| **Cortisol Awakening Response (CAR) Add-on** | HPA axis resiliency<br>Perception of control around chronic stress[17] | Adaptive anticipation of daily stress<br>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**<br>**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**<br>**11:00AM-1:00PM** | Adaptive function of the HPA axis to daily routine | Exercise, blood sugar dysregulation, lifestyle stressors, pain | HPA axis dysfunction |
| **Afternoon**<br>**3:00PM-5:00PM** | Can reflect glycemic control | Exercise, blood sugar dysregulation, lifestyle stressors, pain | HPA axis dysfunction |
| **Evening**<br>**10:00PM- 12:00AM** | Baseline HPA axis function | Stress, alcohol, exercise, lifestyle stressors, pain<br><br>Linked to insomnia and various diseases such as diabetes, cardiovascular disease, hormonally-driven cancers, and osteoporosis[18,20-24] | Optimal |

## Treatment Options:

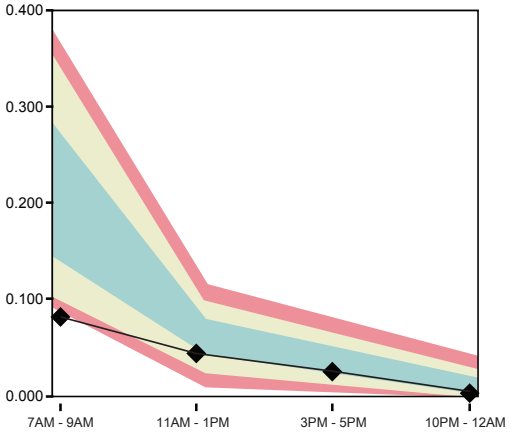
Treatment of abnormal cortisol should be directed at the stressor's root cause. Lifestyle modification with relaxation methods, dietary changes, pain management, and overall HPA axis support with nutrition, adaptogens, and supplements can be helpful.[25]

## Normal Diurnal Slope



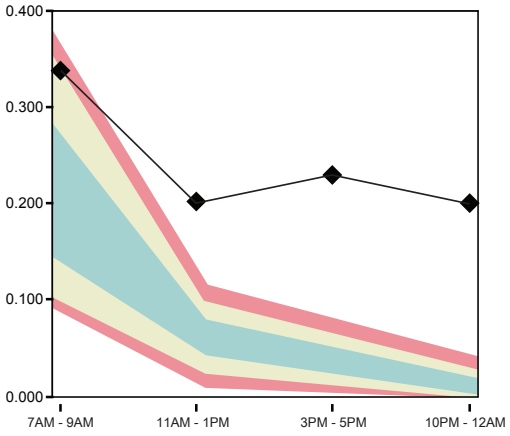

The natural cortisol diurnal rhythm shows a peak within the first hour after awakening, a rapid decline over the morning hours, and then tapering through the rest of the day before reaching its lowest point at night.[2]

## Low Slope



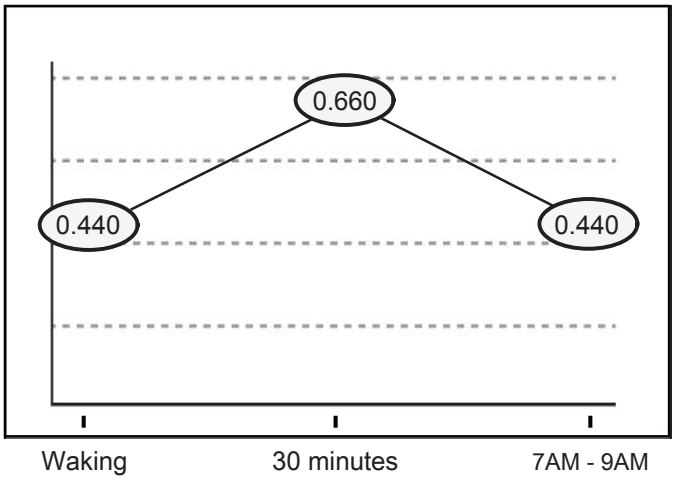

- Chronic stress burden
- Poor psychosocial function
- Lack of HPA axis resiliency
- Lower perceived control over stress
- Post-Traumatic Stress Disorder (PTSD)
- Persistent fatigue, anxiety, and depression
- Predictive of health outcomes, such as increased breast cancer mortality, increased coronary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

## High Slope




- Appropriate response to a major stressor
- Perceived insurmountable challenge[15]

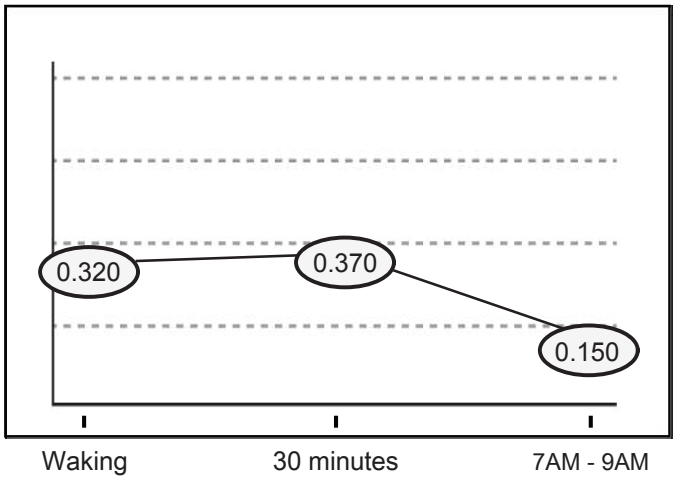
## Cortisol Awakening Response (CAR) Add-On




| Percent Increase | 50 | Expected: >50% |
|---|---|---|

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]

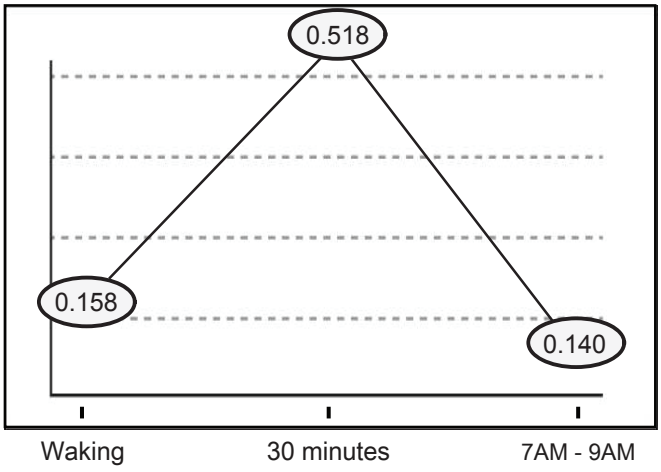
## Blunted CAR




| Percent Increase | 16 | Expected: >50% |
|---|---|---|

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

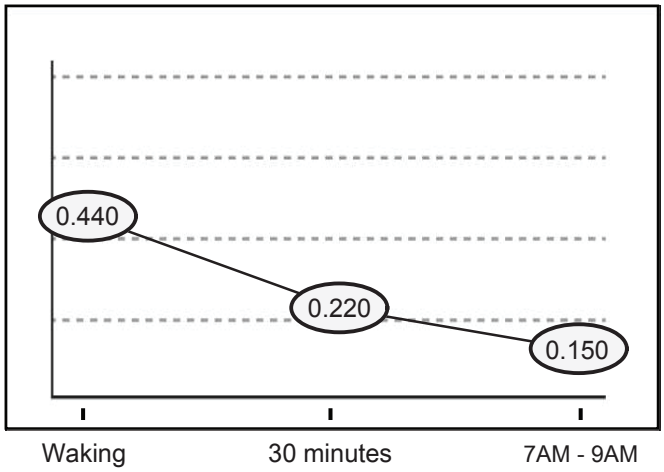
## Elevated CAR




| | | |
|---|---|---|
| **Percent Increase** | **228** | Expected: >50% |

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

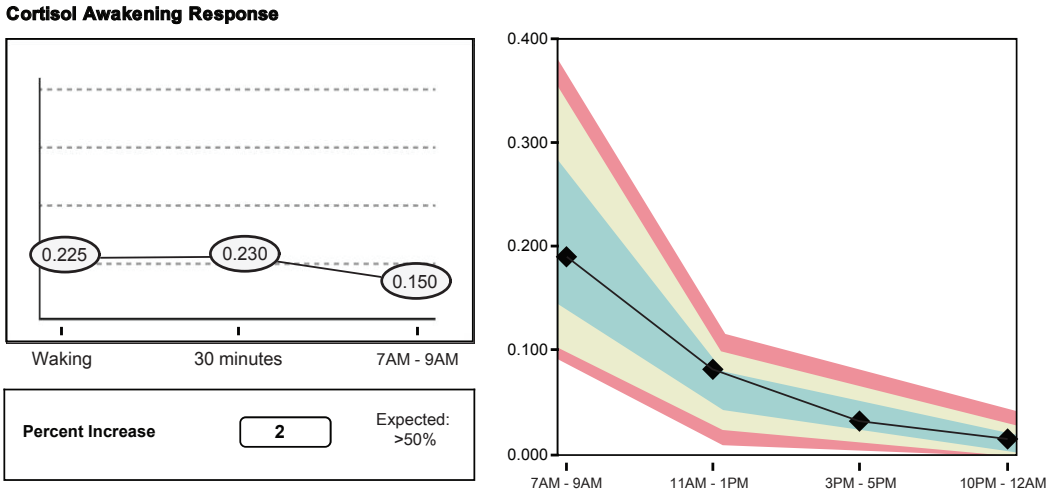
## Negative CAR




| | | |
|---|---|---|
| **Percent Increase** | **-50** | Expected: >50% |

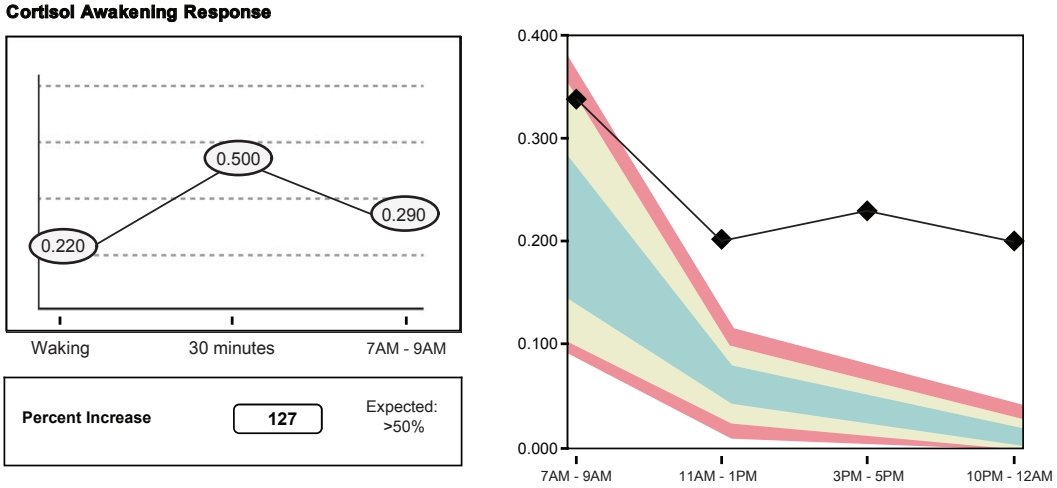
- Ensure there was no delay between waking and obtaining the first sample
- Literature is evolving regarding clinical implications[27,28]
- One hypothesis is that a negative CAR percentage may reflect a blunted CAR with further loss of resiliency

## CAR Non-Response



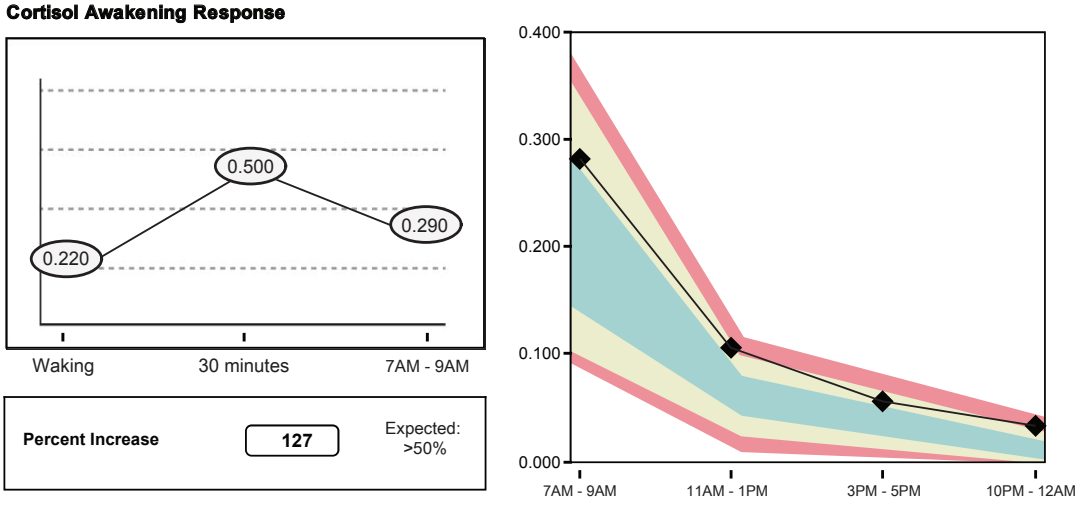

- 25% of healthy adults do not mount a CAR
- Response is defined as an increase of at least 0.09µg/dL above individual baseline with otherwise adequate cortisol diurnal curve
- Ensure proper sampling[17,19]

## CAR Elevated with High Slope



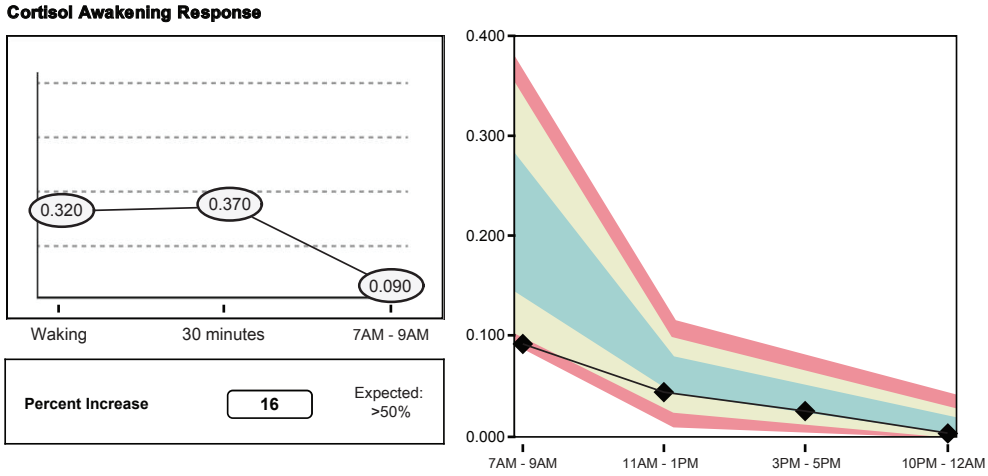

- General HPA axis dysfunction
- Significant stressor, real or perceived[26,29]

## CAR Elevated with Elevated Slope




- 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




- 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:** | Advancing age, chronic stress, HPA axis dysfunction<br>Low DHEA levels have been associated with immune dysregulation, cardiovascular disease, arthritis, osteoporosis, insomnia, declining cognition, depression, fatigue, and decreased libido.[31-35] |

| DHEA:Cortisol Ratio | |
|---|---|
| **HIGH:** | Favors anabolic activity<br>Address specific cortisol and DHEA abnormalities[32,36-38] |
| **LOW:** | Favors catabolic activity<br>Address specific cortisol and DHEA abnormalities[32,36-38] |

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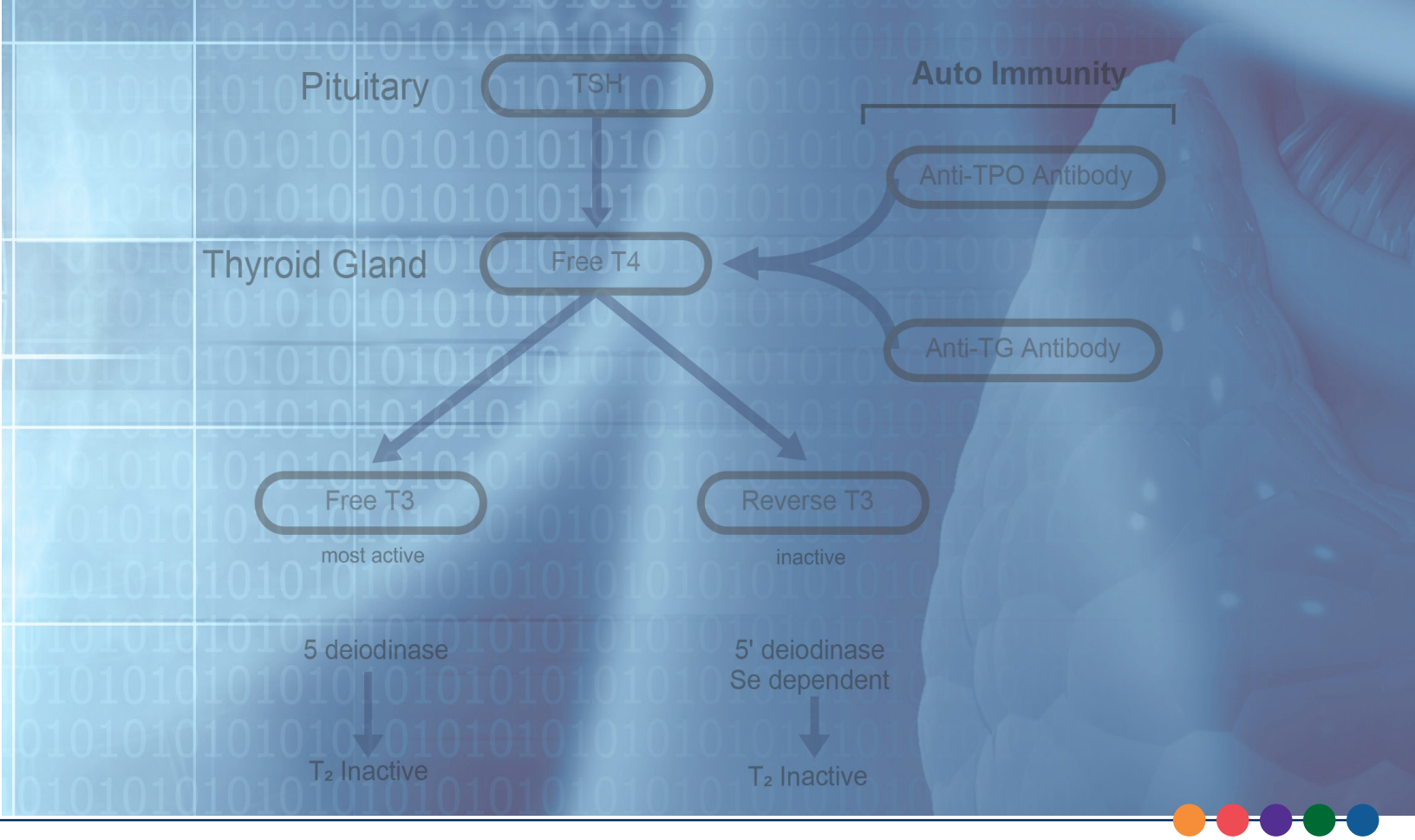


# Comprehensive Thyroid Assessment

## SUPPORT GUIDE

GENOVA DIAGNOSTICS

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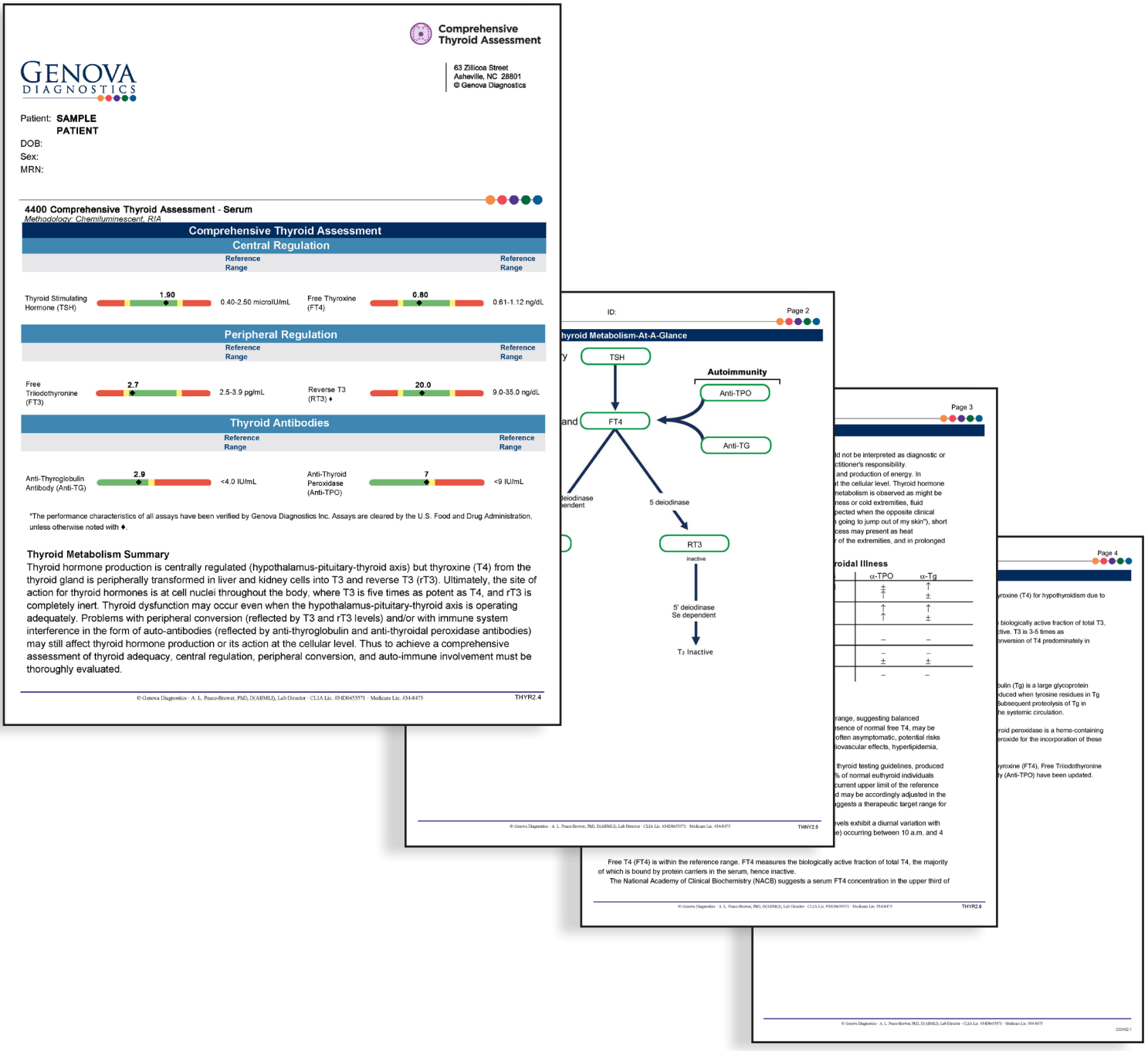
# Comprehensive Thyroid Assessment

Genova Diagnostics' **Comprehensive Thyroid Assessment** provides a thorough analysis of thyroid hormone metabolism. It evaluates central thyroid gland regulation and activity, thyroid hormone production and secretion, peripheral thyroid conversion, and thyroid autoantibodies. This profile allows clinicians to pinpoint common imbalances that underlie a broad spectrum of chronic illness by measuring serum levels of thyroid stimulating hormone (TSH), free thyroxine (free T4), free triiodothyronine (free T3), reverse T3, anti-thyroglobulin antibodies, and anti-thyroid peroxidase antibodies.

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# Thyroid Physiology and Metabolism

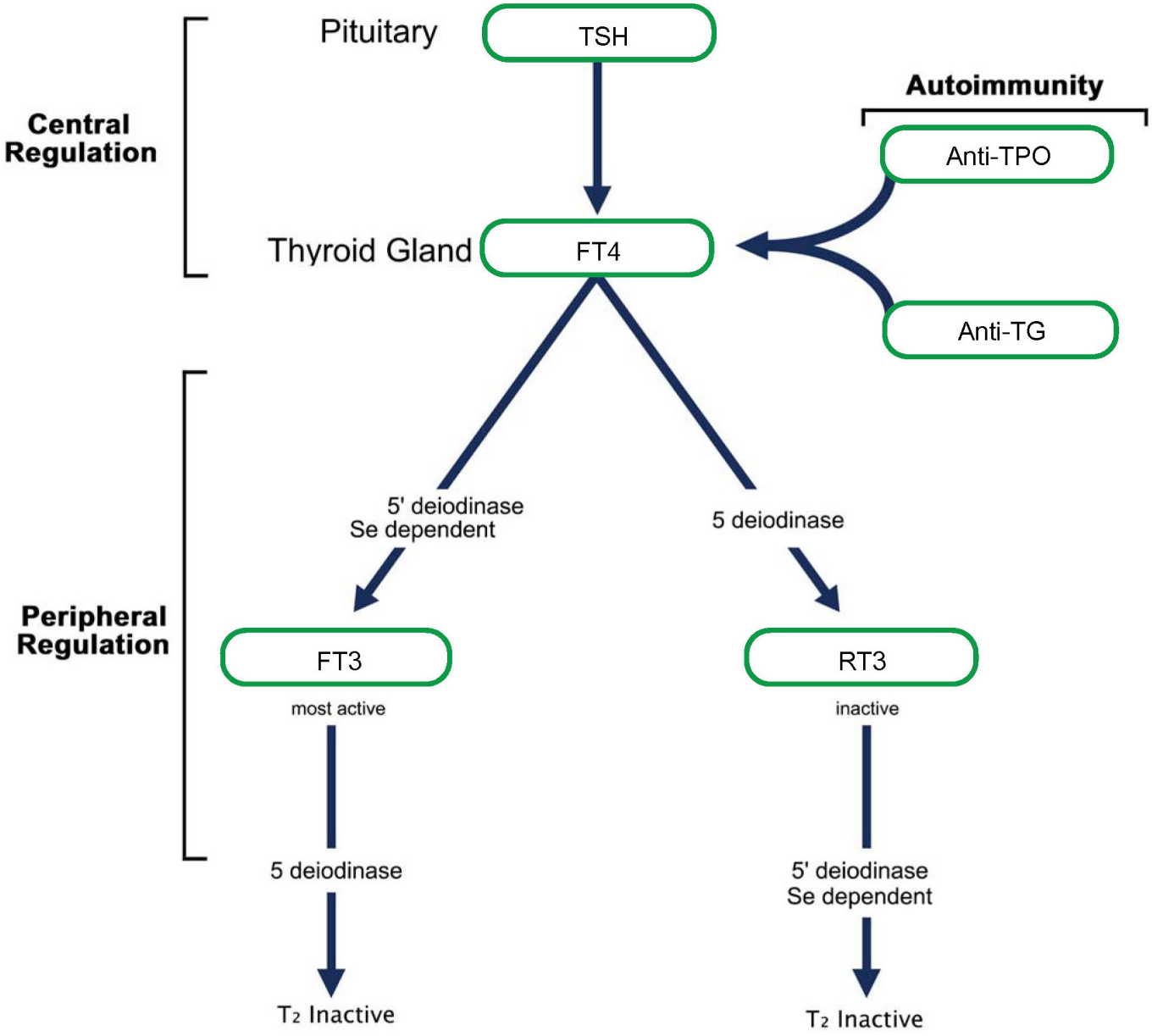
The thyroid gland is a butterfly-shaped organ in the anterior trachea. It is sometimes referred to as the 'thermostat' of the body since thyroid hormones are involved in metabolism, thermogenesis, energy expenditure, growth, and development. Thyroid hormone production affects many organ systems including 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 type1 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

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# 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

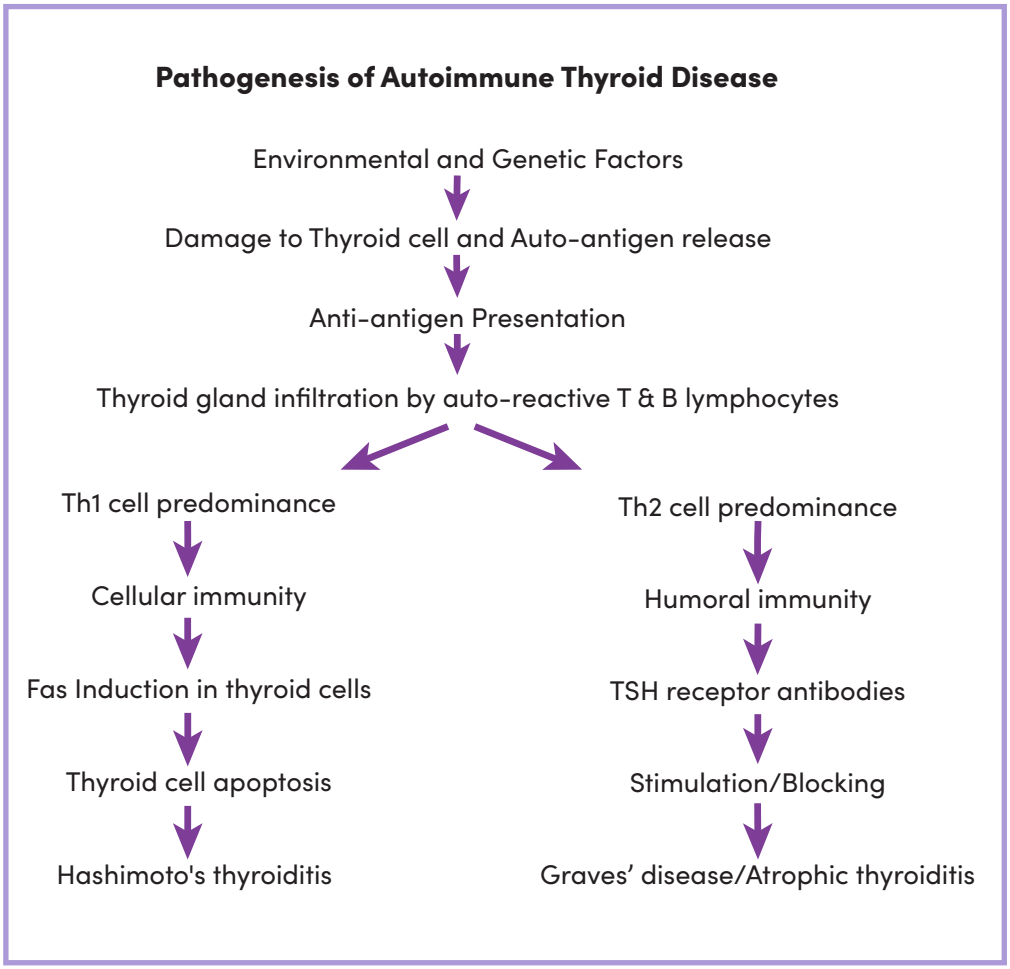
As with all autoimmune diseases, thyroid autoimmune disorders can develop within the triad of increased intestinal permeability, genetic predisposition, and an environmental trigger.

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.

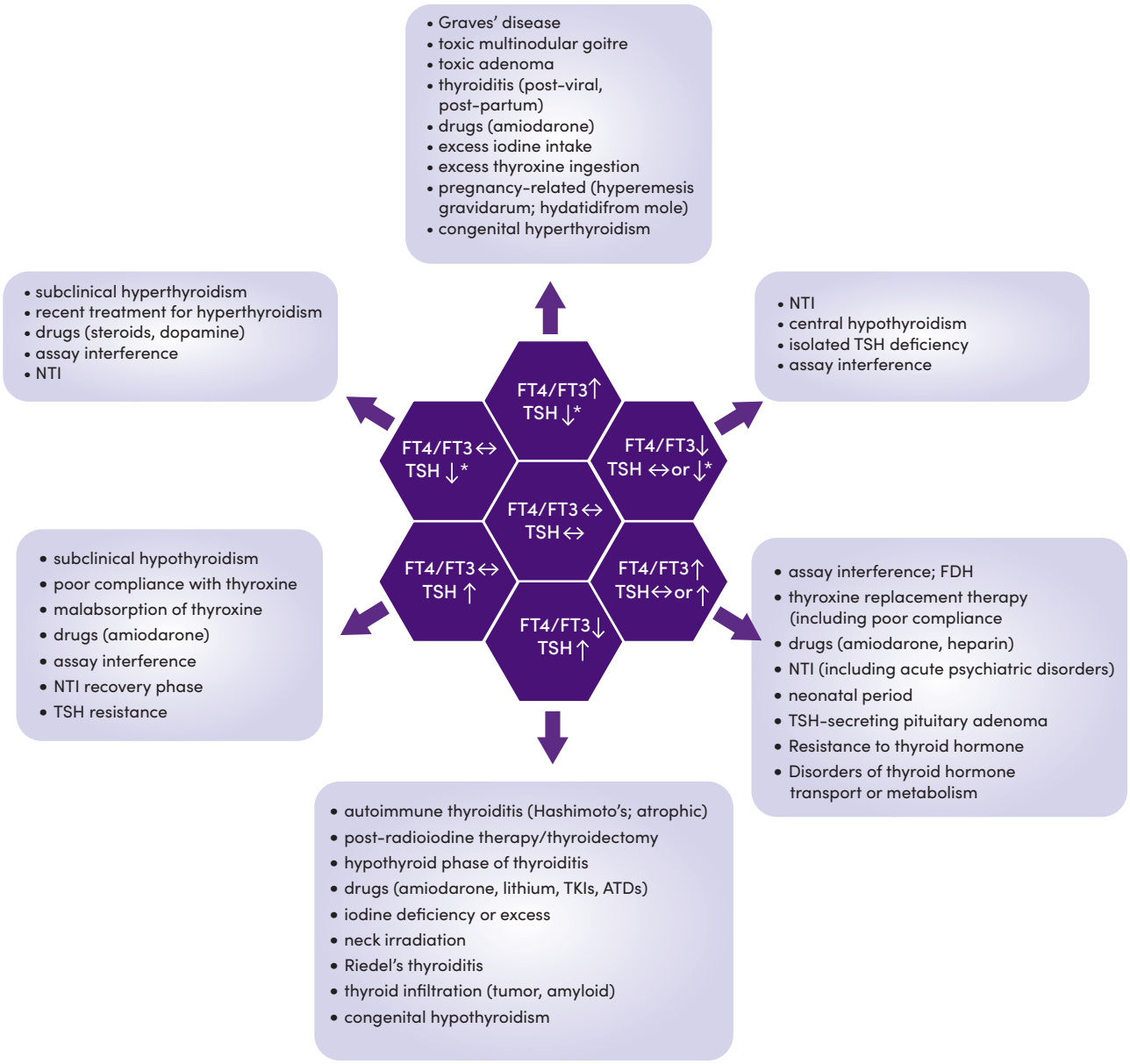
Well-differentiated follicular thyroid cancers include several subtypes: papillary, follicular, and Hurthle cell thyroid cancers. Anaplastic thyroid cancer is a follicular type though can arise from and coexist with differentiated types or occur de novo. Medullary thyroid cancer is derived from the neuroendocrine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

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

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## 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 interferents 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 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 syndrome[53], rheumatic diseases[54], polycystic ovary syndrome[55], type 1 diabetes mellitus[56], 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**

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Saliva • Urine • Blood

# Sex Hormones

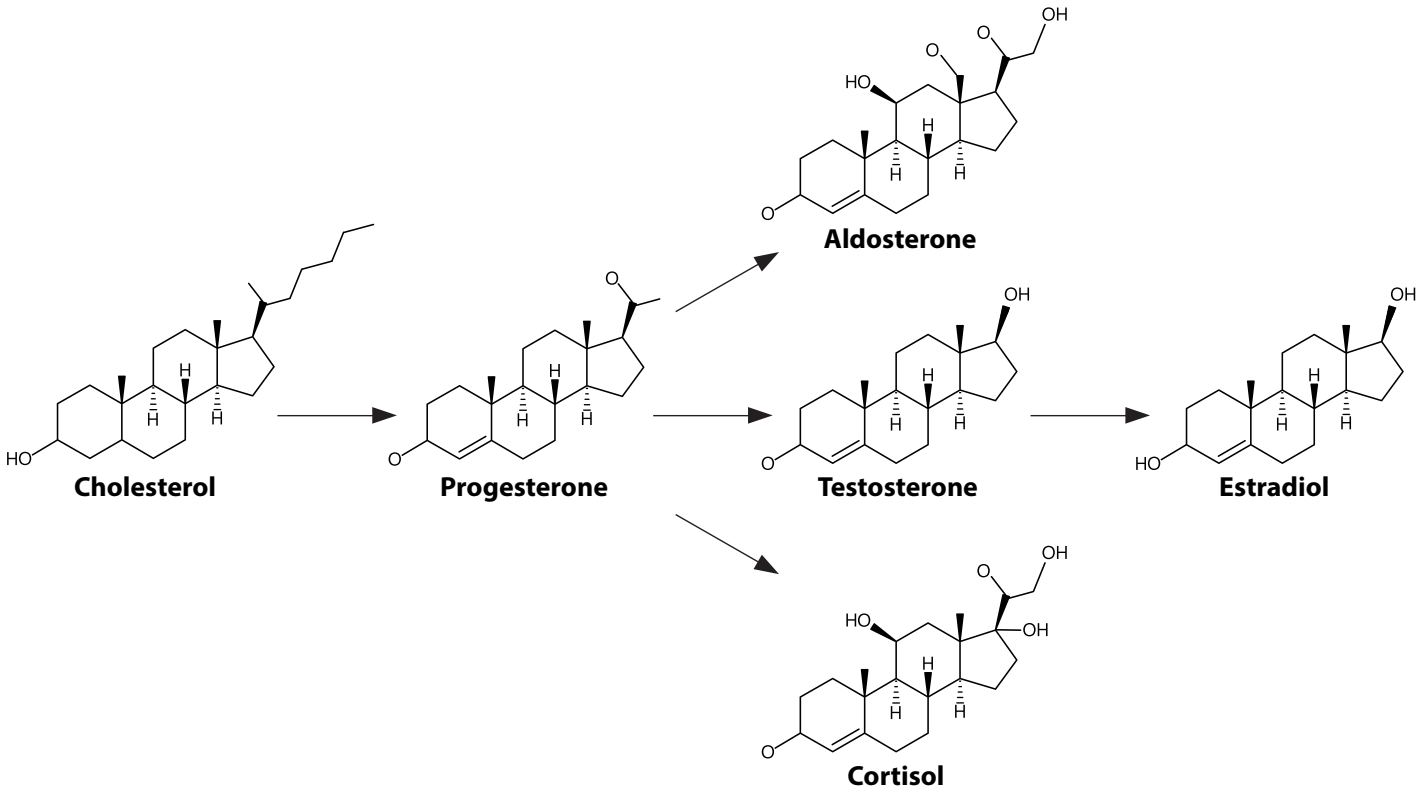
# SUPPORT GUIDE

GENOVA DIAGNOSTICS®

## Table of Contents

## Introduction

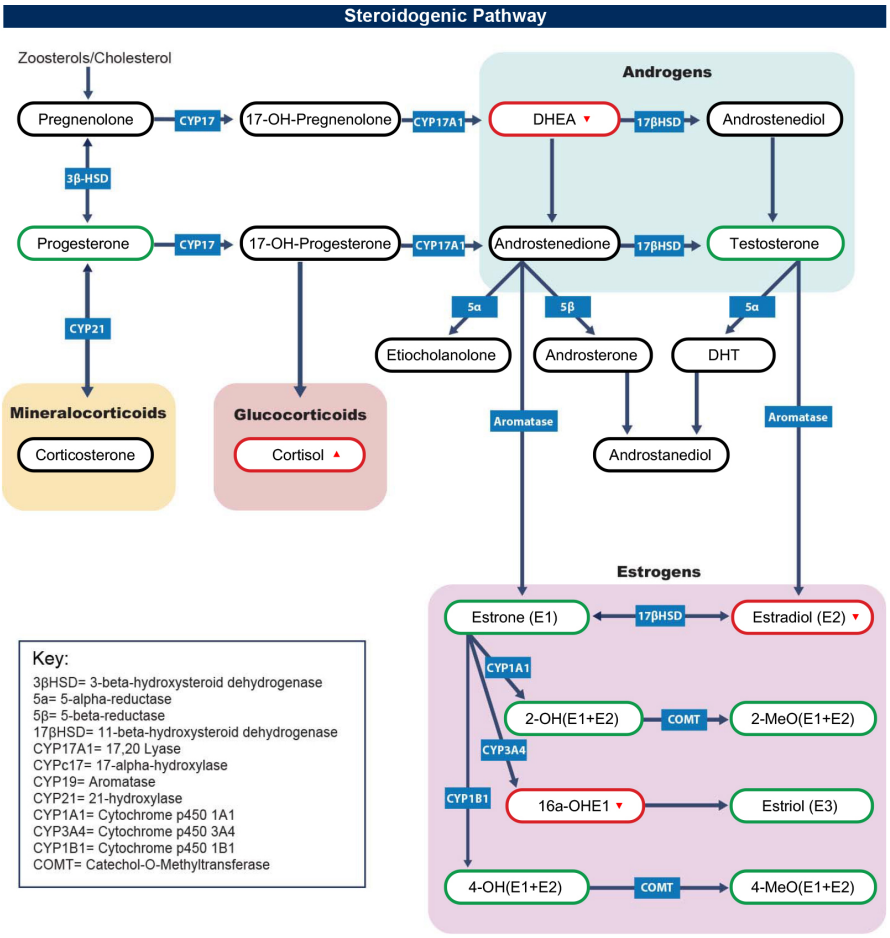
Genova offers a variety of assessments for sex hormones, including serum, saliva, and urine testing for adult males and pre- or postmenopausal females. Parent hormone levels and hormone metabolites provide information about possible causes of a patient's symptoms and health conditions. Measuring hormones 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

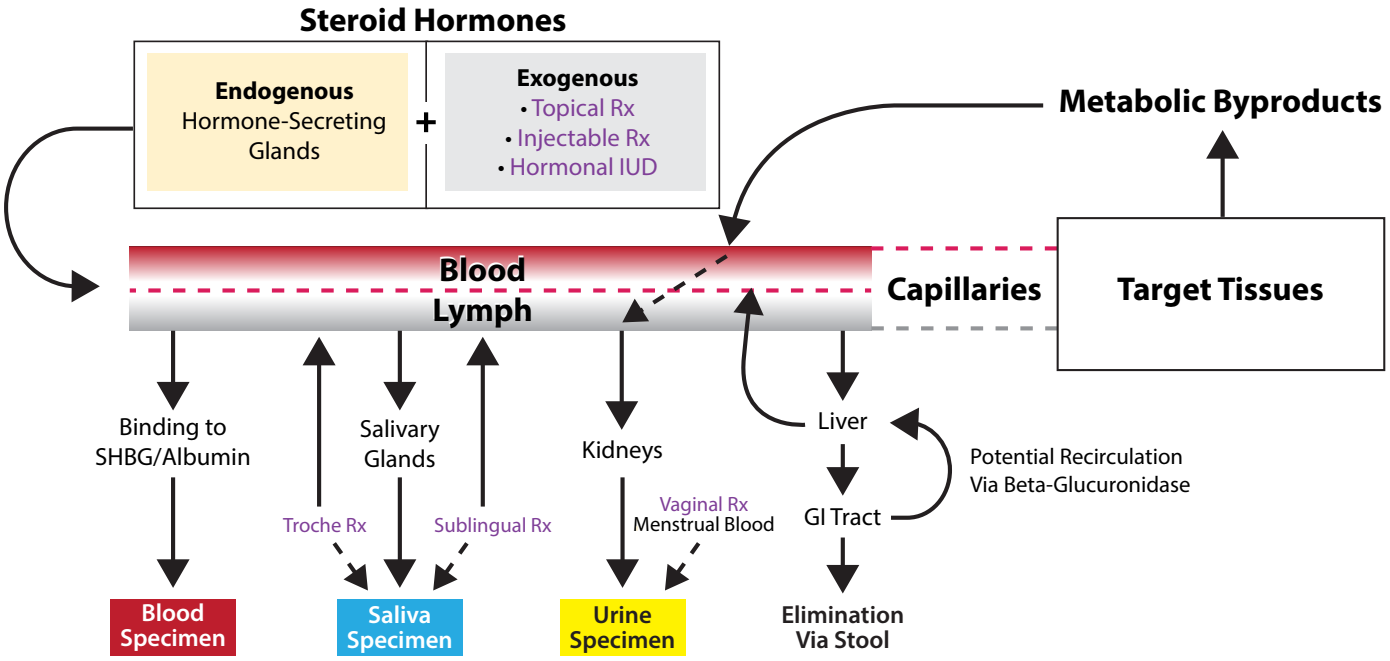
In phase 2, these reactive intermediates undergo conjugation reactions – meaning they combine with other molecules to become water-soluble. These molecules/reactions include glucuronidation, sulfation, methylation, acetylation, and glutathione conjugation. These newly conjugated molecules are now water-soluble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




| | | | |
|---|---|---|---|
| **Represents:** | Total of bound and unbound levels | Unbound bioavailable levels | Unbound metabolites |
| **Benefits:** | • Widely utilized in conventional setting | • Ease of specimen collection<br>• Ease of diurnal testing<br>• Bioavailability | • Information about Estrogen metabolites and disease risk |
| **Limitations:** | • Binding to carrier/ transporter<br>• Potential for under representation | • Not recommended if:<br>- Troche or Sublingual Rx<br>- Poor oral health<br>-Salivary gland pathology | Not recommended if:<br>- Abnormal kidney function<br>- Vaginal Rx<br>- Active menstruation |

## 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

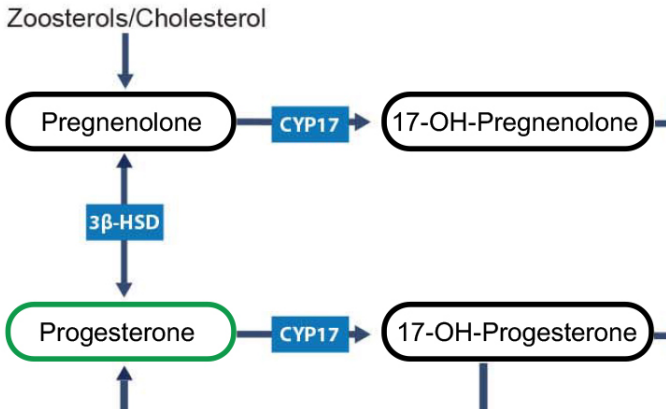
There are a variety of factors that can explain abnormal salivary hormone results. Salivary hormone testing is more prone to factors affecting levels as compared to serum testing.

| Factor | Potential Impact | Notes |
|---|---|---|
| Timing of hormone Rx | • An absorption **peak** or **trough** may be observed, depending on when the Rx was taken prior to specimen collection | • Example: night-time progesterone dosing prior to a morning specimen collection may result in a progesterone absorption peak |
| Dose of hormone Rx | • Ensure proper **dose** was prescribed<br>• Ensure dosing **instructions** are being followed properly by the patient<br>• Consider that steroid hormones are lipophilic and **accumulation** may occur over time | • Example: if dose is 0.1mg topical, ensure the patient isn't applying 1mg |
| Application site of hormone Rx | • **Sublingual** or **troche** hormones may result in extremely high levels in salivary specimen, due to direct entry into saliva<br>• **Topical** hormones applied to upper body, face, or non-fatty tissues may result in uncharacteristically high levels in saliva | • Hormone Rx application in close proximity to salivary glands or directly over lymphatics may introduce concentrated levels into the salivary specimen |
| Synthetic hormone Rx | • **Synthetic** preparations (progestins, AndroGel, etc.) may result in spurious findings (Genova internal analysis) | • Genova hormone assays measure either the bioidentical form of the hormone or its metabolite |

*Continued on next page*

| Factor | Potential Impact | Notes |
|---|---|---|
| Unknown exposures to hormones | • Use of **personal care products**, creams, lotions, contaminated supplements, may result in suspiciously elevated levels<br>• **Transference** from someone else using topical hormones: skin-to-skin or skin-to-surface-to-skin contact | • Higher-than-expected levels of hormones may result |
| Impaired detoxification or excessive recirculation | • Poor **liver health** (impaired methylation, sulfation, glucuronidation, or conjugation)<br>• Constipation<br>• Elevated **beta-glucuronidase** levels in the gut can lead to excessive recirculation of steroid hormones | • Higher-than-expected levels of hormones may result |
| Poor oral health | • **Gingival blood** contamination in saliva may result in uncharacteristically high levels in saliva specimen<br>• **Salivary gland degradation** may prevent proper release of hormones into saliva resulting in low levels[20] | • The health and quality of the saliva are somewhat dependent upon that of the mouth |
| Overweight / obesity | • **Adipose** tissue contains high levels of the aromatase enzyme, which can readily convert androgens to estrogens<br>• 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 | • Progesterone is highly lipophilic and may accumulate in subcutaneous fat cells |
| High secretors of hormones | • **Some individuals** may possess a high- or low- ability to release hormones into the saliva[3,14] | • 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 cytrochrome P450 enzymes and subsequently sulfated and gluruonidated, 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 5a-reductase and 3a-hydroxysteroid dehydrogenase[39-41]
- conjugated with glutathione and excreted[1]

## 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])
- Pregnancy[54]
- Certain types of ovarian cancer[55,56] and ovarian cysts[57]

### 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:

- epilepsy[84]
- obesity[85,86]
- endometrial cancer[87]
- ovarian cancer[88]
- chronic fatigue syndrome[89]
- menopause[35]

**"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 17β-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]

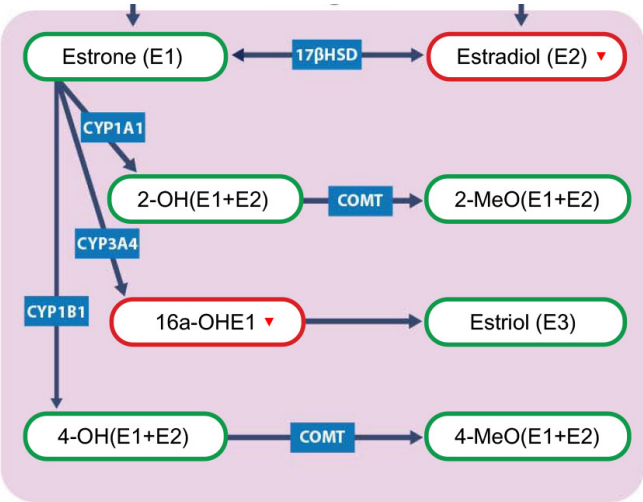
Topical progesterone creams are only available through custom compounded methods versus regulated products and may vary substantially from batch to batch. There is a paucity of literature assessing hormone levels of patients taking compounded formulations, and no known studies assessing long-term safety.[101,105] 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]

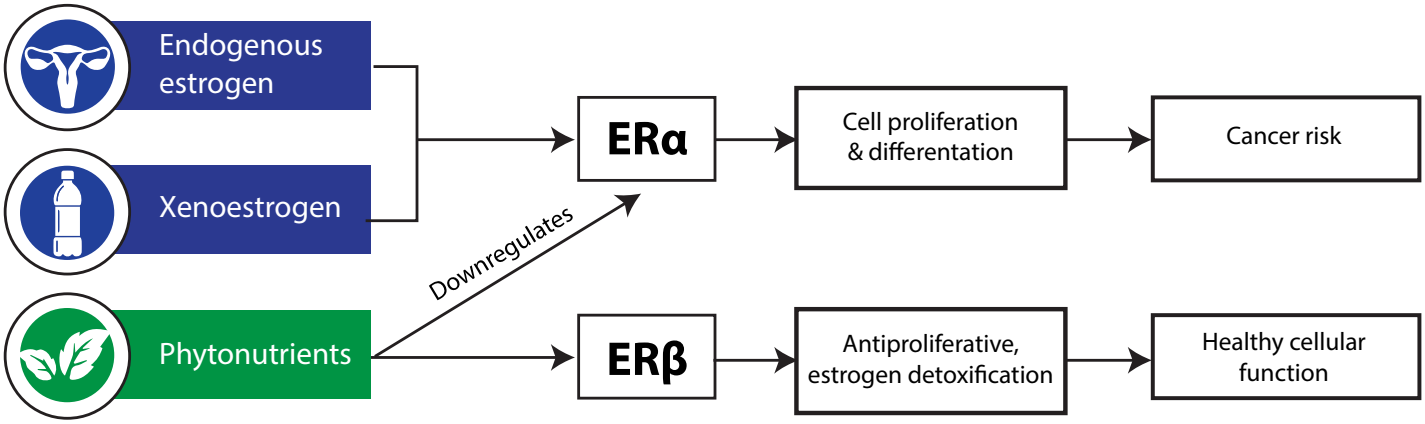
In males, estrogens play a role in healthy sperm function.[110] E2 is important for normal development and function of reproductive and nonreproductive organs. The testes produce approximately 20% of circulating estrogens with the remainder produced by adipose, brain, skin, and bone. Androgens have significant 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]

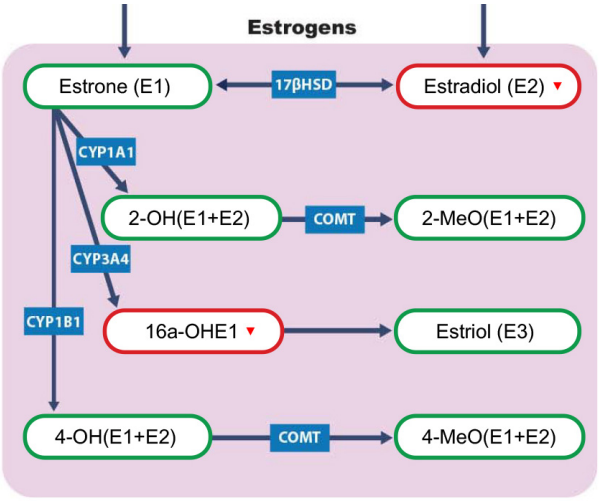
The action of estrogen and other hormones are mediated via their receptors.[34,38] Estrogen receptors (ER), ERα and ERβ are expressed in different tissues and the various estrogens differ in their binding affinity to each receptor. The hormonal potency of the various estrogens is not only determined 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



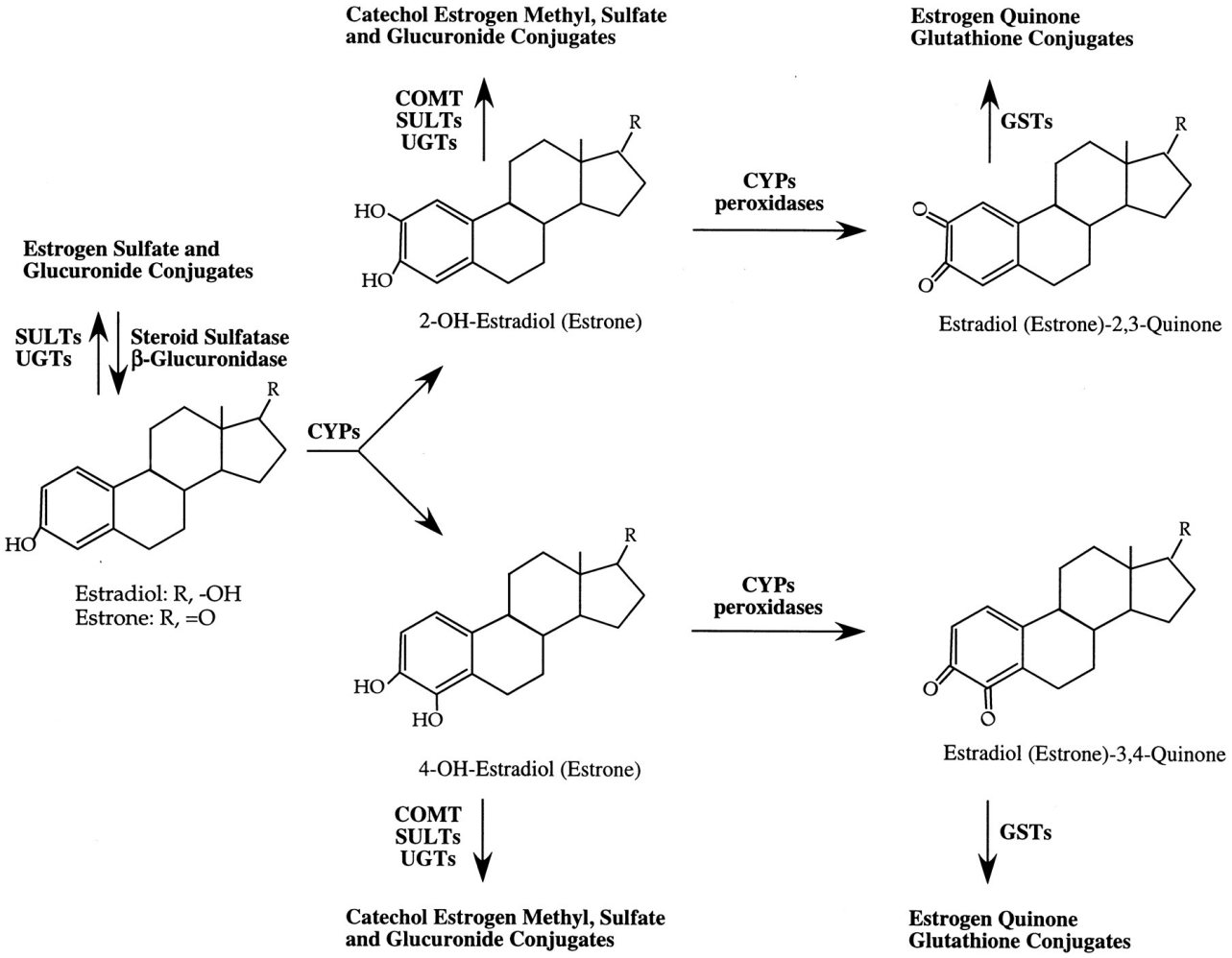
## Estrogen Synthesis and Metabolism

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]



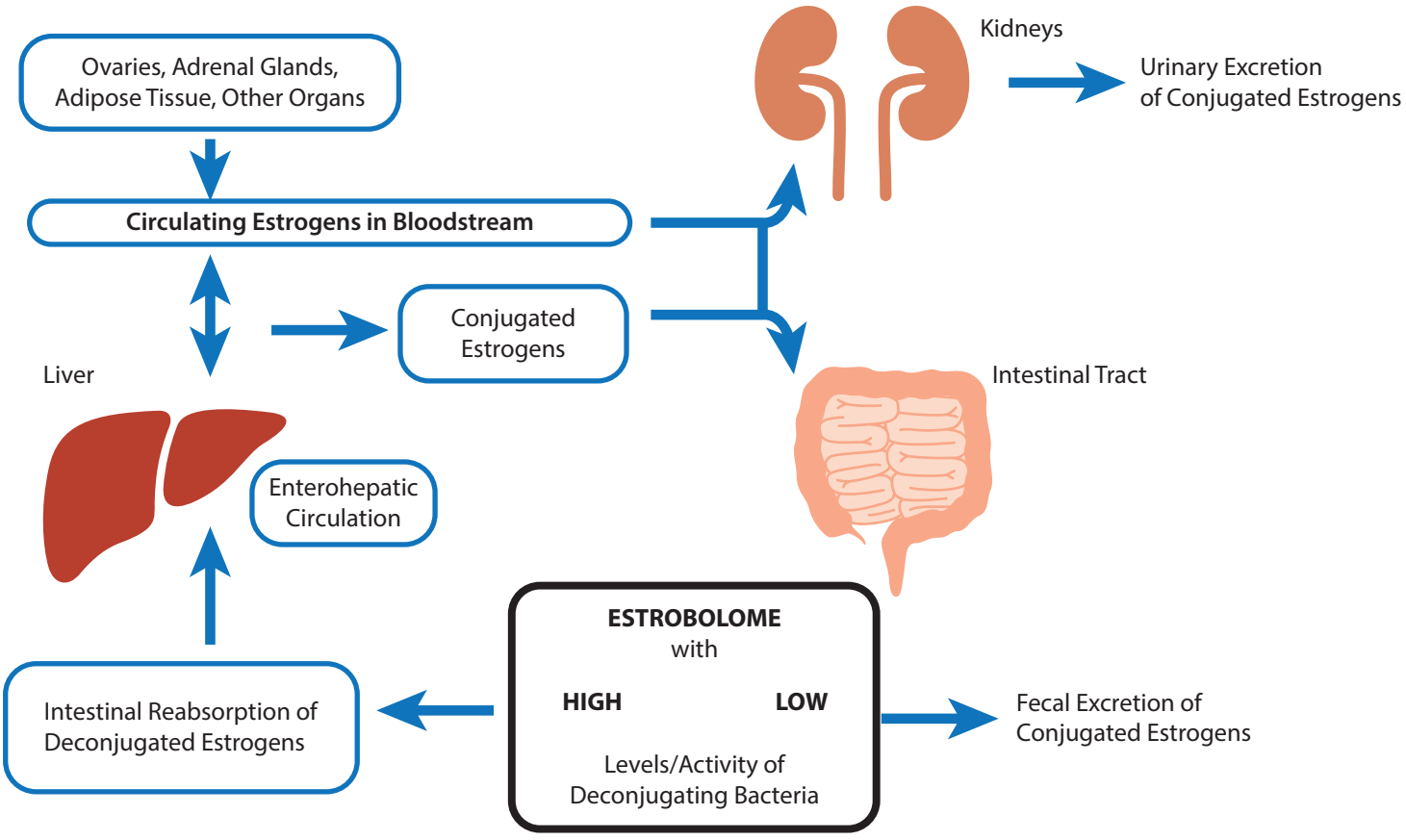

E1 and E2 can be metabolized into 2-hydroxyestrone (2-OHE1), 16α-hydroxyestrone (16α-OHE1), or 4-hydroxyestrone (4-OHE1) via **phase 1 metabolism** through CYP450 pathways.[21] These metabolites may be harmful or protective and are important for assessing risk. Detailed information on estrogen metabolism and urine estrogen metabolites can be found in the "Estrogen Metabolism (Essential Estrogens)" section of the support guide.

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 16α-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 adminstration. The values do not reflect the efficacy or tolerability of the therapies

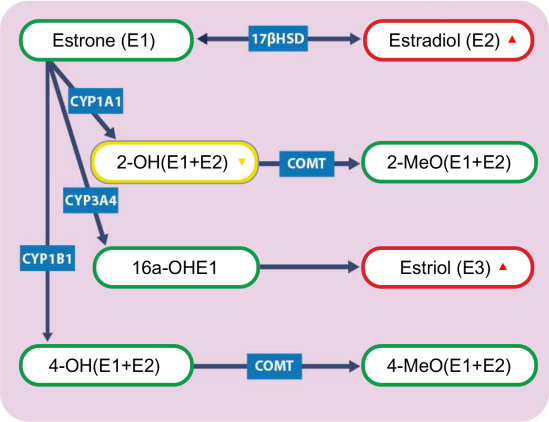
| | Estrone : Estradiol ratio |
|---|---|
| Premenopause | 1 : 2 |
| Postmenopause | 2 : 1 |
| Oral estrone sulfate | 5 : 1 |
| Oral estradiol | 5 : 1 |
| Transdermal estradiol (patch) | 1 : 1 |
| Transdermal estradiol (gel) | 1 : 1 |
| Intraranasal estradiol | 2 : 1 |
| Sublingual estradiol | 1 : 3 |
| Vaginal estradiol | 1 : 5 |
| Subcutancous 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.

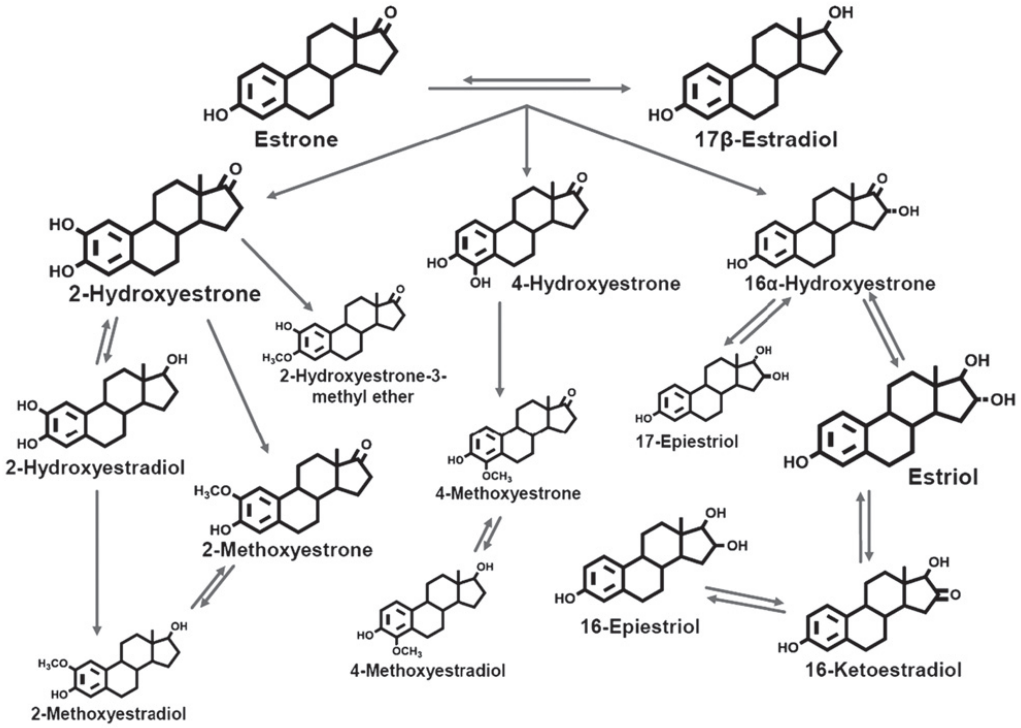
**Phase 1** involves the CYP450 enzymes which hydroxylate estrogen into an active or potentially toxic metabolite.[21,142,186]

- 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.

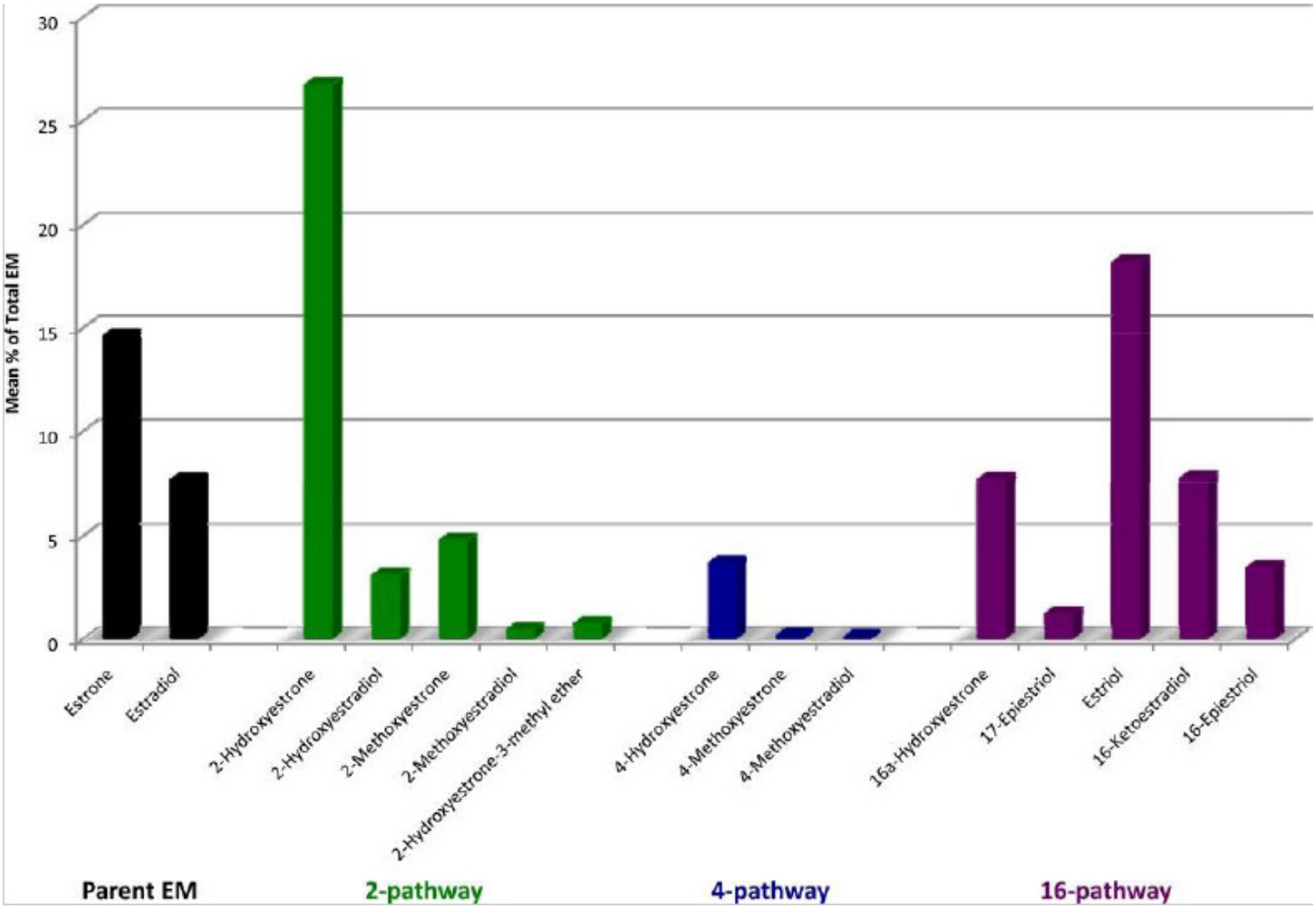
There can also be actual differences in how quickly or efficiently certain cultural populations metabolize parent estrogens.[191]

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.*

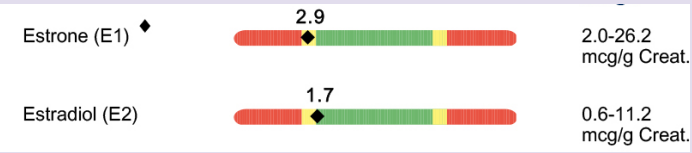
**Relative Metabolite Abundance:**

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 16α-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]

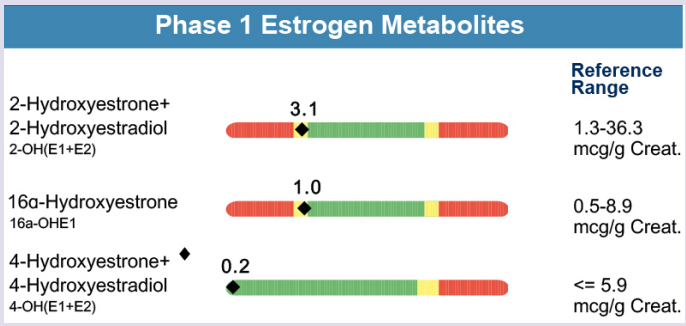
- Low circulating serum estrogen
  - The Shanghai Women's Health Study found that lower urinary parent estrogen levels were associated with decreased breast cancer risk (Postmenopausal women)
- Decreased estrogen metabolism and excretion
- Optimized gut microbiome promoting elimination of detoxified 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

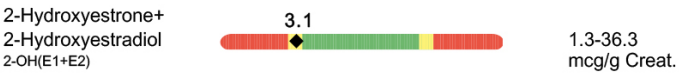
# Urinary Estrogen Metabolites

## 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-hydroylase 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 folinic 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 NutrEval or Metabolomix+ profiles
- Osteoporosis risk assessment
- Stress management

## Low Levels:

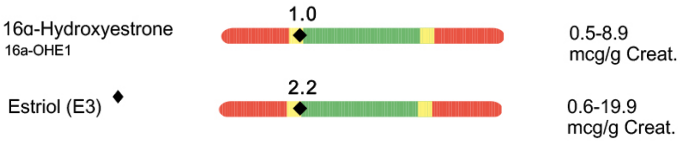
### Considerations and causes of low 2-hydroxyestrogens:
121,176,208

- 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 NutrEval or Metabolomix+ profiles
- If parent hormones are low, refer to section on low estrogen

## 16α-hydroxyestrone (16α-OHE1) and Estriol (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 16α-hydroxyestrone (16α-OH) which can then be converted to estriol (E3). After phase 2 detoxification, these metabolites can be excreted in urine and stool. There is some risk associated with elevations of 16α-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 16αOHE1. These isomers can be reconverted to estriol and 16α-OHE1.[23]

While 16α-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 16α-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]

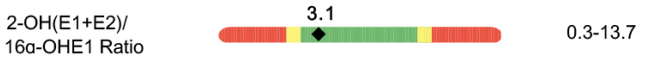
- Low levels of urinary parent hormones
- Decreased metabolism and excretion of circulating serum parent hormones
- Green tea and smoking in premenopausal women
- Aromatase inhibitors
- Antibiotic use

**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




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-methyoxyestrogens and consider methylation support.[116]
  - Genova's Methylation Panel
  - Methylation support 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]
- 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

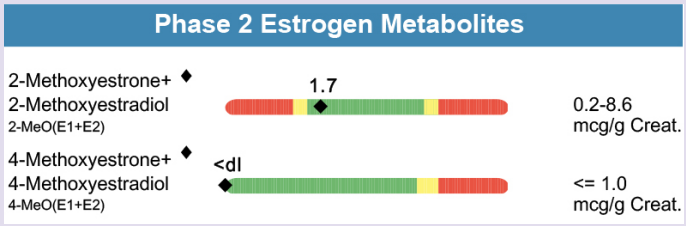
**In general, low 4-OH (E1+E2) levels are considered beneficial due to its association with risk*

### 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 –

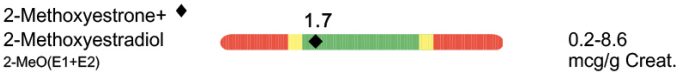
## Phase 2 Estrogen Metabolites




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)




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 (SAMe), 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]

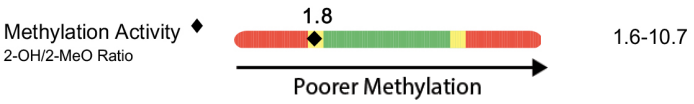
- It is not uncommon to see lower levels of 4-MeO (E1+E2) – healthy populations do not tend to excrete high amounts.[23]
- Genetic SNP in COMT
- Low levels of urinary parent hormones and/or 4-OH estrogens
- Aromatase inhibitors
- Antibiotic use

**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 (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]

## 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.

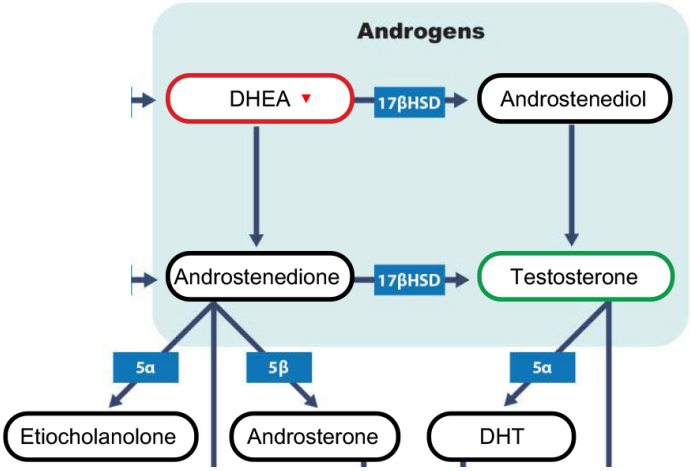
## High P/E2 Ratio:

- 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
- Erythropoesis
- 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 adrenocorticotropic 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 estogens.[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 5α-reductase enzyme into the more potent DHT, which affects the prostate and hair follicles.[242] 5α-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-prepetuating 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 periperhal tissue where they convert to androstenendione, 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 α1 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 & Symptoms**[260-266]

| | |
|---|---|
| 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 NutrEval 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 training [277-280]
- Korean ginseng was shown to increase testosterone levels in young women and DHEA in older women.[281]
- Ashwagandha and fenugreek seed extracts [282] (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 metbolites 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 intitiating 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 exercise [300]
- 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.

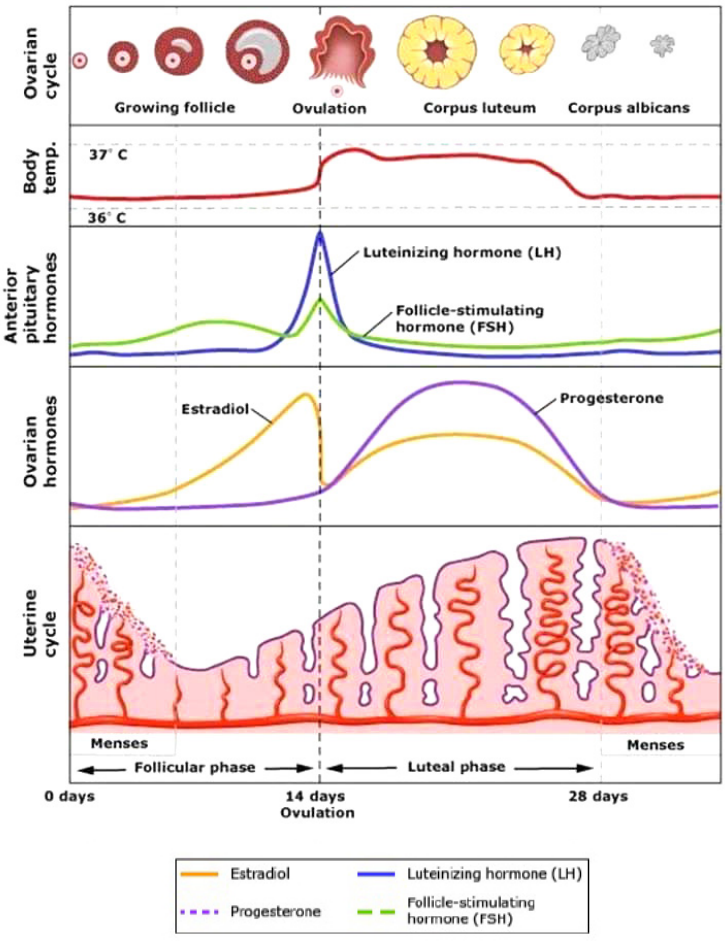
Inside the ovary, multiple follicles grow under the influence of FSH secreted by the pituitary gland. These follicles secrete estrogen, and a dominant follicle emerges. Rising estrogen in the follicular phase indicates that FSH has carried out its function of stimulating the growth of a dominant follicle.

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 oligomennorheic.[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.*

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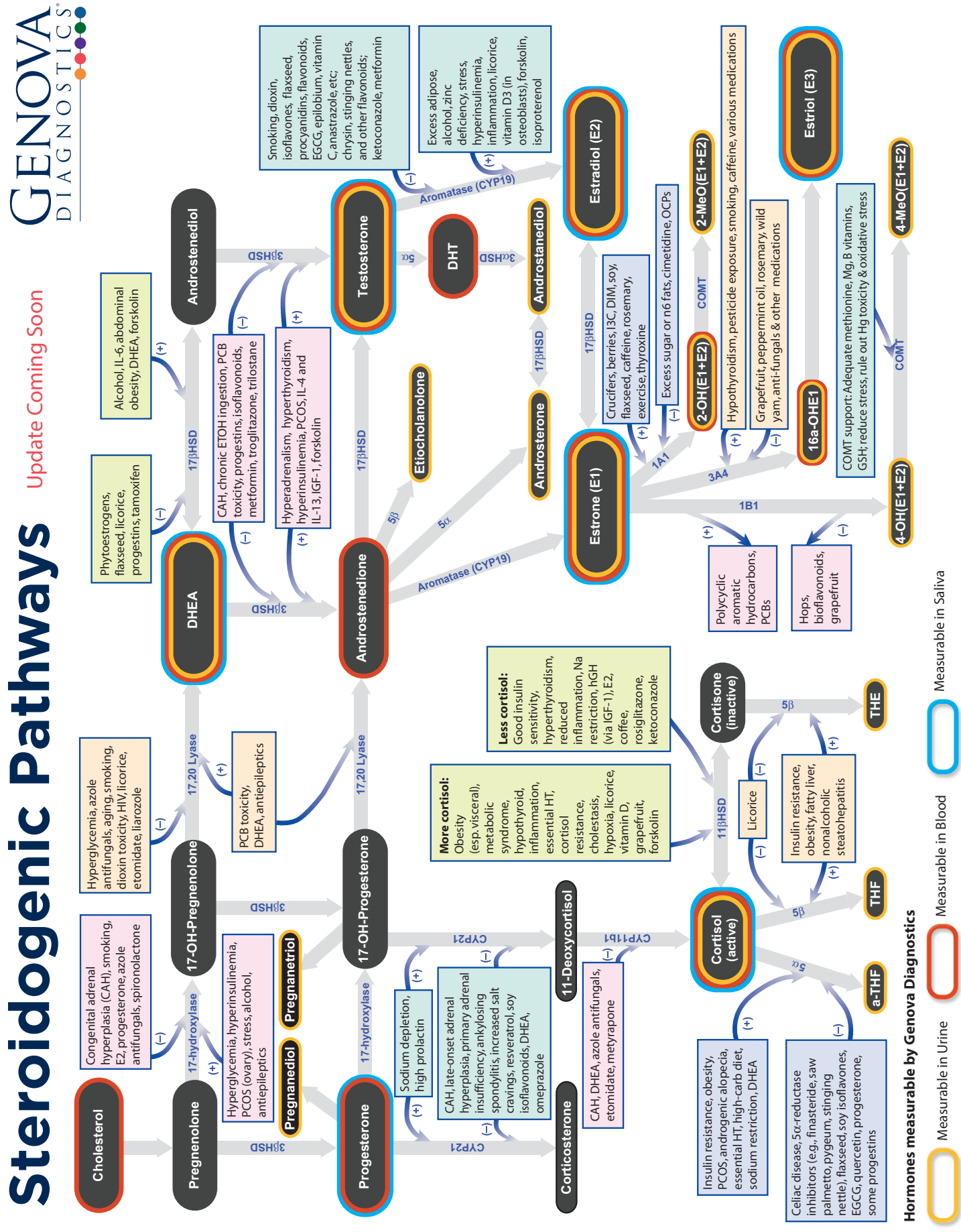
Steroidogenic Pathways
Update Coming Soon
GENOVA DIAGNOSTICS
Cholesterol
Pregnenolone
17-hydroxylase
17,20 Lyase
3βHSD
17-OH-Pregnenolone
Pregnanetriol
Pregnanediol
DHEA
Androstenediol
Progesterone
17-hydroxylase
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17-OH-Progesterone
Androstenedione
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Estradiol (E2)
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3A4
1B1
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CYP21
CYP11b1
Cortisol (active)
11βHSD
Cortisone (inactive)
THE
THF
a-THF
5β
5α
Licorice
Hyperglycemia, azole antifungals, aging, smoking, dioxin toxicity, HIV, licorice, etomidate, liarozole
Congenital adrenal hyperplasia (CAH), smoking, E2, progesterone, azole antifungals, spironolactone
Hyperglycemia, hyperinsulinemia, PCOS (ovary), stress, alcohol, antiepileptics
PCB toxicity, DHEA, antiepileptics
Alcohol, IL-6, abdominal obesity, DHEA, forskolin
Phytoestrogens, flaxseed, licorice, progestins, tamoxifen
CAH, chronic ETOH ingestion, PCB toxicity, progestins, isoflavonoids, metformin, troglitazone, trilostane
Hyperadrenalism, hyperthyroidism, hyperinsulinemia, PCOS, IL-4 and IL-13, IGF-1, forskolin
Smoking, dioxin, isoflavones, flaxseed, procyanidins, flavonoids, EGCG, epilobium, vitamin C, anastrazole, etc; chrysin, stinging nettles, and other flavonoids; ketoconazole, metformin
Excess adipose, alcohol, zinc deficiency, stress, hyperinsulinemia, inflammation, licorice, vitamin D3 (in osteoblasts), forskolin, isoproterenol
Sodium depletion, high prolactin
CAH, late-onset adrenal hyperplasia, primary adrenal insufficiency, ankylosing spondylitis, increased salt cravings, resveratrol, soy isoflavonoids, DHEA, omeprazole
CAH, DHEA, azole antifungals, etomidate, metyrapone
More cortisol: Obesity (esp. visceral), metabolic syndrome, hypothyroid, inflammation, essential HT, cortisol resistance, cholestasis, hypoxia, licorice, vitamin D, grapefruit, forskolin
Less cortisol: Good insulin sensitivity, hyperthyroidism, reduced inflammation, Na restriction, hGH (via IGF-1), E2, coffee, rosiglitazone, ketoconazole
Insulin resistance, obesity, PCOS, androgenic alopecia, essential HT, high-carb diet, sodium restriction, DHEA
Insulin resistance, obesity, fatty liver, nonalcoholic steatohepatitis
Celiac disease, 5α-reductase inhibitors (e.g., finasteride, saw palmetto, pygeum, stinging nettle), flaxseed, soy isoflavones, EGCG, quercetin, progesterone, some progestins
Crucifers, berries, I3C, DIM, soy, flaxseed, caffeine, rosemary, exercise, thyroxine
Excess sugar or n6 fats, cimetidine, OCPs
Hypothyroidism, pesticide exposure, smoking, caffeine, various medications
Grapefruit, peppermint oil, rosemary, wild yam, anti-fungals & other medications
COMT support: Adequate methionine, Mg, B vitamins, GSH; reduce stress, rule out Hg toxicity & oxidative stress
Polycyclic aromatic hydrocarbons, PCBs
Hops, bioflavonoids, grapefruit
Hormones measurable by Genova Diagnostics
Measurable in Urine
Measurable in Blood
Measurable in Saliva

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# Comprehensive Melatonin Profile

## SUPPORT GUIDE

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# Comprehensive Melatonin Support Guide

## Introduction:

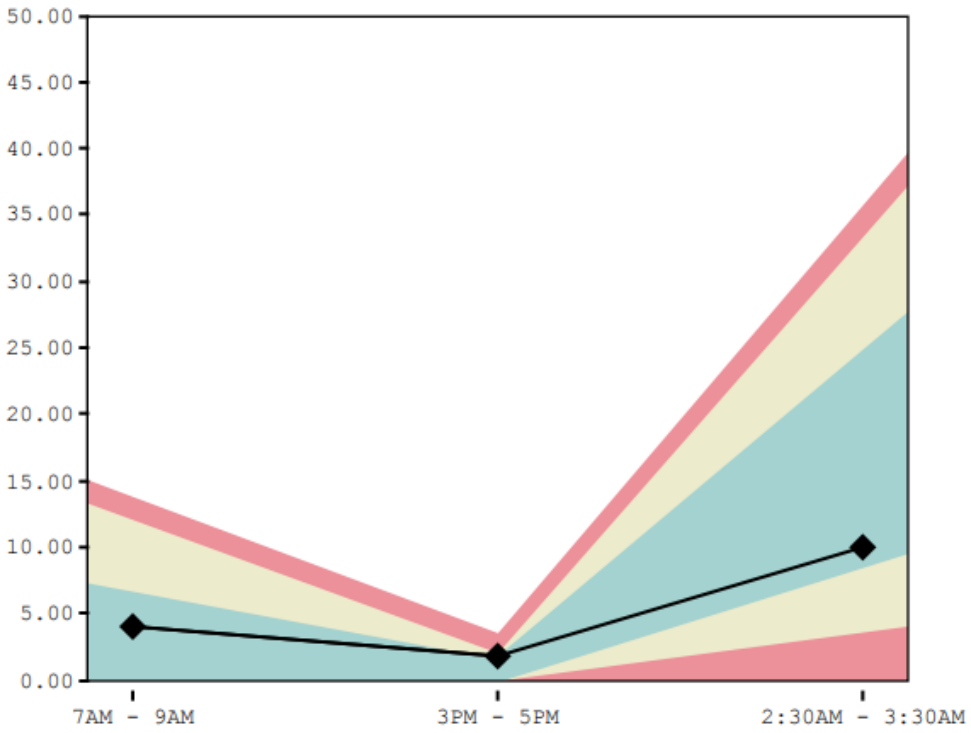
The **Comprehensive Melatonin Profile** is a salivary assessment using three samples collected over 24 hours to capture the levels and circadian rhythm of melatonin. Melatonin is a hormone that affects many bodily functions, regulates the sleep-wake cycle, and is an important systemic antioxidant. Measuring 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) *Based on Collection Times | <=12.12 | <=1.97 | 3.71-33.38 |

## 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]

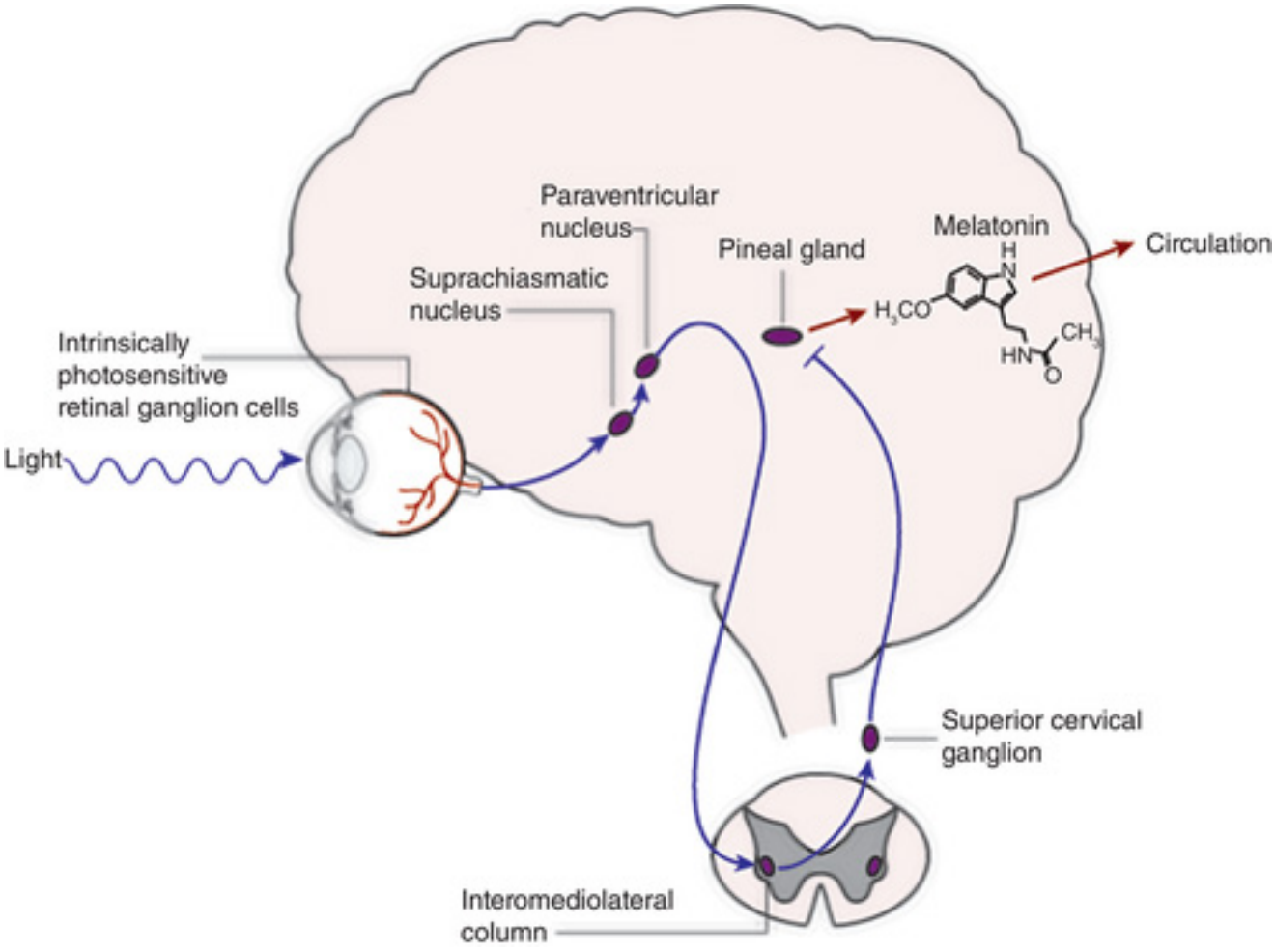
At birth, levels are almost undetectable, the only fetal source being via the placenta. A rhythm appears at around 2-3 months increasing to a lifetime peak in prepubertal children. In fact, melatonin levels may play a role in pubertal Tanner stages of development. Mean adult concentrations in the late 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 derivates (i.e.,6-sulfatoxymelatonin) and 5-30% is converted to glucuronide derivates (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.

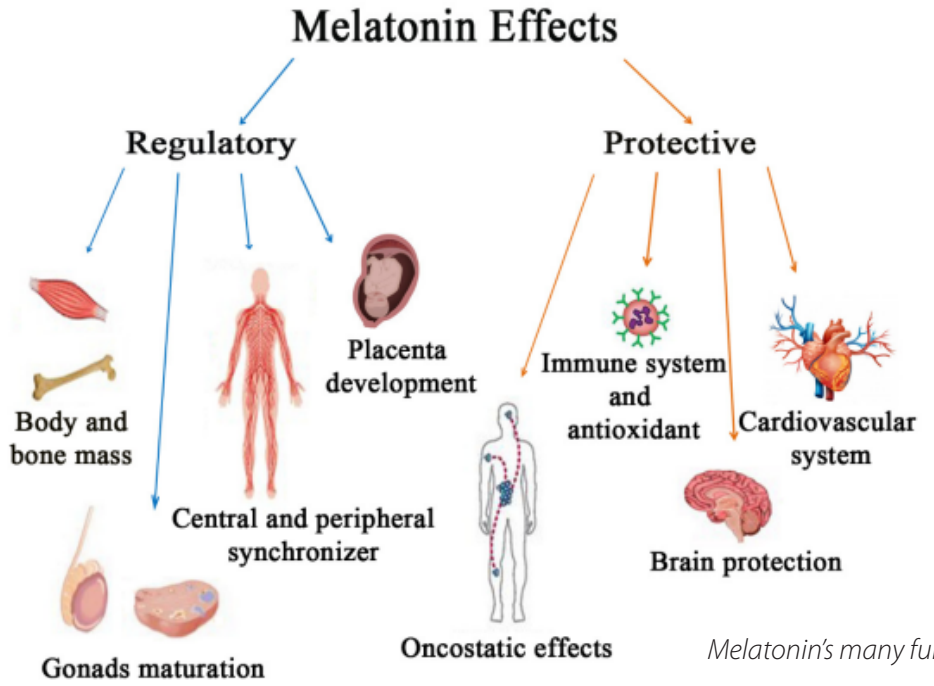
Cortisol and melatonin typically have an inverse relationship. Cortisol helps regulate the body's response to stress and is involved in the body's wakefulness and alertness, while melatonin regulates the sleep-wake cycle and promotes sleep. When cortisol levels are high, melatonin levels are likely low.

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's many functions.*[10]

### 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** | • An **extended dark phase** of the day<br>• Supplementation: **melatonin** (especially sublingual), **tryptophan, 5HTP, cannabis, St. John's Wort**[44,45]<br>• **Medications**: SSRIs, SNRIs, MAO inhibitors[46-48]<br>• **Foods high in *melatonin***: oats, meat, milk, sweet corn, rice, ginger, tomatoes, cherries, berries, bananas, barley, Japanese radish[49]<br>• **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<br>• **Slow metabolism of melatonin by the liver** (hydroxylation, then conjugation with sulphate and glucuronide)[50]<br>• **SNP** of the MTNR1B gene (melatonin receptor), which may extend duration of melatonin production[51]<br>• Compensatory response to chronically **elevated cortisol** (empirical evidence)<br>• Pineal gland **tumor** (excess secretion)<br>- Rare: 0.4-1.0% of brain tumors in adults<br>- Usually leads to headache, vision changes, nausea, vomiting, problems with balance/coordination<br>• **Caffeine** (both stimulatory and inhibitory – delays clearance of melatonin)[52]<br>• **Exercise** Some studies indicate exercise can increase melatonin in women – though studies are mixed[36] | • Consider increasing **morning exposure to bright light** - natural or full-spectrum light source<br>• Check for presence of **medications, supplements,** or **foods** containing melatonin or tryptophan and consider adjusting, if medically appropriate<br>• Consider checking liver function<br>• Consider nutrients that support liver detoxification pathways:<br>• **Sulfation**: allium vegetables, methionine, taurine, cysteine<br>• **Glucuronidation**: cruciferous vegetables (or DIM or I3C)<br>• Consider adrenal adaptogens if elevated cortisol levels<br>• Consider referral for appropriate pineal gland imaging study, especially if chronic headache, vision changes, nausea, vomiting, problems with balance/coordination<br>• Monitor caffeine intake |

*(continued on following page)*

| Result | Potential Contributing Factors | Potential Treatment Strategies |
|---|---|---|
| **Low** | • An extended light phase of the day<br>• Medications: NSAIDS, SSRIs, benzodiazepines, beta-blockers, adrenergics, calcium channel blockers, and steroids[48,53]<br>• Caffeine, tobacco, alcohol, high dose vitamin B12[52,54]<br>• Exercise may suppress melatonin levels for up to 3 hours post-workout and cause sleep phase delays – though studies are mixed[55,56]<br>• Exposure to electromagnetic fields[57]<br>• Decreased production of melatonin by the pineal gland (ex: calcification or damage secondary to head trauma)[58]<br>• Advanced age<br>• **Tryptophan deficiency and lack of cofactors** to make melatonin such as vitamin B6 and folate[7] | • Consider **minimizing bright light exposure** at night<br>• Consider reducing exposure to **electromagnetic fields**<br>• Consider adjusting the timing of **medications**, if medically appropriate<br>• Consider **exercising earlier in the day** to prevent nighttime suppression<br>• Consider single or divided low dose **melatonin supplementation**, if appropriate<br>• Consider increasing **dietary intake** of melatonin and tryptophan containing foods (mentioned above)<br>• Consider **nutrients** required for melatonin production: vitamin B3, vitamin B6, calcium, and magnesium<br>• 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 is 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 supraphysiological levels, though there are no reported overt toxic effects. 1-10mg can raise plasma melatonin levels from 3-60 fold its normal peak[10] 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]

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