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Saliva, Urine, or Blood? Choosing the Right Hormone Test

A Functional Medicine Approach

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Learning Objectives

- Navigate the Doctor's Data hormone testing menu and select the appropriate panel based on clinical presentation, treatment monitoring needs, and cost considerations.
- Explain why morning cortisol is the most clinically significant marker for HPA axis dysregulation and identify evidence-based interventions for elevated nighttime cortisol.
- Describe the gut-gonadal axis and its implications for fertility, menopause, andropause, PCOS, and endometriosis in clinical practice.
- Differentiate between blood, urine, and saliva hormone testing and articulate why serum testing is unreliable for monitoring parenteral hormone therapy.
- Critically evaluate the claim that exogenous testosterone is breast cancer-protective in women and implement superior evidence-based strategies for breast cancer risk reduction.

Course Outline

- Functional Endocrinology — The Journey of the Hormone: the 8-stage framework from production to elimination
- The Clinical Problem — when 'normal' labs don't match the patient
- Methodology Matters — blood, urine, saliva, and why serum fails for parenteral HRT
- The Doctor's Data Hormone Testing Menu — Comprehensive Saliva, HUMAP, and Basic Profile
- HPA Axis Deep Dive — morning cortisol, the CAR, and elevated nighttime cortisol
- The Gut-Gonadal Axis — fertility, menopause, andropause, PCOS, and endometriosis
- Case Study + Forensic Critique — the testosterone-breast cancer myth and what works instead
- Close — Clinical Decision Framework, References, and a path forward

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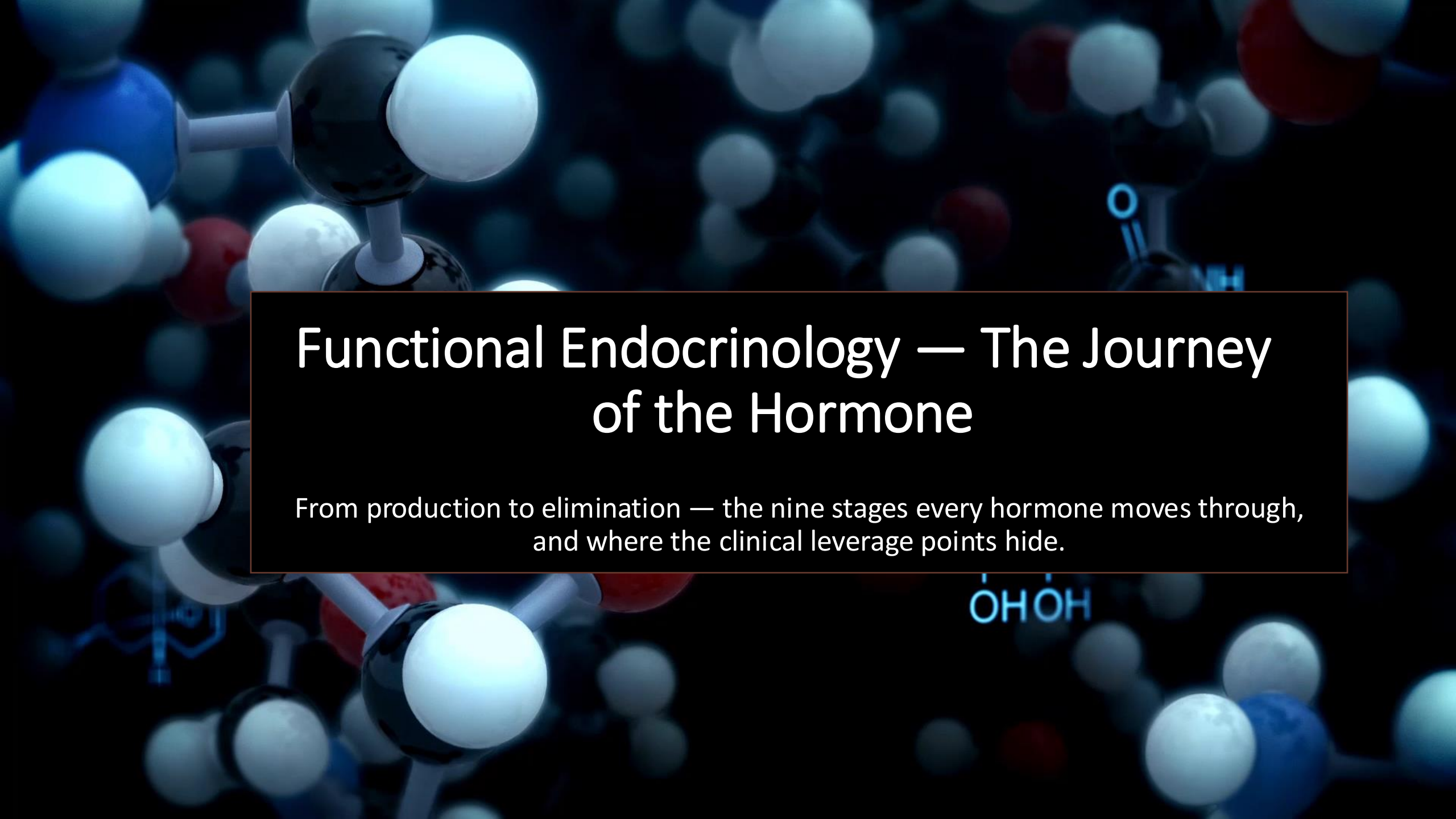


Functional Endocrinology — The Journey of the Hormone

From production to elimination — the nine stages every hormone moves through, and where the clinical leverage points hide.

Functional Endocrinology — A Framework

- Hormones are the communication system of the body — assessed in the context of the Enviro-Neuro-Endo-Immune-Mito-GI crosstalk, never in isolation.
- Functional endocrinology does not simply replace hormones — it repairs and rejuvenates the organs, tissues, and energy systems that produce, transport, and clear them.
- Restorative Medicine is the framework: lifestyle first, then nutraceuticals and botanicals, ***then bio-identicals at the lowest effective dose only when truly indicated.***
- Endocrine dysfunction is rising from conception — driven by endocrine disruptors, dysbiosis, mitochondrial dysfunction, nutrient depletion, sleep loss, and chronic inflammation.
- When bio-identicals are used: short-term where possible, while addressing upstream drivers in parallel. Less is more.
- Test selection follows the same logic: production → blood; metabolism → urine; tissue exposure → saliva. Choose the medium that answers the clinical question.

Stage 1 of 9 — Production & Secretion

- Neuroendocrine regulation: hypothalamic-pituitary feedback loops, circadian rhythm, stress signaling — the upstream control system that decides what gets made.
- Endocrine gland function: how well the gland itself responds to neuroendocrine cues — capacity, reserve, and the cumulative effect of chronic demand.
- Cellular machinery: steroid hormones are synthesized in the smooth endoplasmic reticulum — gland health is downstream of cellular health.
- Mitochondrial integrity is non-negotiable: every steroidogenesis step requires ATP and intact electron transport — mito dysfunction produces measurable hormone deficits.
- Gut microbiome — the overlooked endocrine organ. Dysbiosis directly impairs hormone production through inflammation, nutrient malabsorption, and HPA disruption.
- **Clinical pearl: 'low hormone' is rarely a primary gland failure — it's almost always a downstream signal of mitochondrial, GI, or inflammatory stress.**

Stages 2-5 — Transport, Pre-Receptor, Receptor & Signaling

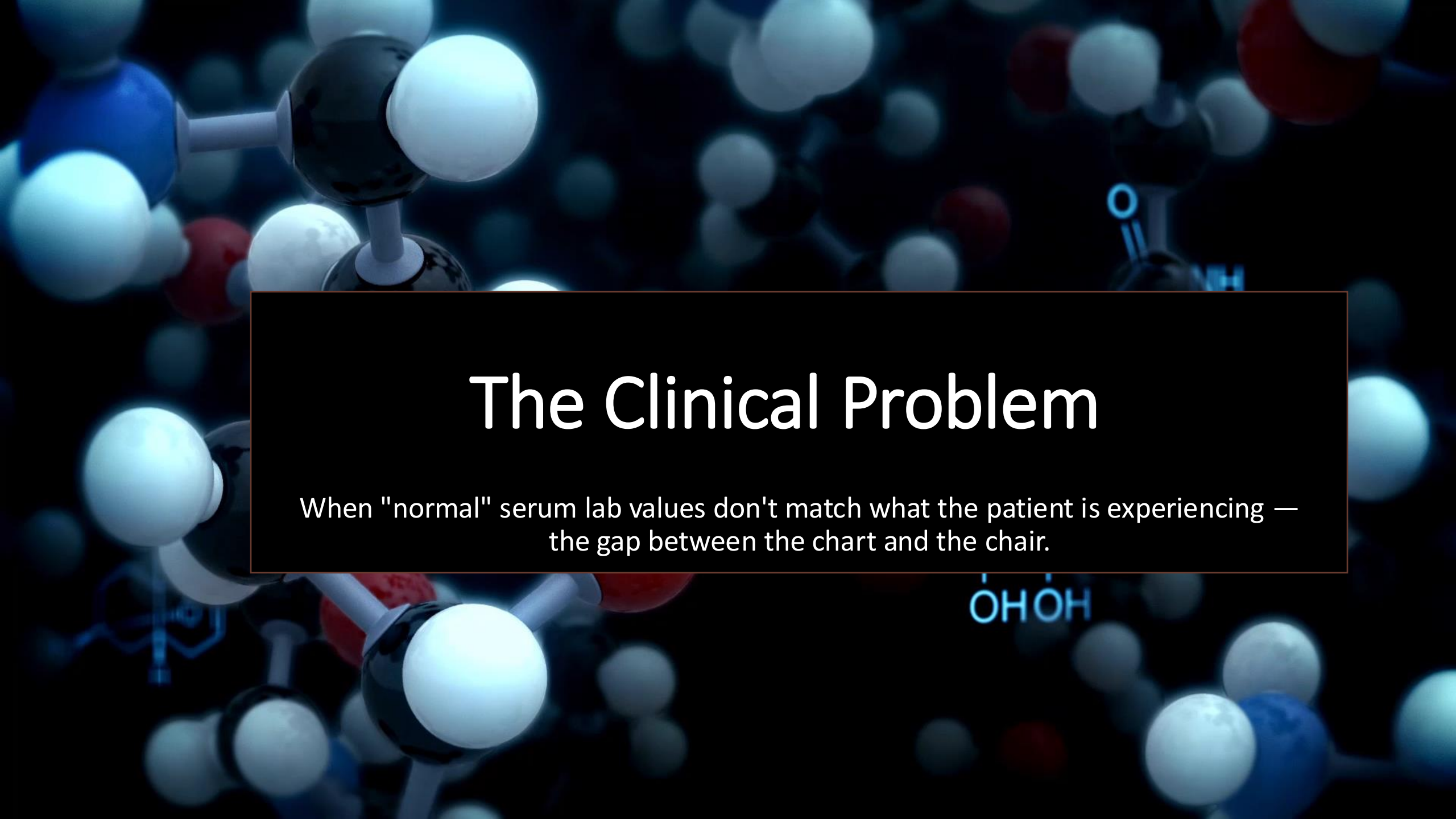
- Transport proteins: SHBG, TBG, albumin, CBG — bind 95–99% of circulating hormone, leaving only the small free fraction biologically active.
- Pre-receptor activation: tissue enzymes (5-deiodinase for T4→T3, 5α-reductase for T→DHT, aromatase for T→E2) determine what the cell actually sees.
- Receptor presence and density: cell-surface receptors (peptide hormones) vs nuclear receptors (steroids, thyroid) — receptor downregulation is a common and missed cause of HRT failure.
- Post-receptor signaling: phosphorylation cascades, cytosolic enzyme activation, second messengers — the molecular work that converts a hormone signal into a cellular response.
- Clinical implication: a 'normal' serum hormone level says nothing about transport binding, pre-receptor conversion, receptor sensitivity, or post-receptor signaling — and patients live in those four layers, not in plasma.
- **Saliva captures the free, transport-independent fraction. HUMAP captures pre-receptor and post-receptor metabolism. Together they expose what serum cannot see.**

Stages 6-9 — Translation, Detox & Elimination

- Translation: nuclear receptor binding activates transcription, protein synthesis, and the downstream cellular response — the actual biological effect of the hormone.
- Self-regulation: counter-balancing feedback, signal modification, stop-genes, and receptor recycling — the system's built-in safeguards against runaway signaling.
- Detoxification & biotransformation: local tissue degradation of hormone and its by-products — the first line of clearance before systemic elimination.
- Hepatic Phase I: cytochrome P450 hydroxylation of estrogens to 2-OH (protective), 4-OH (genotoxic), and 16-OH (proliferative) metabolites — the branch point that determines breast and uterine risk.
- Hepatic Phase II: methylation (COMT), sulfation, glucuronidation, glutathione conjugation — converts active metabolites to water-soluble forms ready for excretion.
- Elimination: biliary (gut/stool), urinary, and the enterohepatic recirculation loop — where the estrobolome decides what gets reabsorbed and what truly leaves the body.

Laboratory Testing for Restorative Endocrinology

- Test free AND bound fractions — total hormone alone is uninterpretable. SHBG, free T, free E2, and bioavailable fractions tell the story serum totals hide.
- Test downstream functional markers, not just hormone levels: HbA1c (more meaningful than insulin alone), MCV/cholesterol (hypothyroid clues), serum electrolytes (adrenal status), reactive markers over static numbers.
- Add organic acids (a-hydroxybutyrate, kynurenate, methylmalonic acid) and amino acids — they reveal mitochondrial function, methylation status, and nutrient sufficiency upstream of hormone biology.
- Test antibodies when autoimmune hormone disorders are suspected: TPO/TgAb for thyroid, 21-OH for adrenal, ovarian/testicular antibodies in primary insufficiency.
- Genetic SNPs — COMT, MTHFR, CYP1A1/B1, GST — provide context for HUMAP findings and explain why certain patients chronically struggle with the same metabolic bottlenecks.



The Clinical Problem

When "normal" serum lab values don't match what the patient is experiencing — the gap between the chart and the chair.

The Patient You've Seen

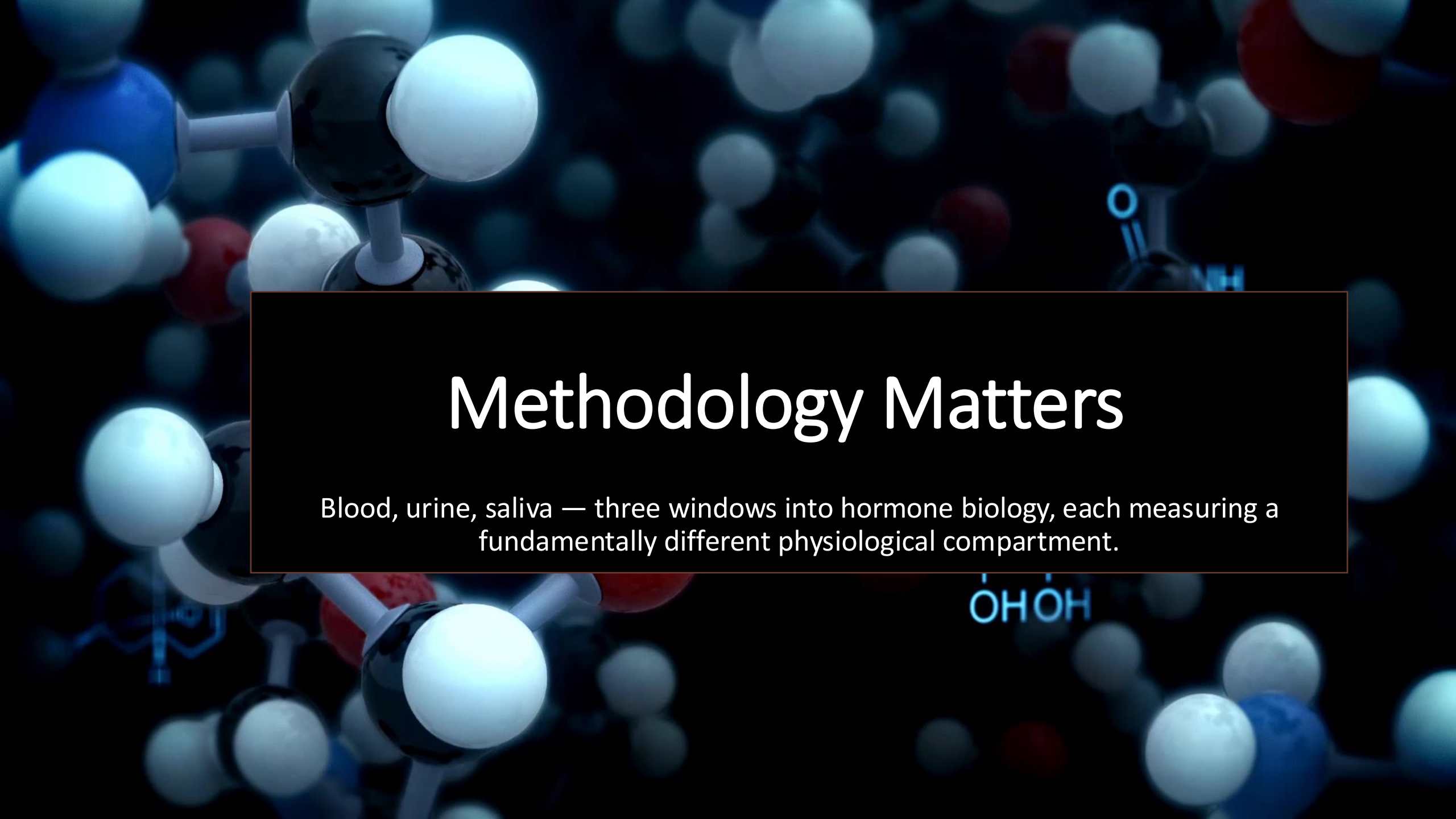
- Patient on HRT presents with real symptoms — anxiety, palpitations, breast tenderness, brain fog, sleep disruption. Maybe they felt better at first, but then symptoms re-appear or new ones emerge.
- Serum labs come back “normal” , or within accepted treatment ranges— yet symptoms persist or worsen. The conventional response is to dose higher, switch products, or dismiss the patient.
- The problem isn't the patient. The problem is the test.

It's Not the Patient — It's the Test, and the Approach

- Fat-soluble hormones (estradiol, testosterone, progesterone) are **lipophilic** — they bind tightly to red blood cell membranes and partition into adipose tissue rather than circulating freely in plasma.
- Transdermal creams, gels, and pellet implants bypass first-pass hepatic metabolism — so serum levels never reflect the dose actually being absorbed and distributed to tissues.
- Standard serum assays measure only the plasma fraction — they completely miss the membrane-bound and tissue-stored hormone pool, which is the clinically active reservoir.
- A patient on pellet therapy can show "low" or "normal" serum testosterone while her tissues are saturated — the result is a symptomatic overdose with deceptively reassuring labs.
- Saliva testing measures the unbound, biologically active hormone fraction that has crossed cell membranes — the only valid medium for monitoring parenteral HRT.
- Urine metabolite testing (HUMAP) adds the methylation and conjugation layer — it reveals what the body actually did with the hormones after absorption, including the production of protective vs. genotoxic estrogen metabolites.

The Three Methods at a Glance

- Saliva — measures the biologically active, unbound hormone fraction at the tissue level
- Urine — reveals the methylation and conjugation metabolites (2-OH, 4-OH, 16-OH estrogens)
- Blood (serum) — useful for general assessment of production/function, SHBG, total T baseline, lipids, and CBC, but a poor choice for monitoring parenteral HRT:
  - Misses the membrane-bound and adipose-stored hormone pool
  - Cannot distinguish therapeutic from supraphysiologic dosing
  - Doesn't reveal estrogen metabolite ratios — blind to 4-OH risk and methylation capacity
  - Cannot capture the cortisol awakening response or circadian rhythm
  - Cheap and widely available — but cheap can be expensive when the answer is wrong



Methodology Matters

Blood, urine, saliva — three windows into hormone biology, each measuring a fundamentally different physiological compartment.

The Four Hormone Compartments

- Plasma fraction (1–3%) — what serum measures: the smallest pool, mostly bound to SHBG and albumin, with only a tiny free fraction that is biologically active.
- Membrane-bound fraction (>50%) — what saliva captures: hormones embedded in red blood cell membranes and partitioned into cell lipid bilayers, reflecting true tissue exposure.
- Adipose-stored fraction (variable) — invisible to routine testing: lipophilic hormones accumulate in body fat as a long-term reservoir, slowly releasing over weeks to months.
- Conjugated metabolites — what HUMAP reveals: glucuronidated, sulfated, and methylated metabolites that show how the body actually processed the parent hormones.
- Each compartment answers a different clinical question — confusing them produces the wrong protocol every time. The right test depends on what you actually need to measure.

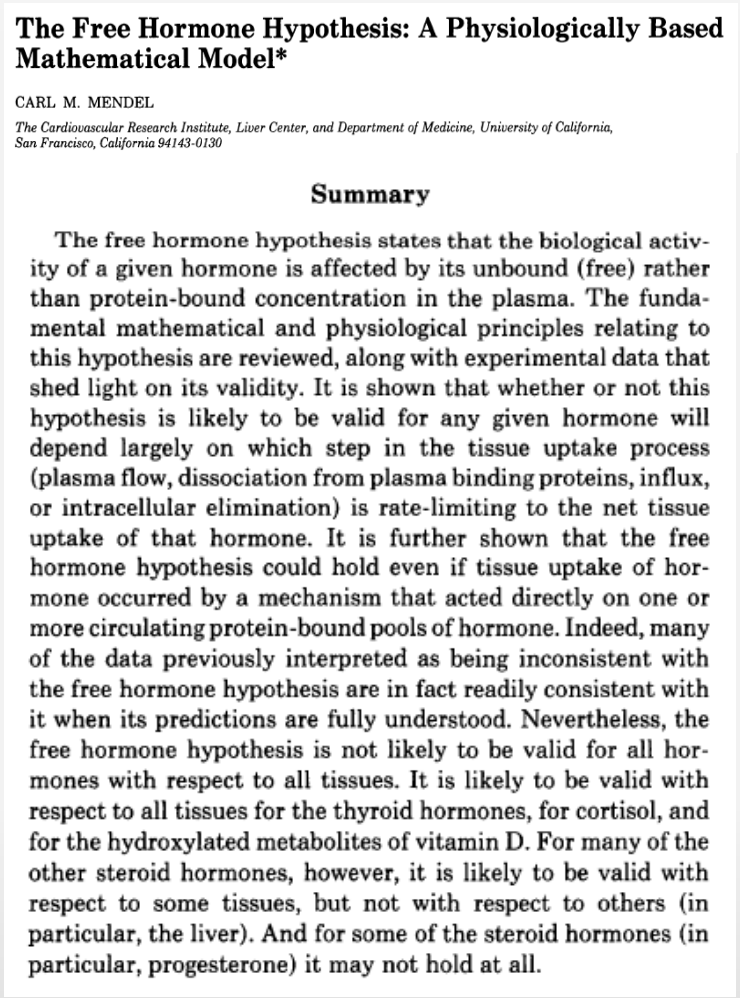
Why Serum Fails for Parenteral HRT

- Pellets release hormone in pulses — serum levels swing wildly across each 24-hour cycle, making any single measurement statistically meaningless.
- Transdermal creams produce a "depot effect" — hormone accumulates in subcutaneous fat over weeks, releasing slowly into tissues without ever showing up in serum.
- SHBG binding masks the active fraction — total serum testosterone can look "normal" while free, bioactive hormone is saturated at supraphysiologic levels.
- The Endocrine Society explicitly does not recommend serum monitoring of parenteral testosterone (pellets, troches, sublingual, creams, gels, IM injections) — yet it remains the dominant practice in mainstream HRT clinics.
- Result: patients are routinely told their hormones are "fine" while metabolic, cognitive, and breast-tissue effects of overdosing accumulate silently.
- The fix: salivary measurement of free hormone, supplemented by HUMAP urinary metabolites for the methylation/conjugation picture serum cannot show.

What Saliva Captures

- Free, unbound hormone — the fraction that has actually crossed cell membranes and is bioavailable to tissues. This is the clinically relevant pool.
- Tissue-equilibrated levels — saliva reflects the diffusion equilibrium between bloodstream and salivary gland tissue, mirroring what is happening at target organs.
- Multiple timepoints in a single day — 4-point cortisol curves, 3-point melatonin profiles, mid-cycle estradiol/progesterone — patterns single-blood-draw testing cannot capture.
- Non-invasive, patient-controlled collection — eliminates phlebotomy stress (which itself spikes cortisol) and enables home collection at physiologically meaningful times.
- The only valid medium for monitoring parenteral HRT (pellets, troches, sublingual, creams, IM injections) — recognized in the medical literature for over four decades, ignored by most conventional practice.
- Limitation: saliva does not show metabolites or methylation patterns. For full estrogen biology, pair with HUMAP urinary testing.

The Free Hormone Hypothesis



The Free Hormone Hypothesis: A Physiologically Based Mathematical Model*

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Summary

The free hormone hypothesis states that the biological activity of a given hormone is affected by its unbound (free) rather than protein-bound concentration in the plasma. The fundamental mathematical and physiological principles relating to this hypothesis are reviewed, along with experimental data that shed light on its validity. It is shown that whether or not this hypothesis is likely to be valid for any given hormone will depend largely on which step in the tissue uptake process (plasma flow, dissociation from plasma binding proteins, influx, or intracellular elimination) is rate-limiting to the net tissue uptake of that hormone. It is further shown that the free hormone hypothesis could hold even if tissue uptake of hormone occurred by a mechanism that acted directly on one or more circulating protein-bound pools of hormone. Indeed, many of the data previously interpreted as being inconsistent with the free hormone hypothesis are in fact readily consistent with it when its predictions are fully understood. Nevertheless, the free hormone hypothesis is not likely to be valid for all hormones with respect to all tissues. It is likely to be valid with respect to all tissues for the thyroid hormones, for cortisol, and for the hydroxylated metabolites of vitamin D. For many of the other steroid hormones, however, it is likely to be valid with respect to some tissues, but not with respect to others (in particular, the liver). And for some of the steroid hormones (in particular, progesterone) it may not hold at all.

- Mathematical model demonstrating that only the free, unbound hormone fraction is biologically active. SHBG- and albumin-bound hormone is biologically inert.
- Standard serum assays measure total hormone — including the inert bound fraction — masking what is actually bioavailable to tissues.
- Saliva captures only the free, unbound fraction — the only fraction that matters clinically. The theoretical foundation for salivary hormone testing.
- Critical for all parenteral HRT (pellets, troches, sublingual, creams, IM): tissue-equilibrated free hormone in saliva can be supraphysiologic while serum total reads "normal."
- Foundational paper that established the scientific basis for the entire saliva-testing field — still cited in 2020s clinical reviews.

Mendel CM, 1989 — PMID: 2673754

Transport of Protein-Bound Hormones into Tissues

Transport of Protein-Bound Hormones into Tissues *in Vivo

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THE STEROID and thyroid hormones circulate in the plasma tightly bound by albumin and by specific plasma globulins (1, 2). Consequently, hormones exist in two states at equilibrium *in vitro*, *i.e.* free (dialyzable) and protein-bound. A widely held hypothesis in endocrinology is that the fraction of hormone that is free *in vitro* is equivalent to the fraction of hormone that is free and available for transport into tissues *in vivo*. Therefore, *in vitro* measurements of free hormones may be commonly used as reliable indices of the free hormone *in vivo* for a variety of clinical states. The purpose of this review is to advance the concept that the large protein-bound moiety of plasma hormone is available for transport into tissues. Consequently, the fraction of plasma hormone that is available for transport *in vivo* may deviate greatly from the free fraction *in vitro*. Under some conditions, changes in the free hormone fraction *in vitro* and in the fraction of plasma hormone that is available for transport *in vivo* will be proportional. However, other clinical situations are expected to be characterized by disproportionate, even opposite, changes between what is available for tissue uptake *in vivo* and the free hormone *in vitro*. It is hoped that this review will assist the reader in future interpretations of the clinical relevance of *in vitro* free hormone measurements.

There are two possible mechanisms by which protein-bound hormone may be transported into tissues: 1) a collision mechanism and 2) a free intermediate mechanism. Data will be presented that indicate that the steroid and thyroid hormones and other hormones or metabolites which have a plasma $t_{1/2}$ on the order of minutes to hours enter tissues via the free intermediate mechanism (Fig. 1). Conversely, compounds such as globulin-bound retinol, 25-hydroxycholecalciferol, or vitamin B_{12}, *i.e.* vitamins with a plasma $t_{1/2}$ on the order of days to weeks, seem to undergo transport into target tissues via a collision mechanism (3–5). The emphasis of this review will be on the transport of those protein-bound hormones that traverse cell membranes via the free intermediate mechanism.

The free intermediate model shown in Fig. 1 emphasizes three primary determinants that regulate the transport of protein-bound hormones: 1) the capillary transit time, 2) the unidirectional dissociation rate (k_{off}), and 3) the membrane permeability-surface area product, hereafter referred to as simply membrane permeability. The capillary transit time is the time of exposure of plasma proteins to a given biological membrane and is a time period that is relatively short compared to the whole organ vessel transit time. The capillary transit time is inversely related to the rate of blood flow and is a function of capillary length, capillary volume, and plasma velocity. There are considerable differences from organ to organ with respect to the duration of the capillary transit time, *e.g.* this period is ~1 sec in brain (6) or skeletal muscle (7), about 2–3 sec in mesenteric capillaries (8), and about 5 sec in liver sinusoidal spaces (9). The plasma capillary transit time is particularly long in liver because, owing to large capillary pores and an absence of any basement membranes, plasma proteins instantaneously equilibrate with the interstitial space of Disse in liver (9). Consequently, the effective capillary volume is quite large and this factor results in a long capillary transit time in liver.

The unidirectional rates of steroid or thyroid hormone dissociation from plasma proteins are variable (Table 1). Ligand dissociation from albumin is characterized by rapid rates, and ligand dissociation from globulin sites is typically a slow process. However, a major point to be emphasized in this review is that the rate of the dissociation reaction should be viewed relative to the capillary transit time of the organ in question. For example, the dissociation of T_3 from thyroid hormone binding globulin (TBG) ($t_{1/2}$ = 4 sec) is slow relative to the brain transit time (~1 sec) but is relatively fast compared to the hepatic transit time (~5 sec). Similarly, estradiol or cortisol dissociation from sex hormone binding globulin (SHBG) or corticosteroid binding globulin (CBG), re-

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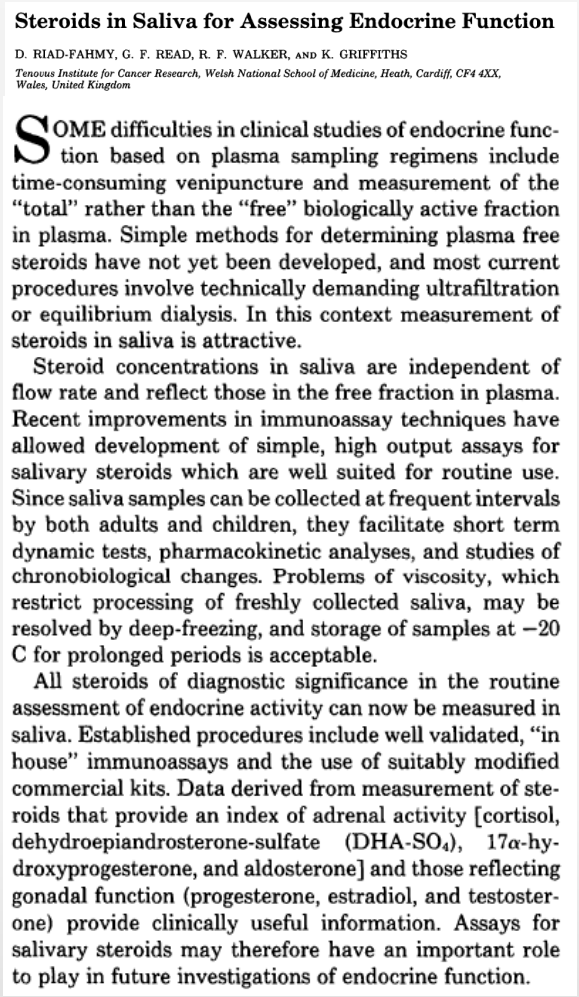
† To whom requests for reprints should be addressed.

- Comprehensive review establishing the pharmacokinetic framework for how steroid and thyroid hormones move from bloodstream into target tissues.
- Demonstrated that protein-bound hormones can transiently dissociate at tissue capillaries, but only the free fraction at the cellular receptor produces biological effect.
- Distinguished between in vitro free hormone (lab measurement) and in vivo bioavailable hormone (tissue exposure) — the gap that explains why serum testing fails for transdermal HRT.
- Showed that lipophilic steroids partition rapidly into red blood cell membranes and adipose tissue, creating a body-burden reservoir invisible to plasma-only assays.
- Foundational 1981 paper — predates and informs Mendel's 1989 mathematical model. Together they form the theoretical pillars supporting saliva for monitoring tissue-bound hormones.

Pardridge WM, 1981 — PMID: 7028469

Steroids in Saliva for Endocrine Function



Steroids in Saliva for Assessing Endocrine Function

D. RIAD-FAHMY, G. F. READ, R. F. WALKER, AND K. GRIFFITHS

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SOME difficulties in clinical studies of endocrine function based on plasma sampling regimens include time-consuming venipuncture and measurement of the "total" rather than the "free" biologically active fraction in plasma. Simple methods for determining plasma free steroids have not yet been developed, and most current procedures involve technically demanding ultrafiltration or equilibrium dialysis. In this context measurement of steroids in saliva is attractive.

Steroid concentrations in saliva are independent of flow rate and reflect those in the free fraction in plasma. Recent improvements in immunoassay techniques have allowed development of simple, high output assays for salivary steroids which are well suited for routine use. Since saliva samples can be collected at frequent intervals by both adults and children, they facilitate short term dynamic tests, pharmacokinetic analyses, and studies of chronobiological changes. Problems of viscosity, which restrict processing of freshly collected saliva, may be resolved by deep-freezing, and storage of samples at −20 C for prolonged periods is acceptable.

All steroids of diagnostic significance in the routine assessment of endocrine activity can now be measured in saliva. Established procedures include well validated, "in house" immunoassays and the use of suitably modified commercial kits. Data derived from measurement of steroids that provide an index of adrenal activity [cortisol, dehydroepiandrosterone-sulfate (DHA-SO_4), 17α-hydroxyprogesterone, and aldosterone] and those reflecting gonadal function (progesterone, estradiol, and testosterone) provide clinically useful information. Assays for salivary steroids may therefore have an important role to play in future investigations of endocrine function.

- The original landmark Endocrine Reviews paper that established salivary steroid testing as a clinically valid alternative to serum.
- Saliva functions as an ultrafiltrate of plasma — only the unbound, lipid-soluble hormone fraction crosses into salivary glands by passive diffusion.
- Validated salivary measurement for cortisol, testosterone, progesterone, estradiol, and DHEA across normal physiology and clinical pathology.
- Demonstrated that salivary steroid concentrations are independent of salivary flow rate — eliminating a major theoretical objection to saliva-based testing.
- Established saliva as the practical, non-invasive alternative to serial blood sampling for circadian and dynamic endocrine assessment — the foundation of modern functional medicine hormone panels.

Riad-Fahmy D et al., 1982 — Endocr Rev — PMID: 6217969

Saliva = Free Serum Cortisol

Salivary cortisol: a better measure of adrenal cortical function than serum cortisol

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SUMMARY Salivary cortisol concentration was found to be directly proportional to the serum unbound cortisol concentration both in normal men and women and in women with elevated cortisol-binding globulin (CBG). The correlation was excellent in dynamic tests of adrenal function (dexamethasone suppression, ACTH stimulation), in normals and patients with adrenal insufficiency, in tests of circadian variation and randomly collected samples. Women in the third trimester of normal pregnancy exhibited elevated salivary cortisol throughout the day. The relationship between salivary and serum total cortisol concentration was markedly non-linear with a more rapid increase in salivary concentration once the serum CBG was saturated. The rate of equilibrium of cortisol between blood and saliva was very fast, being much less than 5 minutes. These data, combined with a simple, stress-free, non-invasive collection procedure, lead us to suggest that salivary cortisol is a more appropriate measure for the clinical assessment of adrenocortical function than is serum cortisol.

- The classic validation: salivary cortisol is directly proportional to free serum cortisol across normal subjects, dynamic testing, and pathological states.
- Excellent correlation in dexamethasone suppression and ACTH stimulation tests — confirming saliva tracks the biologically active fraction during HPA challenge.
- Equilibration between blood and saliva takes less than 5 minutes — near-instantaneous, enabling true real-time HPA monitoring.
- Salivary cortisol is superior to total serum cortisol in patients with elevated CBG (pregnancy, oral contraceptives, estrogen therapy) — where serum is misleadingly high.
- Markedly nonlinear relationship: above CBG saturation, salivary cortisol rises rapidly while serum total plateaus — saliva is the more sensitive marker of biologically active cortisol excess.

Vining RF, McGinley RA, et al., 1983 — PMID: 6316831

Progesterone Cream — Saliva, Not Serum

Salivary, but not serum or urinary levels of progesterone are elevated after topical application of progesterone cream to pre- and postmenopausal women

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(Received 18 June 1999; returned for revision 13 September 1999; finally revised 14 June 2000; accepted 4 August 2000)

Summary

OBJECTIVE The use of topically applied micronised ('natural') progesterone as a substitute for synthetic oestrogens and progestogen preparations is controversial. The aim of this study was to examine the changes in blood and salivary concentrations of progesterone following a single topical application of a progesterone cream.

PATIENTS AND MEASUREMENTS We investigated six premenopausal women in the luteal phase and six postmenopausal women to determine the short-term changes in serum, urinary and salivary progesterone concentrations following a single 64 mg topical application of micronised progesterone.

RESULTS Serum progesterone concentrations did not increase during the first 3 hours after application of progesterone cream, however, salivary values rose significantly in both premenopausal and postmenopausal women, consistent with the view that progesterone is absorbed and transported through the body. Salivary progesterone concentrations were significantly elevated above basal levels by 30–60 minutes and reached peak levels at 1–4 h, with mean levels approximately fivefold higher in premenopausal, than in menopausal women.

CONCLUSIONS Salivary progesterone measurements confirm that topically applied progesterone is absorbed, despite the lack of change in serum progesterone

- Single 64 mg topical progesterone application: salivary progesterone rose dramatically in both pre- and postmenopausal women within 30-60 minutes, peaking at 1-4 hours.
- At the same time points, serum progesterone did NOT rise during the first 3 hours after application — and urinary metabolites were unchanged.
- Salivary peak levels were approximately fivefold higher in premenopausal than postmenopausal women — reflecting differences in tissue distribution and adipose binding.
- Direct clinical proof that serum testing is blind to transdermal progesterone delivery — the cream IS being absorbed and distributed to tissues, just invisibly to plasma assays.
- Definitive evidence that salivary measurement is the only valid monitoring method for compounded progesterone cream — a critical practice point for any clinician prescribing transdermal HRT.

O'Leary P, et al., 2000 — PMID: 11106923

Three Media, Three Different Questions

- Blood (serum) = Production — captures the acute snapshot of recent endogenous secretion or pharmacokinetic peak. The transit lane, not the destination.
- Urine (HUMAP) = Production + Elimination — reveals what was made AND what got conjugated, methylated, or excreted. The metabolic ledger.
- Saliva = Body Burden — reflects steady-state tissue equilibrium of free, biologically active hormone. The actual long-term reality.
- Hormones spend hours in plasma but days to months in tissue — only saliva captures that tissue-equilibrated picture invisible to single blood draws.
- Choose the medium based on the clinical question: production status → blood; metabolism risk → urine; tissue exposure → saliva. Each test answers a different question.
- For HRT monitoring — and especially parenteral HRT (pellets, troches, sublingual, creams, IM injections) — body burden is the question, and saliva is the answer. This is the foundation of every clinical decision in this lecture.

Testosterone Therapy for Women

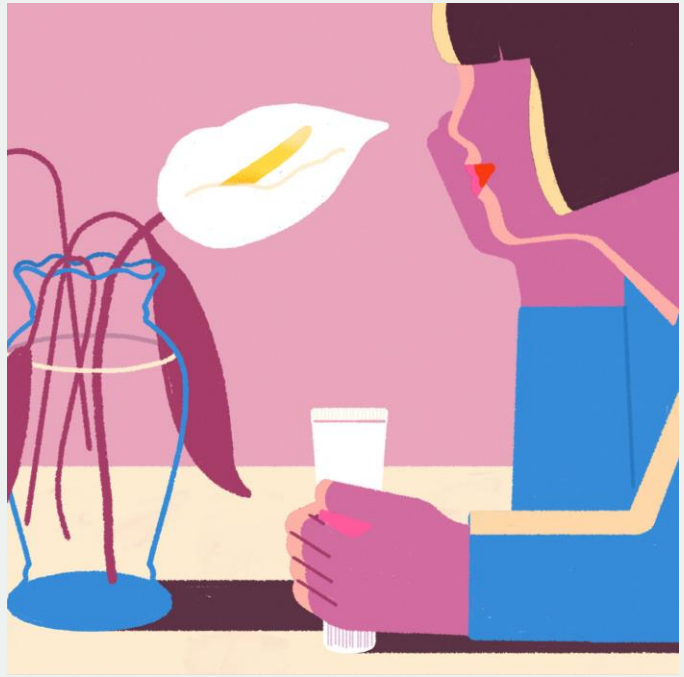
- Testosterone plays an important role in FEMALE sexual health.

- Its use in women, especially for enhancing libido, is surrounded by misconceptions.

- Some evidence suggests benefits in postmenopausal women with hypoactive sexual desire disorder, but its long-term safety and efficacy remain unclear.

- The lack of FDA-approved testosterone formulations for women complicates dosing and monitoring.

- Main cause of low T in post menopausal women = all the same discussed here – mainly effecting the adrenals and ovaries.



The Glaser Testosterone Hypothesis

- The claim: "Testosterone pellets reduce breast cancer risk in postmenopausal women" — widely circulated in the pellet-prescribing community for over a decade.
- Origin: Glaser & Dimitrakakis 2013 retrospective case series from Glaser's own pellet practice. Used as the primary marketing rationale for high-dose pellet therapy in women.
- Has driven massive growth in pellet prescribing over the past decade — including elevated dosing protocols that produce supraphysiologic free testosterone levels.
- The claim is repeated to patients as "testosterone is breast cancer protective" — implying a cancer-prevention benefit that does not exist in the rigorous evidence base.
- This is not an anti-testosterone position — it is a critique of unjustified high-dose pellet use on the basis of methodologically weak evidence in patients who have not been adequately monitored.
- The underlying study requires forensic examination — because a generation of HRT practice has been built on its conclusions.

The Glaser 2013 Paper

Reduced breast cancer incidence in women treated with subcutaneous testosterone, or testosterone with anastrozole: A prospective, observational study

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Implants

ABSTRACT

Objectives: There is evidence that androgens are breast protective and that testosterone therapy treats many symptoms of hormone deficiency in both pre and postmenopausal patients. However, unlike estrogen and progestins, there is a paucity of data regarding the incidence of breast cancer in women treated with testosterone therapy. This study was designed to investigate the incidence of breast cancer in women treated with subcutaneous testosterone therapy in the absence of systemic estrogen therapy.
Study design: This is a 5-year interim analysis of a 10-year, prospective, observational, IRB approved study investigating the incidence of breast cancer in women presenting with symptoms of hormone deficiency treated with subcutaneous testosterone (T) implants or, T combined with the aromatase inhibitor anastrozole (A), i.e., T+A implants. Breast cancer incidence was compared with that of historical controls reported in the literature, age specific Surveillance Epidemiology and End Results (SEER) incidence rates, and a representative, similar age group of our patients used as a 'control' group. The effect of adherence to T therapy was also evaluated.
Results: Since March 2008, 1268 pre and post menopausal women have been enrolled in the study and eligible for analysis. As of March 2013, there have been 8 cases of invasive breast cancer diagnosed in 5642 person-years of follow up for an incidence of 142 cases per 100 000 person-years, substantially less than the age-specific SEER incidence rates (293/100 000), placebo arm of Women's Health Initiative Study (300/100 000), never users of hormone therapy from the Million Women Study (325/100 000) and our control group (390/100 000). Unlike adherence to estrogen therapy, adherence to T therapy further decreased the incidence of breast cancer (73/100 000).
Conclusion: T and/or T+A, delivered subcutaneously as a pellet implant, reduced the incidence of breast cancer in pre and postmenopausal women. Evidence supports that breast cancer is preventable by maintaining a T to estrogen ratio in favor of T and, in particular, by the use of continuous T or, when indicated, T+A. This hormone therapy should be further investigated for the prevention and treatment of breast cancer.

- Title: "Reduced breast cancer incidence in women treated with subcutaneous testosterone, or testosterone with anastrozole: A prospective, observational study" — Maturitas 2013.
- Study design: single-clinic prospective observational case series — 5-year interim analysis of a 10-year observational study from Glaser's own pellet practice (n=1,268).
- Reported: 8 invasive breast cancer cases observed vs SEER-predicted rates for age-matched US women — apparently lower incidence in their pellet patients.
- Authors: Glaser RL, Dimitrakakis C — both clinicians with direct financial interests in pellet implant practice and pellet supply chain.
- This is the paper cited as "the evidence" testosterone pellets are breast cancer protective — driving widespread elevated-dose practice. The methodology must be examined.

Glaser RL, Dimitrakakis C, 2013 — Maturitas — PMID: 24028858

Forensic Critique — Three Fatal Flaws

- Fatal Flaw #1: Sample selection bias — patients self-selected into a wellness-focused pellet practice. Younger, healthier, lower BMI, more health-conscious than SEER averages.
- Fatal Flaw #2: No matched control group — comparison was to general SEER population, not to similar wellness-seeking women not on pellets. The control is the wrong population.
- Fatal Flaw #3: No biomarker monitoring — no free hormone measurement, no estrogen metabolite testing, no methylation status. Effects of overdosing were invisible to the data.
- Cannot conclude testosterone caused the apparent reduction. The wellness-seeking lifestyle of pellet patients is the more parsimonious explanation.
- What this is: apples compared to oranges, with the apples called protective — and an entire prescribing practice built on the conclusion of a single-clinic observational case series.

WHI — Combined HRT and Breast Cancer Risk

Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women

Principal Results From the Women's Health Initiative Randomized Controlled Trial

Writing Group for the Women's Health Initiative Investigators

Context Despite decades of accumulated observational evidence, the balance of risks and benefits for hormone use in healthy postmenopausal women remains uncertain.

Objective To assess the major health benefits and risks of the most commonly used combined hormone preparation in the United States.

Design Estrogen plus progestin component of the Women's Health Initiative, a randomized controlled primary prevention trial (planned duration, 8.5 years) in which 16 608 postmenopausal women aged 50-79 years with an intact uterus at baseline were recruited by 40 US clinical centers in 1993-1998.

Interventions Participants received conjugated equine estrogens, 0.625 mg/d, plus medroxyprogesterone acetate, 2.5 mg/d, in 1 tablet (n=8506) or placebo (n=8102).

Main Outcomes Measures The primary outcome was coronary heart disease (CHD) (nonfatal myocardial infarction and CHD death), with invasive breast cancer as the primary adverse outcome. A global index summarizing the balance of risks and benefits included the 2 primary outcomes plus stroke, pulmonary embolism (PE), endometrial cancer, colorectal cancer, hip fracture, and death due to other causes.

Results On May 31, 2002, after a mean of 5.2 years of follow-up, the data and safety monitoring board recommended stopping the trial of estrogen plus progestin vs placebo because the test statistic for invasive breast cancer exceeded the stopping boundary for this adverse effect and the global index statistic supported risks exceeding benefits. This report includes data on the major clinical outcomes through April 30, 2002. Estimated hazard ratios (HRs) (nominal 95% confidence intervals [CIs]) were as follows: CHD, 1.29 (1.02-1.63) with 286 cases; breast cancer, 1.26 (1.00-1.59) with 290 cases; stroke, 1.41 (1.07-1.85) with 212 cases; PE, 2.13 (1.39-3.25) with 101 cases; colorectal cancer, 0.63 (0.43-0.92) with 112 cases; endometrial cancer, 0.83 (0.47-1.47) with 47 cases; hip fracture, 0.66 (0.45-0.98) with 106 cases; and death due to other causes, 0.92 (0.74-1.14) with 331 cases. Corresponding HRs (nominal 95% CIs) for composite outcomes were 1.22 (1.09-1.36) for total cardiovascular disease (arterial and venous disease), 1.03 (0.90-1.17) for total cancer, 0.76 (0.69-0.85) for combined fractures, 0.98 (0.82-1.18) for total mortality, and 1.15 (1.03-1.28) for the global index. Absolute excess risks per 10000 person-years attributable to estrogen plus progestin were 7 more CHD events, 8 more strokes, 8 more PEs, and 8 more invasive breast cancers, while absolute risk reductions per 10000 person-years were 6 fewer colorectal cancers and 5 fewer hip fractures. The absolute excess risk of events included in the global index was 19 per 10000 person-years.

Conclusions Overall health risks exceeded benefits from use of combined estrogen plus progestin for an average 5.2-year follow-up among healthy postmenopausal US women. All-cause mortality was not affected during the trial. The risk-benefit profile found in this trial is not consistent with the requirements for a viable intervention for primary prevention of chronic diseases, and the results indicate that this regimen should not be initiated or continued for primary prevention of CHD.

THE WOMEN'S HEALTH INITIATIVE (WHI) focuses on defining the risks and benefits of strategies that could potentially reduce the incidence of heart disease, breast and colorectal cancer, and fractures in postmenopausal women. Between 1993 and 1998, the WHI enrolled 161 809 postmenopausal women in the age range of 50 to 79 years into a set of clinical trials (trials of low-fat dietary pattern, calcium and vitamin D supplementation, and 2 trials of postmenopausal hormone use) and an observational study at 40 clinical centers in the United States.[1] This article reports principal results for the trial of combined estrogen and progestin in women with a uterus. The trial was stopped early based on health risks that exceeded health benefits over an average follow-up of 5.2 years. A parallel trial of estrogen alone in women who have had a hysterectomy is being continued, and the planned end of this trial is March 2005, by which time the average follow-up will be about 8.5 years.

The WHI clinical trials were designed in 1991-1992 using the accumulated evidence at that time. The primary outcome for the trial of estrogen plus progestin was designated as coronary heart disease (CHD). Potential cardioprotection was based on generally

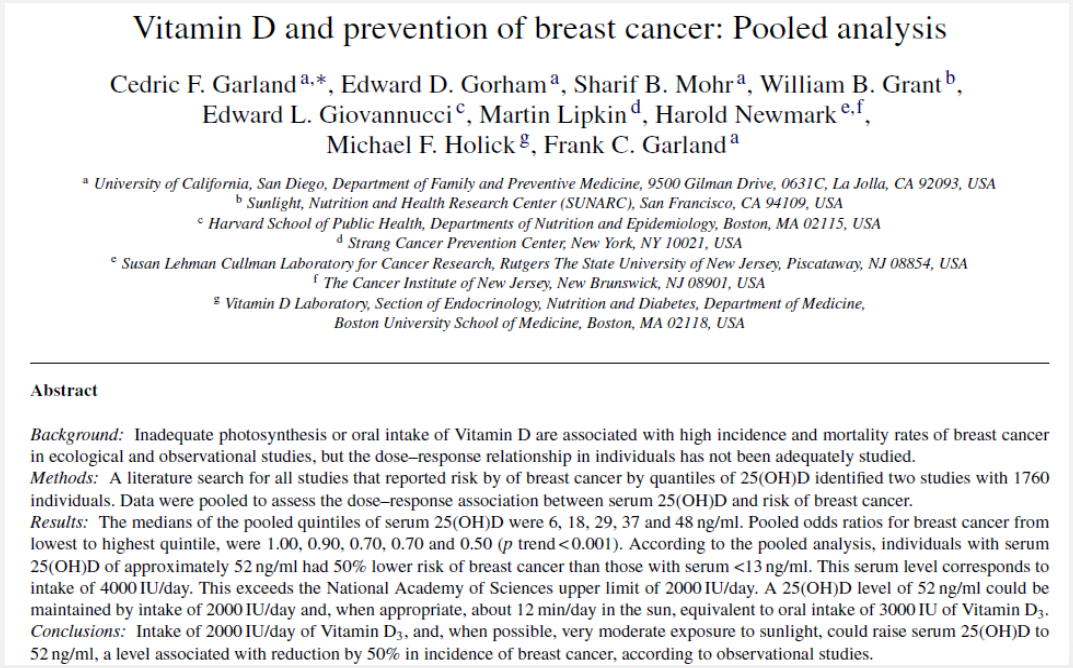
- The Women's Health Initiative — 16,608 postmenopausal women randomized to combined estrogen + progestin vs placebo. Methodologically rigorous, prospective, double-blind.
- Stopped early due to a clear breast cancer signal: HR 1.26 (95% CI 1.00-1.59) — combined hormone therapy increased breast cancer risk by 26% in the active treatment arm.
- This is what rigorous prospective evidence looks like for hormone-cancer relationships — randomized, controlled, prospective, with biomarker monitoring and predefined endpoints.
- Contrasts sharply with the methodological weakness of the Glaser paper — retrospective, uncontrolled, single-clinic, no monitoring, financially conflicted authors.
- Lesson: do not extrapolate clinical practice from retrospective marketing data when rigorous prospective data exists. The methodological hierarchy of evidence matters in clinical decision-making.

Rossouw JE et al., 2002 — JAMA — PMID: 12117397

What Actually Reduces Breast Cancer Risk

- Adequate vitamin D status: serum 25(OH)D >40 ng/mL associated with substantial breast cancer risk reduction across multiple meta-analyses. The strongest single-nutrient intervention.
- Robust melatonin production: oncoprotective via free radical scavenging, ER signaling modulation, and circadian alignment. Salivary melatonin directly measurable on DD's panel.
- Healthy methylation status: adequate 2-MeO-E2 production protects against 4-OH genotoxic damage. HUMAP measures functional capacity directly — a target for intervention.
- Cruciferous vegetables and DIM: shift the 2-OH:16-OH ratio toward protective. Adequate fiber and a healthy estrobolome reduce estrogen recirculation. Modifiable through diet.
- Body composition, sleep, stress, alcohol moderation: the foundational lifestyle interventions that predict breast cancer outcomes more reliably than any single hormone therapy.
- These interventions have prospective evidence — not retrospective marketing data — and they address the actual biology of breast cancer risk, including in patients with adverse family history.

Vitamin D & Breast Cancer Risk



Vitamin D and prevention of breast cancer: Pooled analysis

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Abstract

Background: Inadequate photosynthesis or oral intake of Vitamin D are associated with high incidence and mortality rates of breast cancer in ecological and observational studies, but the dose–response relationship in individuals has not been adequately studied.
Methods: A literature search for all studies that reported risk by of breast cancer by quantiles of 25(OH)D identified two studies with 1760 individuals. Data were pooled to assess the dose–response association between serum 25(OH)D and risk of breast cancer.
Results: The medians of the pooled quintiles of serum 25(OH)D were 6, 18, 29, 37 and 48 ng/ml. Pooled odds ratios for breast cancer from lowest to highest quintile, were 1.00, 0.90, 0.70, 0.70 and 0.50 (p trend < 0.001). According to the pooled analysis, individuals with serum 25(OH)D of approximately 52 ng/ml had 50% lower risk of breast cancer than those with serum <13 ng/ml. This serum level corresponds to intake of 4000 IU/day. This exceeds the National Academy of Sciences upper limit of 2000 IU/day. A 25(OH)D level of 52 ng/ml could be maintained by intake of 2000 IU/day and, when appropriate, about 12 min/day in the sun, equivalent to oral intake of 3000 IU of Vitamin D_3.
Conclusions: Intake of 2000 IU/day of Vitamin D_3, and, when possible, very moderate exposure to sunlight, could raise serum 25(OH)D to 52 ng/ml, a level associated with reduction by 50% in incidence of breast cancer, according to observational studies.

- Garland 2007 pooled analysis: 25(OH)D level of ~52 ng/mL (130 nmol/L) associated with ~50% lower breast cancer risk vs <13 ng/mL.
- Pooled odds ratios across 5 quintiles of serum 25(OH)D: 1.00, 0.90, 0.70, 0.70, 0.50 (p-trend <0.001) — clear dose-response gradient.
- Target serum: ~52 ng/mL achievable with ~2,000 IU/day Vitamin D3 + modest sun exposure (~12 min/day equivalent).
- Mechanistic basis spans cell proliferation, differentiation, apoptosis, and immune surveillance in breast tissue.
- Vitamin D regulates estrogen receptor signaling and modulates inflammatory cascades implicated in tumor initiation and progression.
- Clinical implication: every functional medicine workup should include serum 25(OH)D, target >40-60 ng/mL especially with adverse family history.

Garland CF et al., 2007 — J Steroid Biochem — PMID: 17368188

Melatonin & Breast Cancer Risk

Melatonin: an inhibitor of breast cancer

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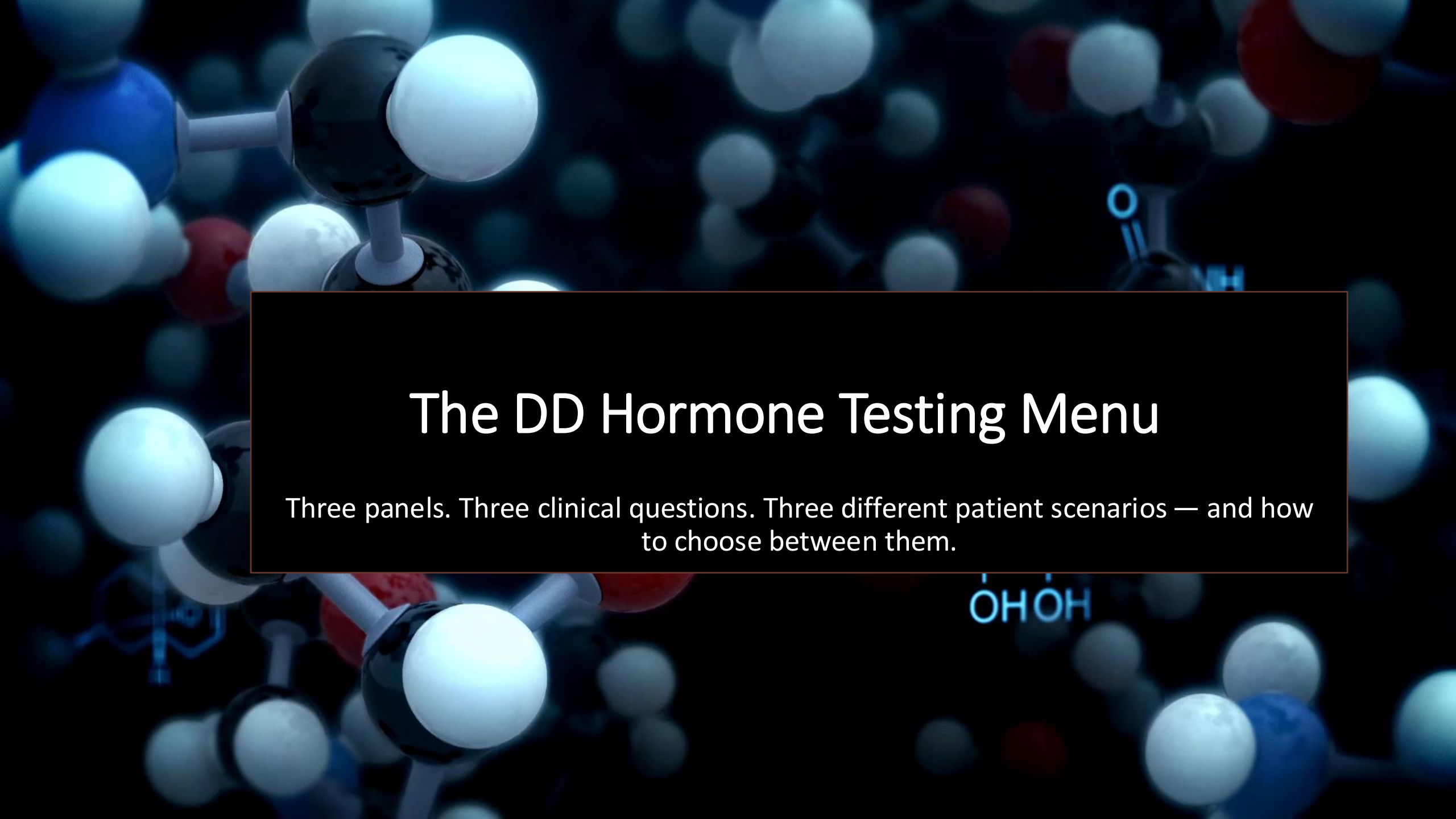
Abstract

The present review discusses recent work on melatonin-mediated circadian regulation, the metabolic and molecular signaling mechanisms that are involved in human breast cancer growth, and the associated consequences of circadian disruption by exposure to light at night (LEN). The anti-cancer actions of the circadian melatonin signal in human breast cancer cell lines and xenografts heavily involve MT_1 receptor-mediated mechanisms. In estrogen receptor alpha (ERα)-positive human breast cancer, melatonin suppresses ERα mRNA expression and ERα transcriptional activity via the MT_1 receptor. Melatonin also regulates the transactivation of other members of the nuclear receptor superfamily, estrogen-metabolizing enzymes, and the expression of core clock and clock-related genes. Furthermore, melatonin also suppresses tumor aerobic metabolism (the Warburg effect) and, subsequently, cell-signaling pathways critical to cell proliferation, cell survival, metastasis, and drug resistance. Melatonin demonstrates both cytostatic and cytotoxic activity in breast cancer cells that appears to be cell type-specific. Melatonin also possesses anti-invasive/anti-metastatic actions that involve multiple pathways, including inhibition of p38 MAPK and repression of epithelial–mesenchymal transition (EMT). Studies have demonstrated that melatonin promotes genomic stability by inhibiting the expression of LINE-1 retrotransposons. Finally, research in animal and human models has indicated that LEN-induced disruption of the circadian nocturnal melatonin signal promotes the growth, metabolism, and signaling of human breast cancer and drives breast tumors to endocrine and chemotherapeutic resistance. These data provide the strongest understanding and support of the mechanisms that underpin the epidemiologic demonstration of elevated breast cancer risk in night-shift workers and other individuals who are increasingly exposed to LEN.

- Decades of research establishing melatonin's oncoprotective effects in breast tissue — Hill, Davis, Reiter, and others have built a comprehensive evidence base.
- Mechanism: melatonin inhibits estrogen receptor signaling at physiologic levels and exerts direct anti-proliferative and pro-apoptotic effects on breast cancer cells in vitro.
- Free radical scavenging: melatonin is one of the most potent endogenous antioxidants — directly protecting DNA from oxidative damage caused by 4-OH catechol estrogen quinones.
- Epidemiologic evidence: night-shift workers (chronically suppressed melatonin) show elevated breast cancer rates — IARC classified shift work as a probable carcinogen on this basis.
- This is precisely why DD's saliva panel includes 3-point melatonin — it is not just a sleep marker but a quantitative cancer-risk biomarker. A core reason to choose comprehensive saliva over basic profiles.

Hill SM et al., 2015 — Endocr Relat Cancer — PMID: 25876649



The DD Hormone Testing Menu

Three panels. Three clinical questions. Three different patient scenarios — and how to choose between them.

Comprehensive Plus (Saliva) + Melatonin

- The flagship panel for the functional medicine clinic — six steroid hormones plus melatonin and the full HPA cortisol curve in a single home-collection kit.
- Best for the patient who needs the full picture: perimenopause workup, fatigue with mood changes, parenteral HRT monitoring, and complex HPA dysfunction.
- Adds the circadian dimension via 3-point melatonin and 4-point cortisol — essential for sleep, fatigue, and oncoprotective melatonin assessment.
- Six steroid hormones (E1, E2, E3, Pg, T, DHEA) plus 7 cortisol/melatonin time points. Most clinically dense panel DD offers.
- Almost every new functional medicine patient benefits from this baseline — the default starting panel for any first workup.
- Single home collection kit, mailed back, results in 7-10 days. Cost: approximately $300–$350 cash-pay.

HUMAP — The Urine Metabolite Profile

- HUMAP measures 24-hour urinary estrogen metabolites — the methylation and conjugation picture saliva cannot show.
- Quantifies the three estrogen metabolic pathways: 2-OH (protective), 4-OH (genotoxic), and 16-OH (proliferative). Each tells a different clinical story.
- The 2-OH:16-OH ratio is a validated breast cancer risk biomarker — backed by Nurses' Health Study, Bradlow data, and modern prospective cohorts.
- Critical when patient has family history of breast cancer, fibrocystic disease, endometriosis, or any estrogen-sensitive condition.
- Identifies methylation bottlenecks: poor 2-MeO-E2 production, elevated 4-OH risk pattern, sulfation/glucuronidation insufficiency.
- Pairs with Comp Saliva for the complete estrogen biology picture — production (saliva) plus elimination/transformation (urine).

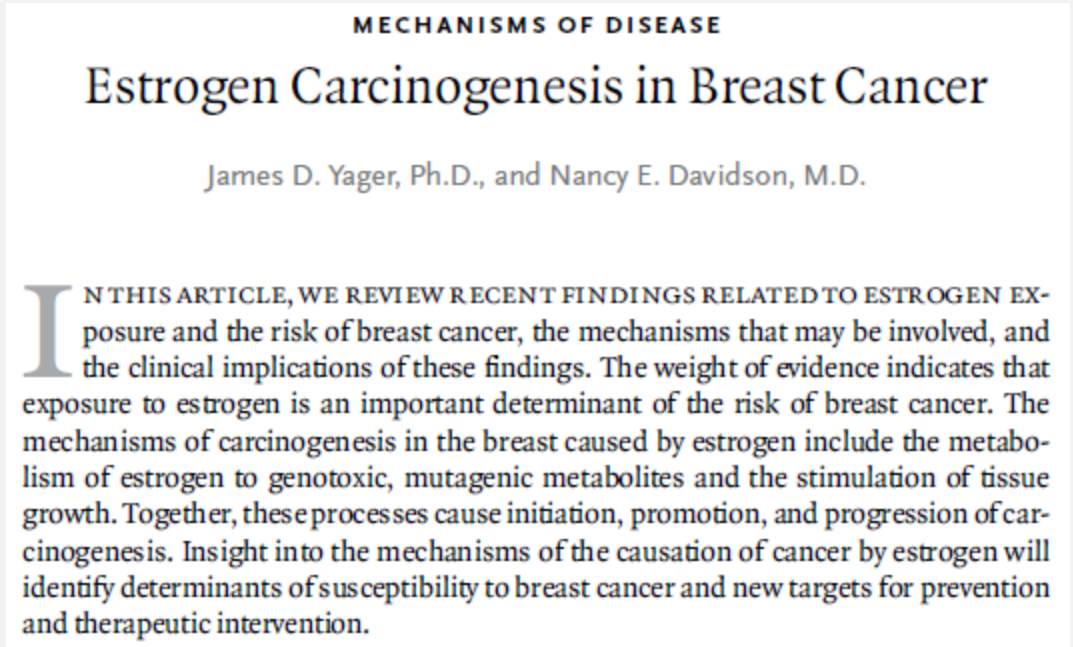
What HUMAP Reveals

- 2-OH estrogens — the protective metabolic pathway. Higher levels associated with reduced breast cancer risk in multiple cohorts.
- 4-OH estrogens — genotoxic, DNA-damaging metabolites. Elevation requires immediate methylation support and risk-reduction protocols.
- 16-OH estrogens — proliferative pathway. Drives tissue growth in breast and uterus; elevation correlates with estrogen-dominant symptom patterns.
- Methylation capacity via the 2-MeO-E2:2-OH ratio — measures functional COMT activity and SAMe sufficiency. Low ratio = methylation bottleneck.
- Phase II conjugation status — sulfation and glucuronidation of all estrogen metabolites. Identifies impaired excretion patterns.
- Direct guide to clinical intervention: DIM, calcium-D-glucarate, methylation support, sulforaphane — each addresses a specific HUMAP finding.

Basic Hormone Profile

- Cost-effective follow-up panel for monitoring established treatment — designed for serial use after initial Comp Saliva baseline.
- Single morning saliva collection — measures core hormones at peak diurnal time without the multi-timepoint complexity of the full panel.
- Includes: E2, Pg, T, DHEA, AM cortisol — the essentials for routine HRT or wellness monitoring.
- Best for: routine follow-up after initial workup, treatment monitoring on stable HRT, and simpler clinical questions that don't require the full circadian picture.
- Lower cost (~$200) makes it accessible for serial monitoring — quarterly or semi-annual tracking without the financial burden of full Comp Saliva each time.
- Use when you don't need the CAR, multi-time melatonin, or three-estrogen breakdown — but still need real, tissue-active hormone data.
- Morning Cortisol is the most important data point for HPA axis

Estrogen Carcinogenesis in Breast Cancer



MECHANISMS OF DISEASE

Estrogen Carcinogenesis in Breast Cancer

James D. Yager, Ph.D., and Nancy E. Davidson, M.D.

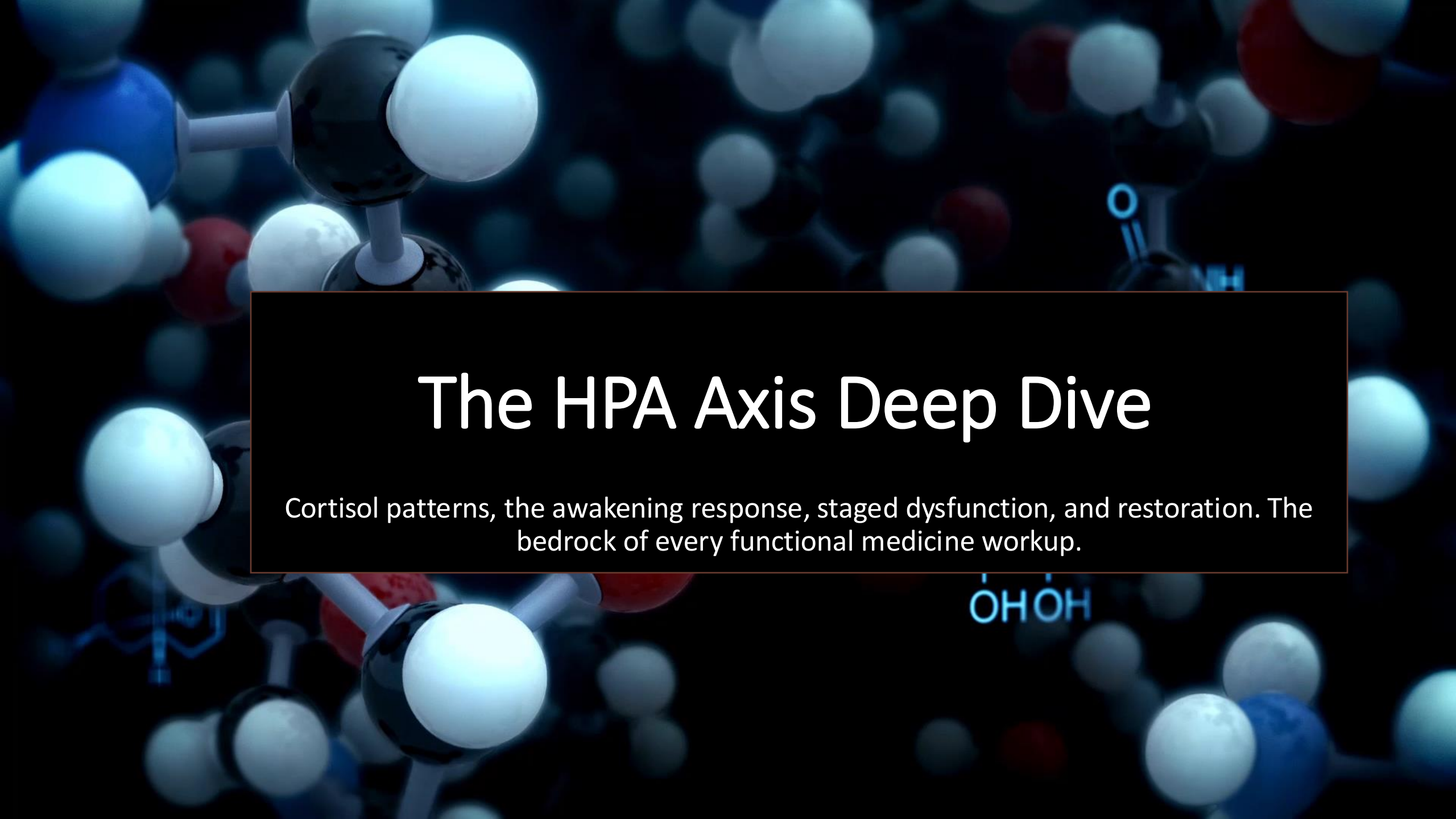
IN THIS ARTICLE, WE REVIEW RECENT FINDINGS RELATED TO ESTROGEN EXposure and the risk of breast cancer, the mechanisms that may be involved, and the clinical implications of these findings. The weight of evidence indicates that exposure to estrogen is an important determinant of the risk of breast cancer. The mechanisms of carcinogenesis in the breast caused by estrogen include the metabolism of estrogen to genotoxic, mutagenic metabolites and the stimulation of tissue growth. Together, these processes cause initiation, promotion, and progression of carcinogenesis. Insight into the mechanisms of the causation of cancer by estrogen will identify determinants of susceptibility to breast cancer and new targets for prevention and therapeutic intervention.

- Comprehensive NEJM mechanism review establishing the molecular pathway of estrogen-induced breast cancer.
- Documented that 4-OH catechol estrogen quinones form covalent DNA adducts → depurination → mutation → cancer initiation.
- Established the two-pronged mechanism: hormonal (ER-mediated proliferation) AND genotoxic (4-OH metabolite-mediated DNA damage).
- Provides the mechanistic biology linking metabolite ratios to clinical outcomes — why measuring 4-OH and 2-OH:16-OH ratios is not just academic.
- Cited in modern breast cancer prevention and risk-reduction guidelines — the definitive reference for explaining estrogen metabolite testing to patients and skeptical colleagues.

Yager JD, Davidson NE, 2006 — N Engl J Med — PMID: 16421368

Decision Tree by Clinical Scenario

- New functional medicine patient → Comp Saliva + Melatonin — full baseline including HPA, gonadal, and circadian assessment.
- Established patient on stable HRT, routine monitoring → Basic Hormone Profile — efficient serial tracking at lower cost.
- Family history of breast cancer or estrogen-sensitive condition → add HUMAP to whichever saliva panel — methylation and metabolite data are non-negotiable here.
- Complex HPA dysfunction, fatigue, anxiety, or sleep patterns → Comp Saliva is mandatory — the 4-point cortisol and melatonin curves are diagnostic.
- Methylation/COMT concerns (anxiety, estrogen dominance, MTHFR variants) → HUMAP for the 2-MeO-E2:2-OH ratio — directly measures functional methylation capacity.
- Pregnancy, postpartum, or fertility workup → Comp Saliva with timing-specific protocols. Saliva is uniquely suited for repeated mid-cycle and longitudinal measurement.



The HPA Axis Deep Dive

Cortisol patterns, the awakening response, staged dysfunction, and restoration. The bedrock of every functional medicine workup.

Why the HPA Axis Matters in HRT Practice

- HPA dysfunction precedes and predicts nearly every gonadal hormone problem you will encounter — including infertility, perimenopause severity, andropause, PCOS, and treatment failure.
- The cortisol-DHEA balance is the master regulator of metabolic adaptation. Disrupted patterns drive insulin resistance, weight gain, immune dysfunction, and accelerated aging.
- Sleep, mood, and cognitive symptoms almost always reflect HPA dysregulation before they reflect anything else. Address HPA first, and many "psychiatric" symptoms resolve.
- Pregnenolone Steal / Cortisol Shunt is real: chronic cortisol demand depletes substrate for sex hormone synthesis — directly causing low progesterone, low testosterone, and low DHEA.
- Salivary 4-point cortisol + 3-point melatonin + DHEA gives the most diagnostically dense single panel in functional medicine — the foundation of every workup.
- Failure to assess HPA before initiating HRT is a common cause of treatment non-response — and a leading reason patients abandon protocols and lose trust in the practitioner.

The 4-Point Cortisol Curve

- Morning sample (within 30 min of waking) — the gateway marker. Captures the cortisol peak that drives wakefulness, attention, and metabolic activation for the day.
- Noon sample — captures the first descent from morning peak. A flat or sluggish slope here indicates blunted HPA reactivity and early-stage dysfunction.
- Evening sample (4-5pm) — the rest-and-digest marker. Should be moderately low. Elevated evening cortisol predicts insomnia, anxiety, and elevated nighttime catabolism.
- Bedtime sample — the recovery marker. Should be the lowest value of the day. Elevation here is the single most predictive marker of poor sleep architecture.
- The diurnal slope (morning ÷ evening) — a flatter slope predicts mortality across multiple cohort studies independently of total cortisol load.
- Pattern recognition over absolute numbers — the shape of the curve tells the story, not just whether values are "in range." This is where saliva testing decisively beats serum.

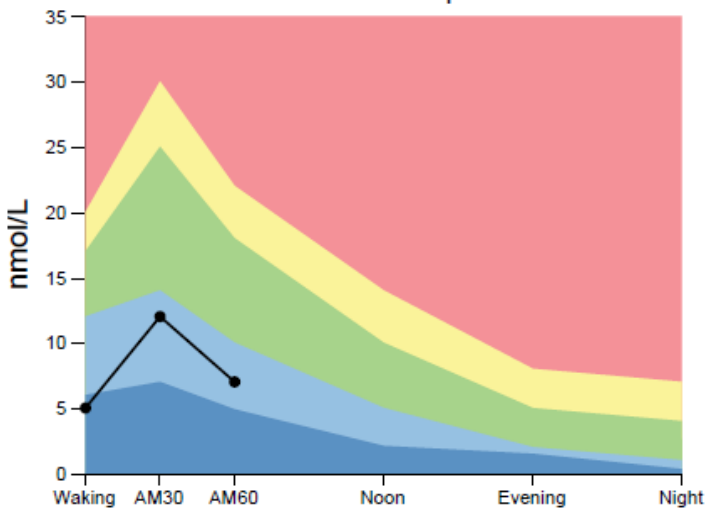
The Cortisol Awakening Response (CAR)

- The CAR is the 30-50% rise in cortisol within 30 minutes of waking — a discrete physiological event distinct from the diurnal rhythm.
- A robust CAR signals healthy HPA reactivity, intact circadian function, and adequate adrenal reserves. Blunted CAR predicts depression, burnout, and chronic fatigue.
- Exaggerated CAR (>75% rise) signals anticipatory stress, anxiety, and overreactive HPA — common in early-stage adrenal dysfunction and chronic worry patterns.
- Measured by paired waking and 30-minute post-waking samples — a critical reason proper morning sampling protocol must be followed exactly.
- Patients with elevated nighttime cortisol AND blunted CAR have the worst clinical prognosis — this combination predicts treatment-resistant depression and metabolic dysfunction.
- A clinically robust CAR is one of the best markers of HPA recovery during functional medicine treatment — track it serially to confirm protocol effectiveness.

The Cortisol Awakening Response (CAR)

Analyte	Result	Unit	L	WRI	H	Optimal Range	Reference Interval
Cortisol Waking	5.0	nmol/L	↓			12 – 17	6.0 – 20
Cortisol AM30	12	nmol/L		◆		14.0 – 25.0	7.0 – 30.0
Cortisol AM60	7.0	nmol/L		◆		10.0 – 18.0	4.9 – 22.0
CAR Rise *(Calculated)*	140.0	%			↑		35 – 60
CAR Decline *(Calculated)*	40.00	%			↑		-33 – 0

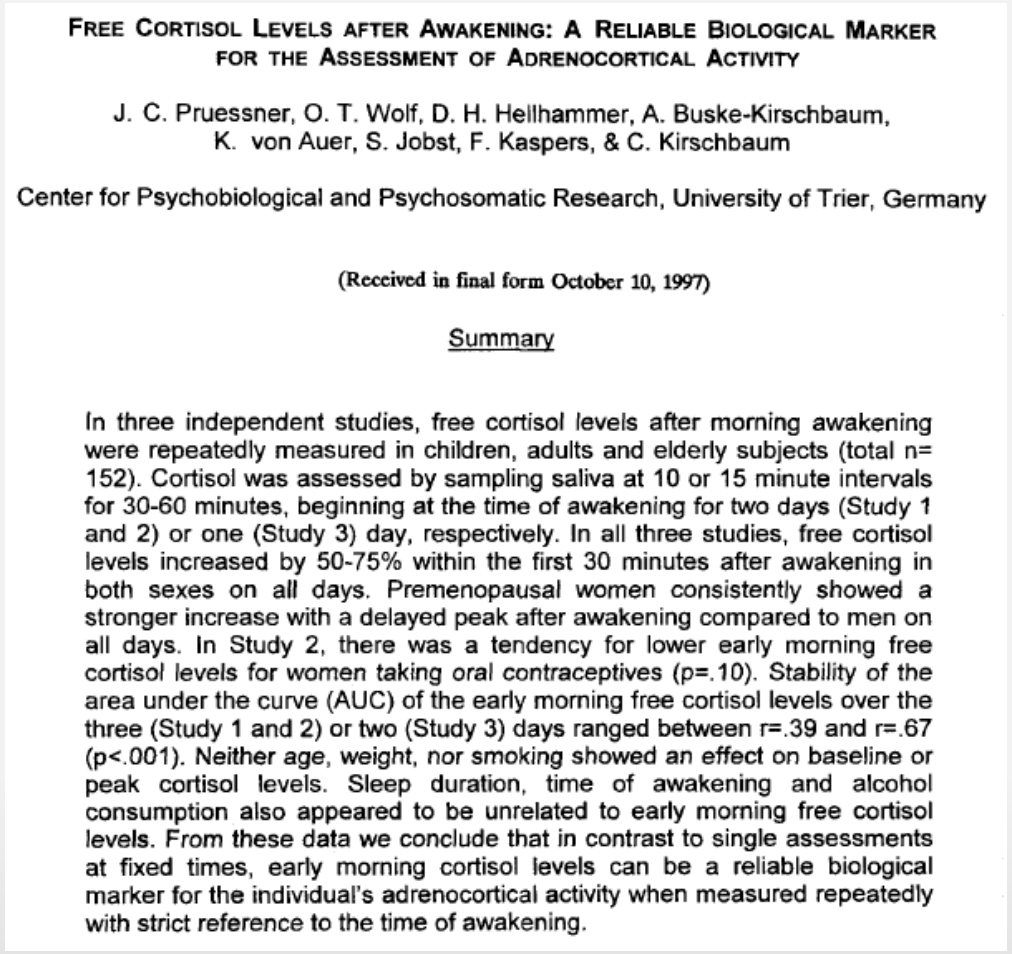
Cortisol Graph



Hormone Comments

- The AM cortisol level is suboptimal. Additional cortisol testing is a consideration.
- Cortisol Awakening Response (CAR) is the expected maximum rise in cortisol levels observed at 30 minutes (AM30) post awakening (approximately 35-60% above the waking value).This is followed by an expected decline sixty minutes after waking. The behavior of cortisol is a critical marker to understand the HPA axis' physiologic responsiveness and is a key indicator of HPA axis adaptability and reactivity.
- This patient's *CAR Rise* (the percent change from waking to AM30) exceeds the expected degree of increase.
- This patient's *CAR Decline* (the percent change from waking to AM60) is observed as an unexpected increase.

Original CAR Characterization



FREE CORTISOL LEVELS AFTER AWAKENING: A RELIABLE BIOLOGICAL MARKER FOR THE ASSESSMENT OF ADRENOCORTICAL ACTIVITY

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Center for Psychobiological and Psychosomatic Research, University of Trier, Germany

(Received in final form October 10, 1997)

Summary

In three independent studies, free cortisol levels after morning awakening were repeatedly measured in children, adults and elderly subjects (total n= 152). Cortisol was assessed by sampling saliva at 10 or 15 minute intervals for 30-60 minutes, beginning at the time of awakening for two days (Study 1 and 2) or one (Study 3) day, respectively. In all three studies, free cortisol levels increased by 50-75% within the first 30 minutes after awakening in both sexes on all days. Premenopausal women consistently showed a stronger increase with a delayed peak after awakening compared to men on all days. In Study 2, there was a tendency for lower early morning free cortisol levels for women taking oral contraceptives ($p=.10$). Stability of the area under the curve (AUC) of the early morning free cortisol levels over the three (Study 1 and 2) or two (Study 3) days ranged between $r=.39$ and $r=.67$ ($p<.001$). Neither age, weight, nor smoking showed an effect on baseline or peak cortisol levels. Sleep duration, time of awakening and alcohol consumption also appeared to be unrelated to early morning free cortisol levels. From these data we conclude that in contrast to single assessments at fixed times, early morning cortisol levels can be a reliable biological marker for the individual's adrenocortical activity when measured repeatedly with strict reference to the time of awakening.

- The foundational paper characterizing the cortisol awakening response — distinguished CAR as a discrete physiological event from the broader diurnal cortisol rhythm.
- Demonstrated that the CAR is reproducible within individuals and reflects HPA reactivity to the demands of the upcoming day, not just baseline secretion.
- Documented a 50-75% rise within 30 minutes of waking as the normal physiological pattern in healthy adults — the reference standard still used today.
- Identified that blunted CAR correlates with depression, burnout, and post-traumatic stress — making the awakening response a sensitive psychiatric biomarker.
- Provides the scientific foundation for the morning + 30-min sampling protocol used in Doctors Data and other functional medicine cortisol panels worldwide.

Pruessner JC et al., 1997 — Life Sci — PMID: 9416776

Elevated Nighttime Cortisol — Causes & Patterns

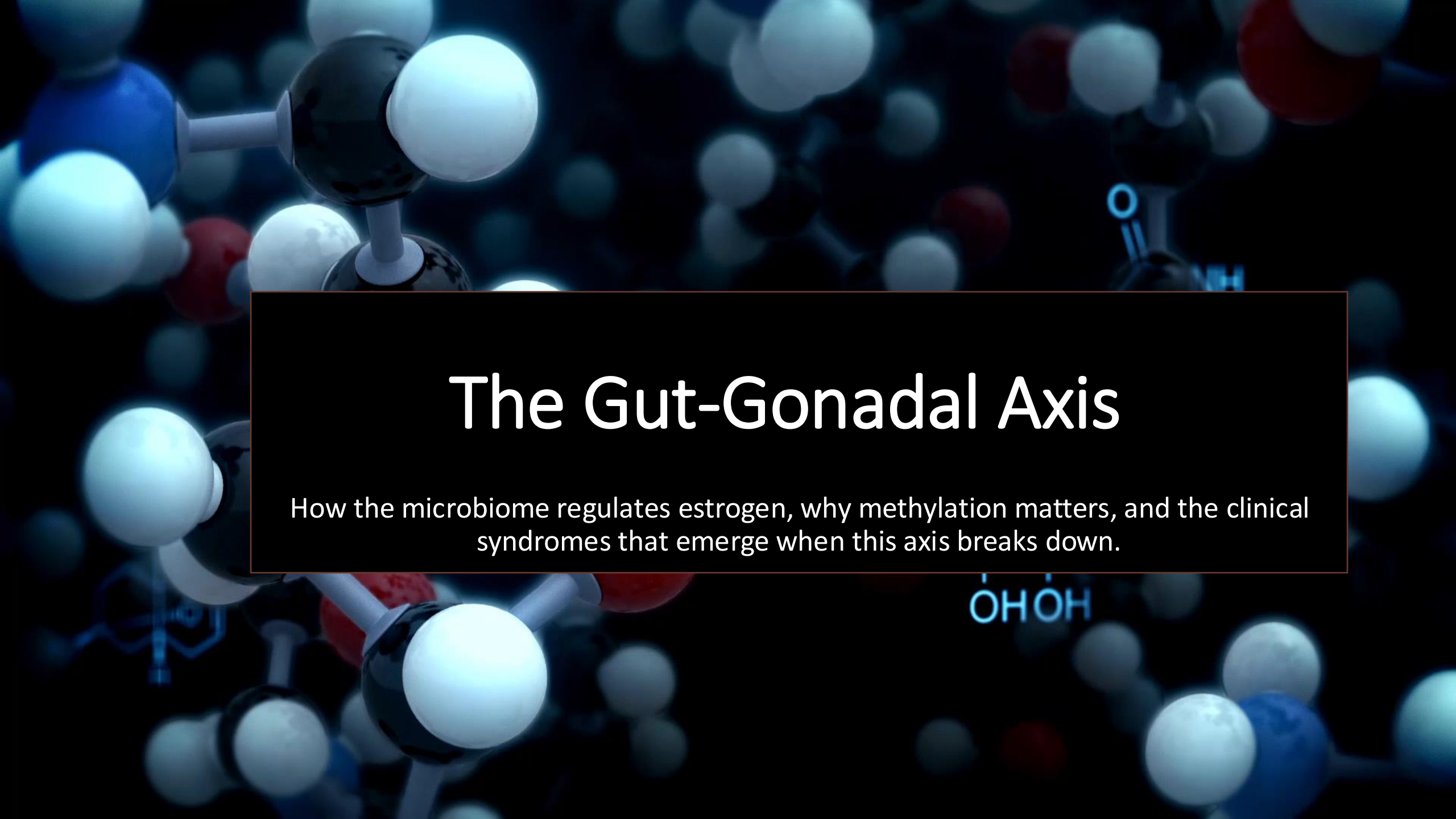
- The single most common abnormality on functional medicine cortisol panels — elevated bedtime cortisol is found in 60-70% of new patients presenting with insomnia.
- Primary drivers: chronic anticipatory stress, blue light exposure, late caffeine, late meals, blood sugar dysregulation — almost all addressable through behavioral interventions.
- Less obvious drivers: hidden infections, mold exposure, GI dysbiosis, untreated sleep apnea — when the "obvious" causes don't resolve the pattern, look here.
- Mechanism: elevated nighttime cortisol suppresses melatonin (DD's 3-point melatonin captures this), disrupts sleep architecture, and prevents tissue repair during the night.
- Cascade effect: high nighttime cortisol → poor sleep → blunted morning CAR → daytime fatigue → coffee/sugar dependency → repeat. Breaking this loop is the foundation of HPA restoration.
- Clinical priority: address nighttime cortisol before any HRT or thyroid intervention — otherwise downstream therapies fail and patients lose confidence in the protocol.

Staged HPA Dysfunction

- Stage 1 — Alarm: elevated total cortisol output across the day, robust or exaggerated CAR, normal or high DHEA. The young, driven, anxious patient. Reversible with stress management alone.
- Stage 2 — Resistance: mixed pattern — high morning, blunted slope, elevated evening/bedtime, normal-to-low DHEA. The classic "tired but wired" patient. Requires active intervention.
- Stage 3 — Exhaustion: flattened curve, low total output, low DHEA, blunted CAR. The chronic fatigue, fibromyalgia, late-stage burnout patient. Requires the deepest intervention and longest recovery timeline.
- Each stage requires different therapeutic approach — adaptogens for Stage 1, glandulars and full circadian intervention for Stage 2, cautious low-dose hydrocortisone or licorice for Stage 3.
- Misidentifying the stage is the most common HPA treatment error: giving stimulating adaptogens to an exhausted patient worsens symptoms and erodes adrenal reserves further.
- Track stage transitions over time — patients should move from 3→2→1 over months of treatment, with serial salivary panels confirming the recovery trajectory.

HPA Restoration Framework

- Foundation: sleep, blood sugar, light hygiene, and stress management — these four pillars precede any supplement intervention. Without them, no protocol works.
- Adaptogens by stage: rhodiola and ginseng for Stage 1; ashwagandha, holy basil, and reishi for Stage 2; licorice (with monitoring) and pantothenic acid for Stage 3.
- Adrenal glandulars and DHEA — use cautiously based on actual lab values (DD's panel measures DHEA directly). Never empirically supplement DHEA without testing.
- Phosphatidylserine (PS) at bedtime — the single most useful intervention for elevated nighttime cortisol. Direct evidence base, near-universal patient response.
- Address the upstream drivers: infections, GI dysfunction, mold, sleep apnea, food sensitivities. Pure HPA support fails when there is unaddressed "demand" coming from elsewhere.
- Track recovery with serial salivary cortisol curves at 3-6 month intervals — both for protocol confirmation and for patient motivation. Visible progress sustains compliance.



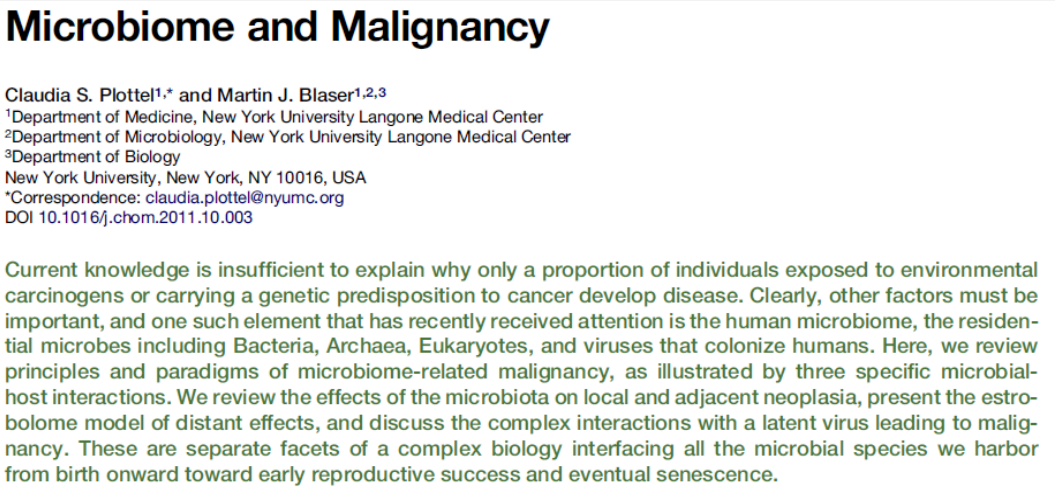
The Gut-Gonadal Axis

How the microbiome regulates estrogen, why methylation matters, and the clinical syndromes that emerge when this axis breaks down.

The Estrobolome — Gut Microbiome and Estrogen

- The estrobolome is the collection of gut microbial genes encoding β-glucuronidase — the enzyme that deconjugates estrogens excreted into bile, allowing reabsorption into circulation.
- Healthy estrobolome activity regulates circulating estrogen levels through enterohepatic recirculation. Disrupted activity drives both estrogen excess and estrogen deficiency syndromes.
- Dysbiosis — particularly overgrowth of β-glucuronidase-producing bacteria (Clostridia, certain E. coli) — increases reabsorption and circulating estrogen burden.
- Antibiotics, alcohol, processed foods, and chronic stress alter the estrobolome within days — directly affecting circulating hormone levels measurable on saliva and HUMAP.
- Calcium-D-glucarate works precisely here: it inhibits microbial β-glucuronidase, reducing estrogen reabsorption — a mechanistically targeted intervention for estrogen-dominant patterns.
- Implication: every estrogen-related complaint must be evaluated through a gut lens — fibrocystic breast, endometriosis, fibroids, perimenopause severity, even certain breast cancers.

Microbiome and Malignancy — Estrobolome Coined



Microbiome and Malignancy

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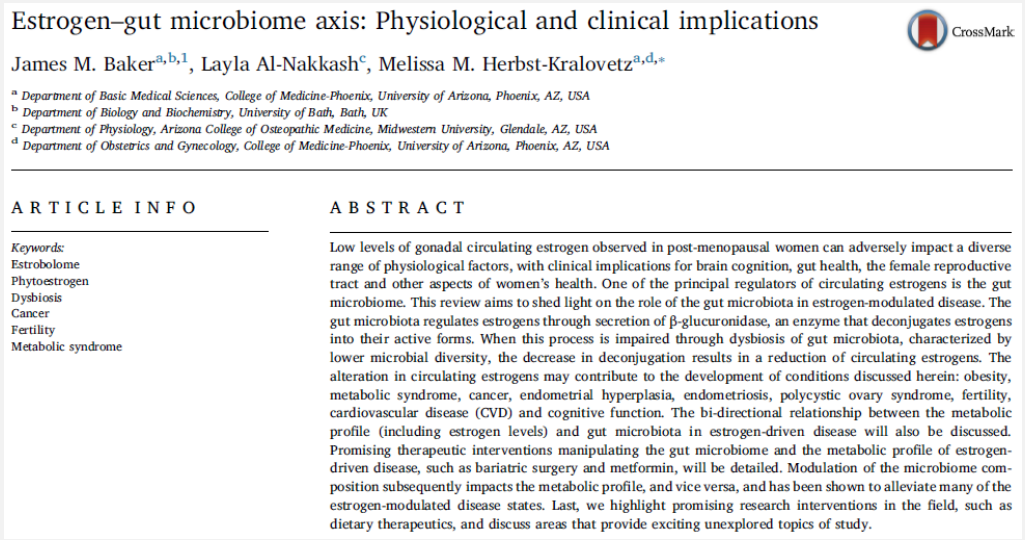
Current knowledge is insufficient to explain why only a proportion of individuals exposed to environmental carcinogens or carrying a genetic predisposition to cancer develop disease. Clearly, other factors must be important, and one such element that has recently received attention is the human microbiome, the resident microbes including Bacteria, Archaea, Eukaryotes, and viruses that colonize humans. Here, we review principles and paradigms of microbiome-related malignancy, as illustrated by three specific microbial-host interactions. We review the effects of the microbiota on local and adjacent neoplasia, present the estrobolome model of distant effects, and discuss the complex interactions with a latent virus leading to malignancy. These are separate facets of a complex biology interfacing all the microbial species we harbor from birth onward toward early reproductive success and eventual senescence.

- The foundational paper that coined the term "estrobolome" and established the conceptual framework for microbial regulation of host estrogen biology.
- Reviewed evidence linking gut bacterial composition to circulating estrogen levels through the β-glucuronidase deconjugation pathway and enterohepatic recirculation.
- Established the mechanistic link between gut dysbiosis and estrogen-related malignancies — breast, endometrial, ovarian — providing a microbiome-based risk modification target.
- Proposed the estrobolome as a modifiable cancer prevention target through diet, prebiotics, probiotics, and microbiome-directed interventions.
- Sparked the modern field of integrative oncology and microbiome-hormone research — central reference for explaining the gut-hormone connection in clinical practice.

Plottel CS, Blaser MJ, 2011 — Cell Host Microbe — PMID: 22018233

The Estrogen-Gut Microbiome Axis



Estrogen–gut microbiome axis: Physiological and clinical implications

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ABSTRACT

Low levels of gonadal circulating estrogen observed in post-menopausal women can adversely impact a diverse range of physiological factors, with clinical implications for brain cognition, gut health, the female reproductive tract and other aspects of women's health. One of the principal regulators of circulating estrogens is the gut microbiome. This review aims to shed light on the role of the gut microbiota in estrogen-modulated disease. The gut microbiota regulates estrogens through secretion of β-glucuronidase, an enzyme that deconjugates estrogens into their active forms. When this process is impaired through dysbiosis of gut microbiota, characterized by lower microbial diversity, the decrease in deconjugation results in a reduction of circulating estrogens. The alteration in circulating estrogens may contribute to the development of conditions discussed herein: obesity, metabolic syndrome, cancer, endometrial hyperplasia, endometriosis, polycystic ovary syndrome, fertility, cardiovascular disease (CVD) and cognitive function. The bi-directional relationship between the metabolic profile (including estrogen levels) and gut microbiota in estrogen-driven disease will also be discussed. Promising therapeutic interventions manipulating the gut microbiome and the metabolic profile of estrogen-driven disease, such as bariatric surgery and metformin, will be detailed. Modulation of the microbiome composition subsequently impacts the metabolic profile, and vice versa, and has been shown to alleviate many of the estrogen-modulated disease states. Last, we highlight promising research interventions in the field, such as dietary therapeutics, and discuss areas that provide exciting unexplored topics of study.

- Comprehensive 2017 review covering bidirectional regulation between estrogen and the gut microbiome across the female lifespan and across multiple disease states.
- Documented that estrogen itself shapes microbiome composition — explaining microbiome shifts during puberty, menstrual cycle, pregnancy, and menopause.
- Reviewed evidence linking estrobolome dysregulation to endometriosis, PCOS, polycystic ovaries, and infertility — extending the framework beyond breast cancer.
- Established that gut barrier integrity (leaky gut) directly affects estrogen metabolism — providing the rationale for combined GI repair + hormone support protocols.
- The reference paper for explaining the gut-hormone connection to patients, colleagues, and skeptics. Cited in modern functional medicine and integrative gynecology curricula.

Baker JM et al., 2017 — Maturitas 103:45-53 — PMID: 28778332

Female Reproductive Implications

- Perimenopause: estrobolome dysregulation amplifies estrogen dominance during the years when natural progesterone production is declining — driving severity of vasomotor, mood, and sleep symptoms.
- PCOS: elevated circulating estrogens with low progesterone (anovulation) plus relative androgen excess. The gut-hormone axis often drives the persistent estrogen dominance.
- Endometriosis: gut-driven estrogen excess feeds extra-uterine endometrial tissue. Treating gut alongside hormones produces dramatically better outcomes than hormone therapy alone.
- Fibroids and fibrocystic breast disease: classic estrogen-dominant tissue patterns. Always investigate gut, methylation, and HUMAP findings — surgery without addressing root cause leads to recurrence.
- Infertility (unexplained, recurrent miscarriage): often a multi-axis dysfunction — HPA + gut + methylation + thyroid + nutrient status. Single-axis interventions rarely succeed.
- Postpartum mood disorders: rapid hormone shift on a baseline of gut and HPA dysfunction produces severe presentations. Pre-conception axis stabilization is the most powerful prevention.

Male Implications — Andropause and Gut Health

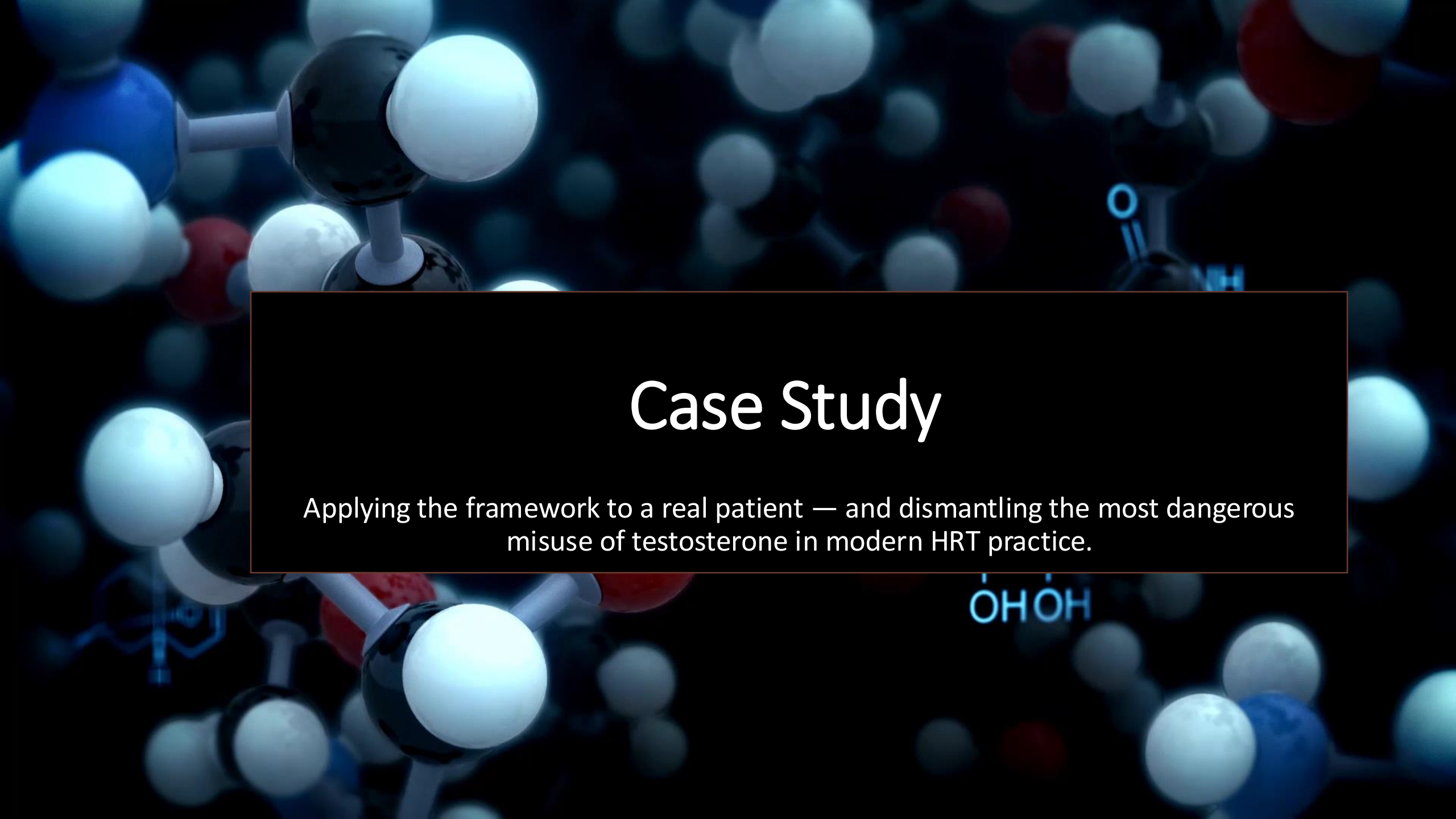
- Estrobolome matters in men too: aromatase converts testosterone to estradiol in adipose tissue, and estrobolome regulates the resulting estrogen burden.
- Andropause typically presents with low testosterone AND elevated estradiol — an aromatization-driven pattern visible on saliva. Treating only with testosterone often worsens this ratio.
- Gut dysbiosis in men drives chronic systemic inflammation that suppresses HPG axis function — causing secondary hypogonadism that does not respond to testosterone therapy alone.
- Visceral adiposity is both a cause and consequence of male hormonal dysfunction — increased aromatization, elevated SHBG-binding inflammation, and gut dysbiosis form a vicious cycle.
- The functional medicine intervention: gut healing + body composition + adaptogenic HPA support precedes testosterone replacement for the best long-term outcomes.
- When TRT is appropriate, monitor with saliva (free hormone) plus HUMAP (estrogen metabolites) — especially watching the estrogen metabolite ratios for elevated 4-OH risk.

PCOS (PMOS) Hormone Patterns

- Classic salivary pattern: elevated testosterone, low progesterone, high estrone:estradiol ratio. The Pg/E2 ratio is often very low, reflecting chronic anovulation Elevated DHEA.
- Insulin resistance is the central metabolic driver — hyperinsulinemia stimulates ovarian androgen production AND suppresses SHBG, increasing free testosterone.
- HUMAP findings: relatively elevated 16-OH and 4-OH metabolites, often with poor methylation capacity. Addresses why PCOS women have elevated long-term cancer risk.
- Gut dysfunction: PCOS women consistently show altered microbiome composition — supporting estrobolome as both contributor and target for intervention.
- HPA dysfunction is nearly universal: elevated daytime cortisol with disrupted slope — often the missed driver of treatment-resistant PCOS.
- Treatment hierarchy: insulin sensitivity → gut → HPA → cycle restoration. Trying to treat hormones first without metabolic foundation produces unreliable results.

Endometriosis Hormone Patterns

- Salivary pattern: estrogen dominance with low to normal progesterone, often with mid-cycle and luteal-phase E2 elevation. The Pg/E2 ratio guides progesterone supplementation.
- HUMAP signature: elevated 16-OH metabolites driving tissue proliferation, often with elevated 4-OH and methylation insufficiency — explaining the inflammatory cascade and pain.
- Estrobolome: endometriosis patients show characteristic gut dysbiosis with elevated β-glucuronidase activity — making calcium-D-glucarate and microbiome support core therapies.
- Inflammation is the bridge: gut-driven systemic inflammation fuels endometrial implant growth. Resolving gut dysfunction often produces dramatic pain reduction within 3-6 months.
- Functional medicine outperforms hormone-only approaches: combined gut + HPA + estrogen + immune intervention produces sustained remission in many patients otherwise headed for surgery.
- Saliva + HUMAP enables serial monitoring of treatment response — track Pg/E2 ratio improvement, 2-OH:16-OH shift, and 2-MeO-E2 production as objective markers of recovery.



Case Study

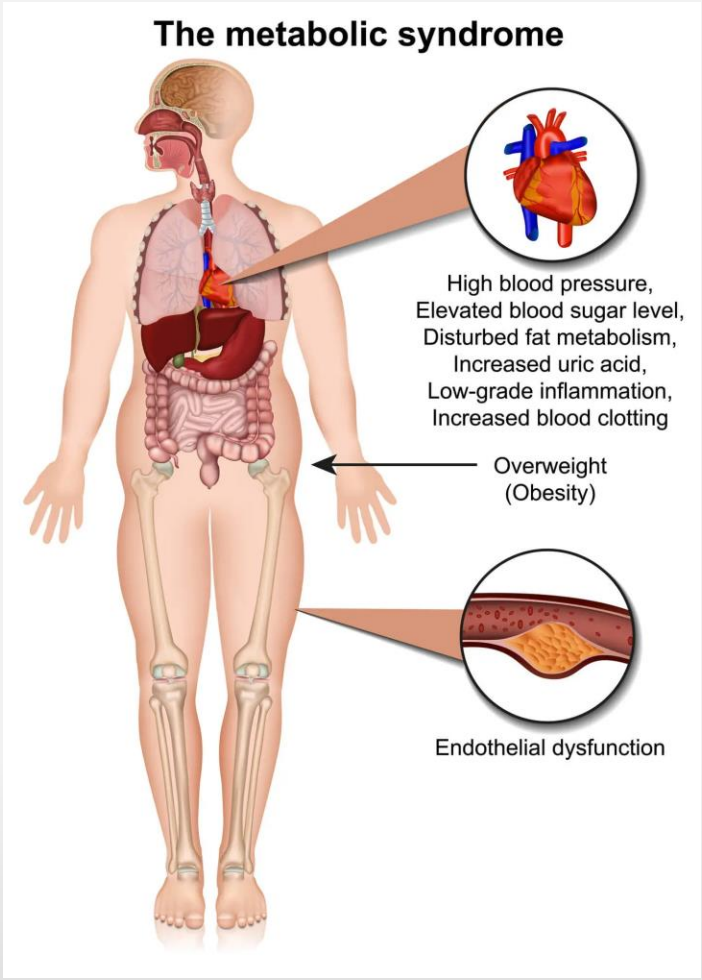
Applying the framework to a real patient — and dismantling the most dangerous misuse of testosterone in modern HRT practice.

Allie — Presentation

- 49-year-old perimenopausal female. LMP Nov–Dec 2025. 5'7", 290 lbs at intake (BMI 45).
- Chief complaints: profound fatigue, overweight, insulin resistance with worsening A1c, joint pain, recurrent illness, low libido, mood lability, sleep disruption.
- 5+ year history of bio-identical HRT — 2mg estradiol daily, 100-300mg cycling progesterone, 20mg/mL testosterone cream, 10mg DHEA.
- Hyperthyroidism (Graves' disease) on methimazole — titrated 15→30→40→20→10→5 mg across 18 months + low-dose naltrexone 4.5mg.
- Family history: heart disease, hypertension, obesity, diabetes, stroke, fatty liver, autoimmune disease.
- Goal: overcome insulin resistance, taper off methimazole, rebalance hormones, address root causes.

Allie — Baseline Metabolic Panel (June 2024)

- Hemoglobin A1c: 6.2% (pre-diabetic, up from 5.4 18 months earlier).
- Fasting insulin: 25 µIU/mL (target <12) — confirming insulin resistance.
- Fasting glucose: ~100-102 mg/dL — borderline pre-diabetic.
- HOMA-IR (calculated): consistent with metabolic syndrome.
- Liver enzymes mildly elevated — developing NAFLD.
- Clinical picture: full-blown metabolic syndrome trending toward Type 2 diabetes.



Allie — Baseline Lipids (June 2024)

Lipids

Test name	In Control	Moderate	High Risk	In Control Range	Moderate Range	High Risk Range	Previous
Cholesterol, Total (mg/dL)	147			≤199	200~240	≥241	
LDL Calculation (mg/dL)	69			≤99	100~129	≥130	
HDL Direct (mg/dL)			40	≥66	45~65	≤44	
Cholesterol/HDL Ratio		3.7		≤3.5	3.6~4.9	≥5.0	
Triglyceride (mg/dL)		191		≤149	150~200	≥201	

- HDL: 40 mg/dL — low; protective cholesterol fraction depleted.
- Triglycerides: 191 mg/dL — moderate range (150-200); contributes to metabolic syndrome lipid pattern.
- Total cholesterol: 147 mg/dL — within range, but ratios matter more than totals.
- Classic metabolic syndrome dyslipidemia: low HDL + elevated triglycerides drives insulin resistance.
- Pattern reflects upstream insulin resistance, not primary lipid disorder.
- Treatment target: address insulin resistance first; lipids normalize as upstream metabolic dysfunction resolves.

Allie — Baseline Thyroid (June 2024)

Thyroid

Test Name	Current	Reference Range	Previous
Free T3 (pg/mL)	3.3	2.0~4.4	
Free T4 (ng/dL)	1.3	0.9~1.7	
TSH (µIU/mL)	0.639	0.111~4.910	
Anti-TPO (IU/mL)	**85 H**	≤34	
Reverse T3* (ng/dL)	22	7~23	
Anti-TG (IU/mL)	23.8	≤115.0	

- Free T3: 3.3 pg/mL (ref 2.0–4.4) — upper half of range.
- Free T4: 1.3 ng/dL (ref 0.9–1.7) — mid-range.
- TSH: 0.639 µIU/mL (ref 0.111–4.91) — suppressed-side of normal.
- Anti-TPO: 85 H (ref ≤34) — autoimmune thyroid activity.
- Reverse T3: 22 ng/dL (ref 7–23) — high-normal, peripheral conversion impairment.
- Anti-TG: 23.8 IU/mL (ref ≤115) — negative.
- Pattern: poorly-controlled Graves' on methimazole 15 mg. TPO antibodies active, TSH suppressed by endogenous overproduction.

Allie — Baseline Hormones (June 2024)

Hormones

Test Name	Current	Reference Range	Previous
Estradiol (pg/mL)	61.1		
FSH (mIU/mL)	4.9		
DHEA-S (µg/dL)	241.0	35.4~256.0	
LH (mIU/mL)	5.9		
SHBG (nmol/L)	59.1	24.6~122.0	
Cortisol (µg/dL)	8.5	A.M.: 6.2-19.4 P.M.: 2.3-11.9	
Testosterone, Total (ng/dL)	35.9	4.5~269.2	
Free Testosterone (ng/dL)	0.44	0.03~2.56	
Progesterone (ng/mL)	1.590		
Pregnenolone (ng/mL)	0.47	0.31~3.80	

Estradiol

Phase	Reference Range
FOLLICULAR	12.4 - 233 pg/mL
OVULATION	41.0 - 398 pg/mL
LUTEAL	22.3 - 341 pg/mL
Postmenopause	<5 - 138 pg/mL
PREGNANCY 1st trimester	154 - 3243 pg/mL
PREGNANCY 2nd trimester	1561 - 21280 pg/mL
PREGNANCY 3rd trimester	8525 - >30000 pg/mL

FSH

Phase	Reference Range
FOLLICULAR	3.5 - 12.5 mIU/mL
OVULATION	4.7 - 21.5 mIU/mL
LUTEAL	1.7 - 7.7 mIU/mL
Postmenopause	25.8-134.8 mIU/mL

LH

Phase	Reference Range
FOLLICULAR	2.4 - 12.6 mIU/mL
OVULATION	14-95.6 mIU/mL
LUTEAL	1 - 11.4 mIU/mL
Postmenopause	7.7-58.5 mIU/mL

Progesterone

Phase	Reference Range
FOLLICULAR	0.057 - 0.893 ng/mL
OVULATION	0.121 - 12.0 ng/mL
LUTEAL	1.83 - 23.9 ng/mL
Postmenopause	0 - 0.126 ng/mL
Pregnant women (trimester)	
1st	11.0 - 44.3 ng/mL
2nd	25.4 - 83.3 ng/mL
3rd	58.7 - 214 ng/mL

- Estradiol: 61.1 pg/mL — on 2 mg estradiol daily.
- Progesterone: 1.590 ng/mL — modest, on 100–300 mg cycling progesterone.
- Total testosterone: 35.9 ng/dL — at upper end of female range despite 'low-dose' cream.
- Free testosterone: 0.44 ng/dL.
- DHEA-S: 241 µg/dL — at the top of range.
- Cortisol AM: 8.5 µg/dL — low-normal morning.
- SHBG: 59.1 nmol/L — mid-range.
- Pregnenolone: 0.47 ng/mL — low. Hormone precursor pool depleted.

Allie — Baseline Inflammation (June 2024)

Rheumatoid Arthritis

Test name	In Control	Moderate	High Risk	In Control Range	Moderate Range	High Risk Range	Previous
hs-CRP (mg/L)			7.8	≤0.9	1.0~3.0	≥3.1	

For Physician: Please fill in the Score column and calculate DAS Score using the following criteria.

Joint Distribution (0-5 points)	Points	Score
1 large joint	0 point	
2-10 large joints	1 point	
1-3 small joints (large joints not counted)	2 points	
4-10 small joints (large joints not counted)	3 points	
>10 joints (at least one small joint)	5 points	
Acute Phase Reactants (0-1 point)		
In Control hs-CRP	0 point	
Abnormal hs-CRP	1 point	
Serology (0-3 points)		
In Control RF and In Control CCP	0 point	
Moderate RF	2 points	
Moderate CCP	2 points	
High Risk RF	3 points	
High Risk CCP	3 points	
Symptom Duration (0-1 point)		
< 6 weeks	0 point	
>= 6 weeks	1 point	
Total Score		

If the sum of all points is greater than or equal to 6, there is likely diagnosis of Rheumatoid Arthritis

- hs-CRP: 7.8 mg/L (ref ≤0.9) — frank systemic inflammation, ~9× upper reference.
- Cardiovascular risk: HIGH (≥3.1).
- Joint complaints with negative RA screen — CRP elevation is broader systemic inflammation from thyroid + IR + dysbiosis + HRT load.
- Combined with autoimmune thyroid (TPO 85 H) and metabolic syndrome — chronic inflammatory state requiring multi-system intervention.

Allie — Sept 2024 Thyroid: Thyrotoxic Breakthrough

Thyroid

Test Name	Current	Previous	Result	Reference
T3 - Triiodothyronine (ng/mL)	2.0		0 0.7 2	0.8-2.0
T4 - Thyroxine (µg/dL)	11.7		0 4.4 9.8	4.5-9.8
Free T3 (pg/mL)	4.5	3.3 (06-10-2024)	0 1.9 4.4	2.0-4.4
Free T4 (ng/dL)	1.7	1.3 (06-10-2024)	0 0.8 1.7	0.9-1.7
TSH (µIU/mL)	0.005	0.639 (06-10-2024)	0 0.11 4.91	0.111-4.91
Anti-TPO (IU/mL)	63	85 (06-10-2024)	0 34	≤34.0
Reverse T3* (ng/dL)	27	22 (06-10-2024)	0 6 23	7.0-23.0
Anti-TG (IU/mL)	17.4	23.8 (06-10-2024)	0 115	≤115.0

Hormones (all)

Test Name	Current	Previous	Result	Reference
SHBG (nmol/L)	78.4	59.1 (06-10-2024)	0 24.5 122	24.6-122.0

Other Markers

Test Name	Current	Previous	Result	Reference
ESR (Erythrocyte Sedimentation Rate)* (mm/hour)	32		0 20	≤20.0

- T4 (total): 11.7 µg/dL H (ref 4.5–9.8) — frankly elevated.
- Free T4: 1.7 ng/dL — at the upper limit.
- T3 (total): 2.0 ng/mL — at ceiling.
- Free T3: 4.5 pg/mL H (ref 2.0–4.4) — over the top.
- TSH: 0.005 µIU/mL — fully suppressed.
- Anti-TPO: 63 H (improved from 85) — autoimmune still active.
- Reverse T3: 27 H — peripheral shunt away from active T3.
- Clinical: methimazole 15 mg has lost the battle. Patient is thyrotoxic — drives cortisol, inflammation, IR. Methimazole doubled to 30 mg Oct 2024.

Allie — Sept 2024 Metabolic Status

Beta Cell Function

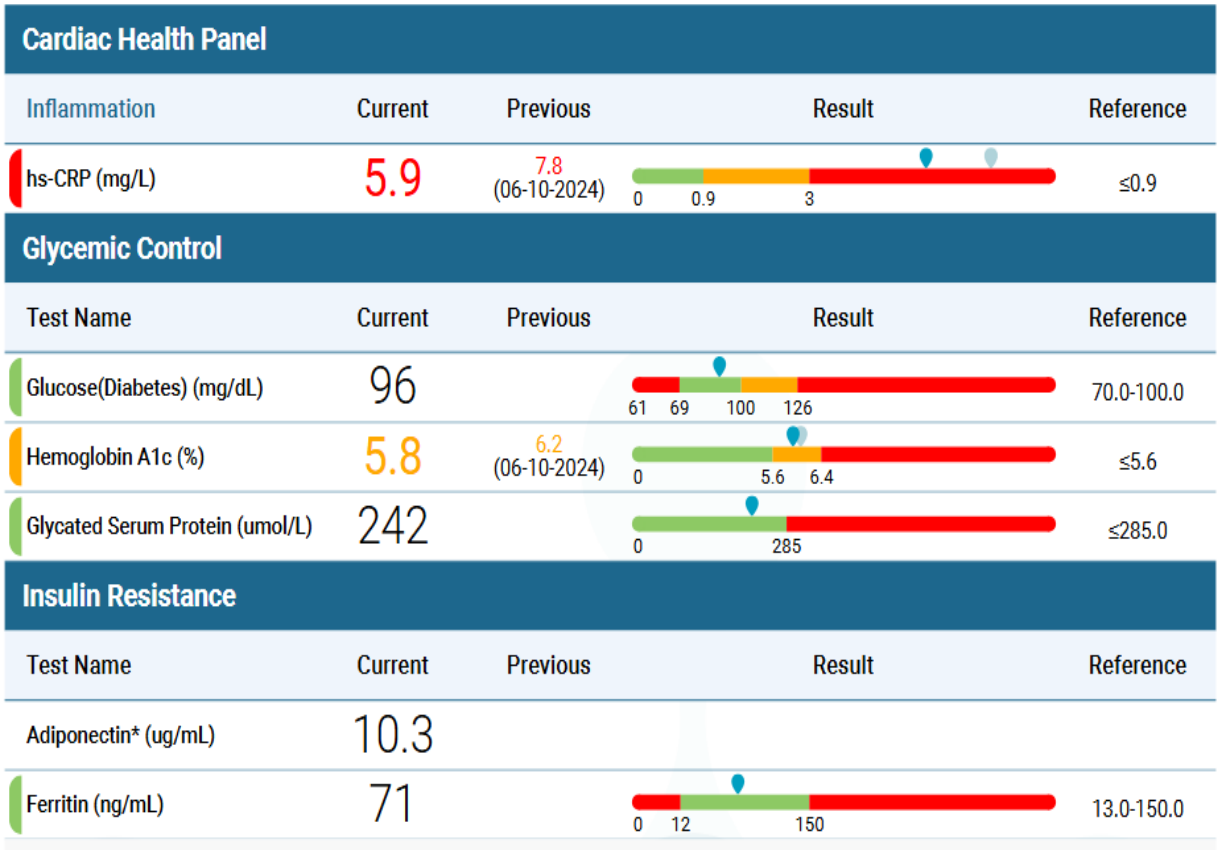
Test Name	Current	Previous	Result	Reference
Insulin (µU/mL)	23.5	25.0 (06-10-2024)	0 2.5 24.9	2.6-24.9

Hepatic Function Panel

Test Name	Current	Previous	Result	Reference
Albumin (g/dL)	4.1	4.4 (06-10-2024)	0 3.4 5.2	3.5-5.2

- Insulin: 23.5 µIU/mL (June: 25.0) — minimal change, insulin resistance persistent.
- Hemoglobin A1c: 5.8% (June: 6.2) — improving even at intake before pharmacology changes.
- Fasting glucose: 96 mg/dL.
- Albumin: 4.1 g/dL (June: 4.4) — slight drop, monitor.
- ESR: 32 mm/hr H (ref ≤20) — systemic inflammation.
- Glycated Serum Protein: 242 µmol/L (ref ≤285) — confirms glycemic trajectory.
- Clinical: dietary + supplement intervention alone has begun moving glycemic markers before any prescription changes.

Allie — Inflammation Markers (Sept 2024)



Cardiac Health Panel

Inflammation	Current	Previous	Result	Reference
hs-CRP (mg/L)	5.9	7.8 (06-10-2024)	0 0.9 3	≤0.9

Glycemic Control

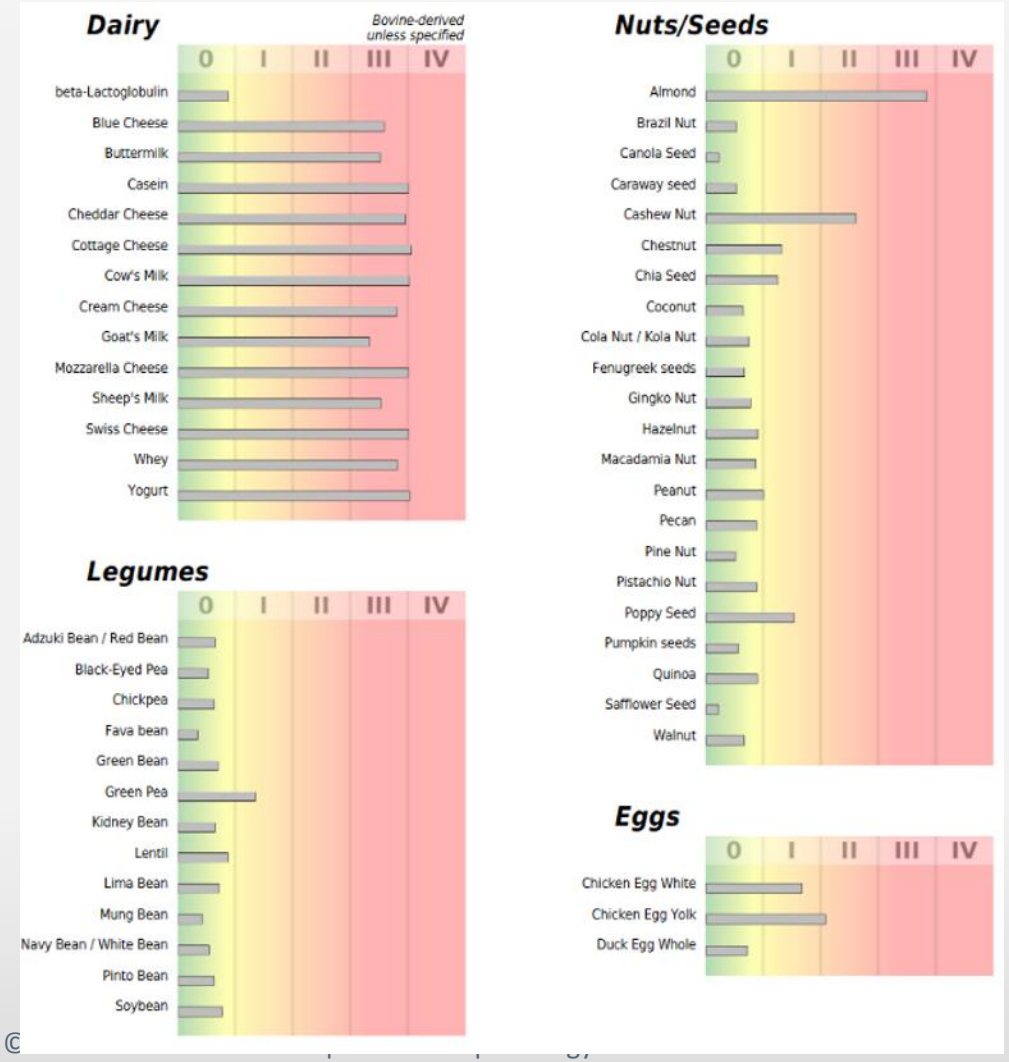
Test Name	Current	Previous	Result	Reference
Glucose(Diabetes) (mg/dL)	96		61 69 100 126	70.0-100.0
Hemoglobin A1c (%)	5.8	6.2 (06-10-2024)	0 5.6 6.4	≤5.6
Glycated Serum Protein (umol/L)	242		0 285	≤285.0

Insulin Resistance

Test Name	Current	Previous	Result	Reference
Adiponectin* (ug/mL)	10.3			
Ferritin (ng/mL)	71		0 12 150	13.0-150.0

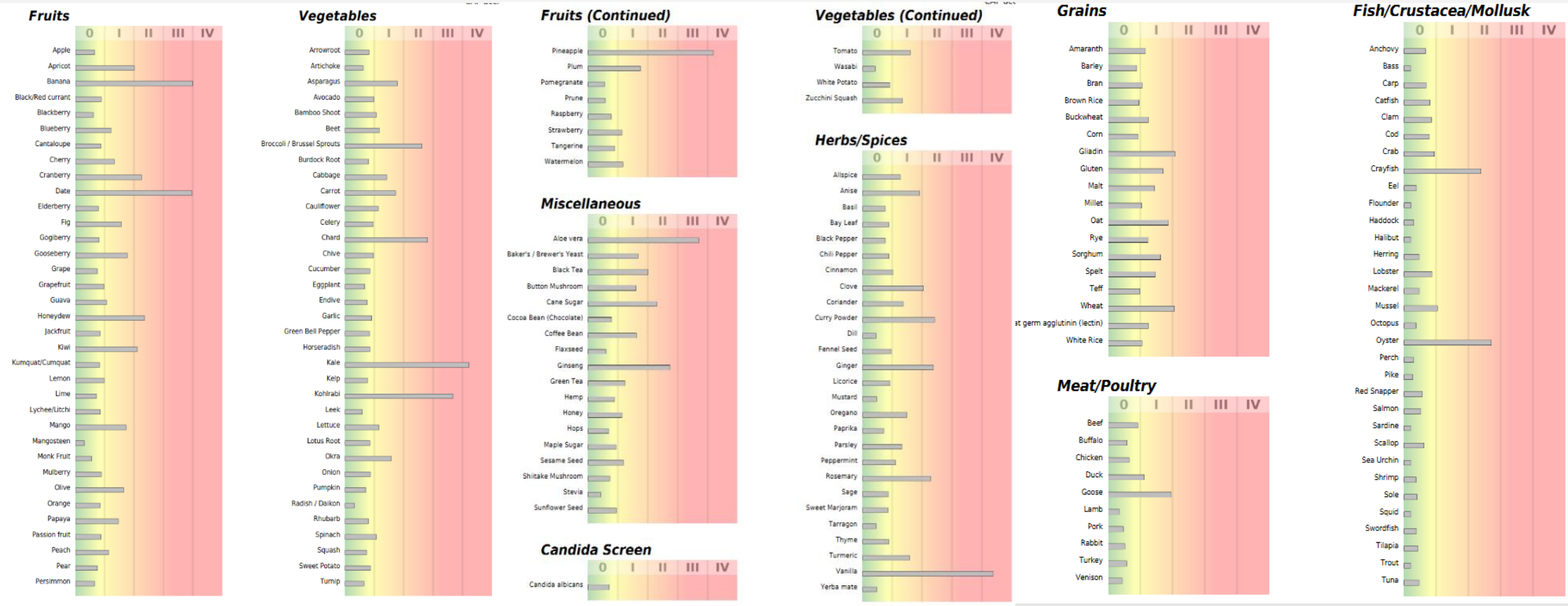
- hs-CRP: 5.9 mg/L (HIGH, ref ≤0.9) — improved from 7.8 in June 2024 but still 6x reference.
- Hemoglobin A1c: 5.8% — improved from 6.2% baseline, still high-normal pre-diabetic range.
- Glucose: 96 mg/dL (fasting) — borderline elevated.
- Homocysteine: elevated (~13-14) — methylation insufficiency confirmed.
- Inflammation is the upstream driver of insulin receptor dysfunction, weight gain, and HPA disruption.
- Source: gut dysbiosis, food sensitivities, exogenous hormone burden, mitochondrial dysfunction.
- Despite directional improvement, CRP and A1c remain above target — must reduce before HRT recalibration.

Allie — IgG Food Sensitivity (Sept 2024)

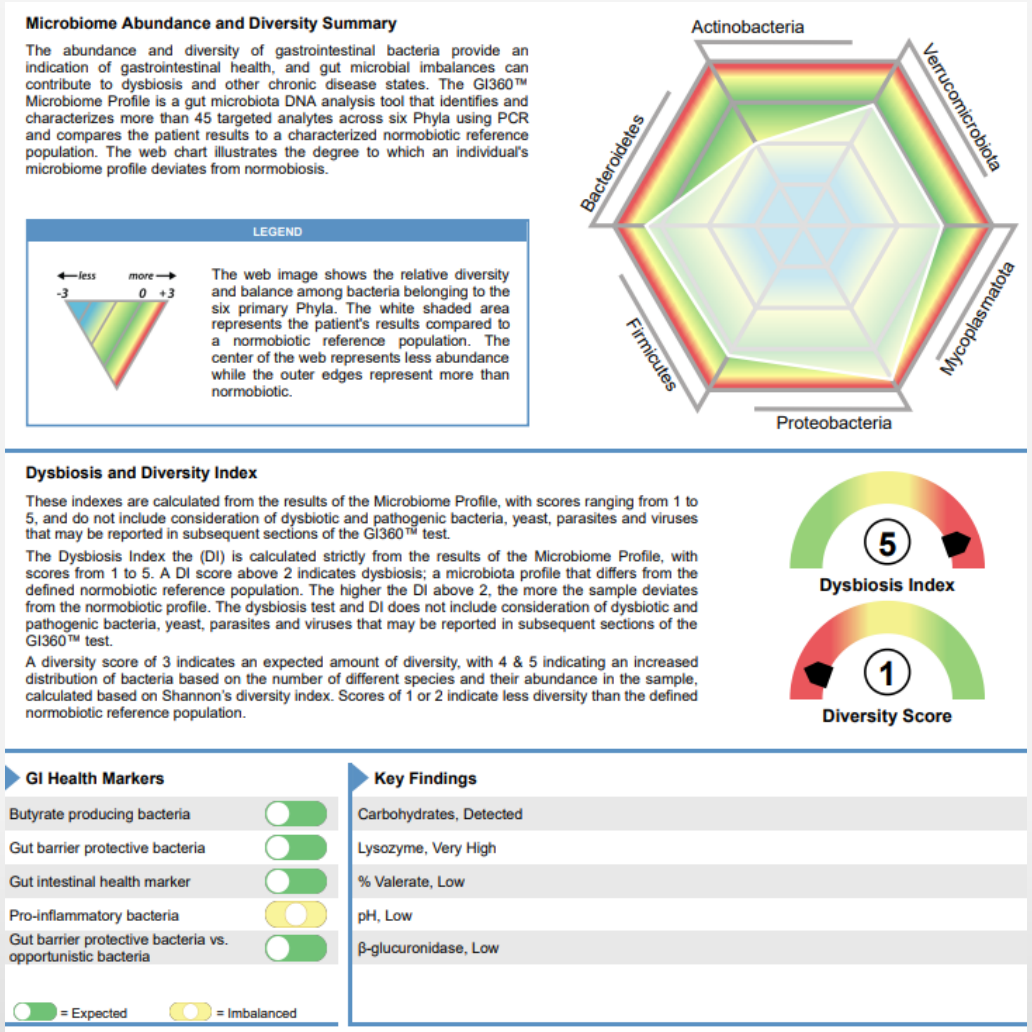


- US BioTek 240-food IgG panel — measures delayed (Type III) hypersensitivity reactions.
- High reactivity: dairy (all forms), pineapple, almonds, cashews, select vegetables.
- IgG food reactions drive chronic systemic inflammation, leaky gut, and autoimmune flares.
- Mechanism: undigested protein antigens cross compromised intestinal barrier → immune complex formation → tissue inflammation.
- Clinical decision: full elimination of all 1+ reactive foods for minimum 90 days.
- Pair with gut barrier repair to enable safe future reintroduction trials.

Allie — IgG Food Sensitivity (Sept 2024)



Allie — GI 360 Microbiome Overview



Microbiome Abundance and Diversity Summary

The abundance and diversity of gastrointestinal bacteria provide an indication of gastrointestinal health, and gut microbial imbalances can contribute to dysbiosis and other chronic disease states. The GI360™ Microbiome Profile is a gut microbiota DNA analysis tool that identifies and characterizes more than 45 targeted analytes across six Phyla using PCR and compares the patient results to a characterized normobiotic reference population. The web chart illustrates the degree to which an individual's microbiome profile deviates from normobiosis.

LEGEND

less ← -3 0 +3 → more

The web image shows the relative diversity and balance among bacteria belonging to the six primary Phyla. The white shaded area represents the patient's results compared to a normobiotic reference population. The center of the web represents less abundance while the outer edges represent more than normobiotic.

Dysbiosis and Diversity Index

These indexes are calculated from the results of the Microbiome Profile, with scores ranging from 1 to 5, and do not include consideration of dysbiotic and pathogenic bacteria, yeast, parasites and viruses that may be reported in subsequent sections of the GI360™ test.

The Dysbiosis Index the (DI) is calculated strictly from the results of the Microbiome Profile, with scores from 1 to 5. A DI score above 2 indicates dysbiosis; a microbiota profile that differs from the defined normobiotic reference population. The higher the DI above 2, the more the sample deviates from the normobiotic profile. The dysbiosis test and DI does not include consideration of dysbiotic and pathogenic bacteria, yeast, parasites and viruses that may be reported in subsequent sections of the GI360™ test.

A diversity score of 3 indicates an expected amount of diversity, with 4 & 5 indicating an increased distribution of bacteria based on the number of different species and their abundance in the sample, calculated based on Shannon's diversity index. Scores of 1 or 2 indicate less diversity than the defined normobiotic reference population.

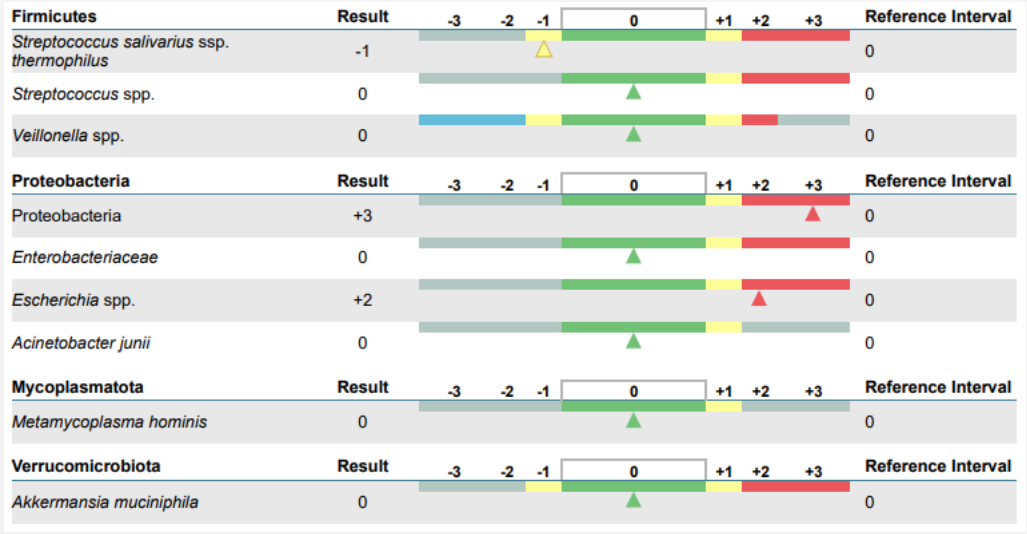
GI Health Markers	
Butyrate producing bacteria	
Gut barrier protective bacteria	
Gut intestinal health marker	
Pro-inflammatory bacteria	
Gut barrier protective bacteria vs. opportunistic bacteria	

= Expected = Imbalanced

Key Findings
Carbohydrates, Detected
Lysozyme, Very High
% Valerate, Low
pH, Low
β-glucuronidase, Low

- Dysbiosis Index: 5/5 (maximum dysbiosis). Bacterial Diversity Score: 1/5 (critically low).
- Carbohydrate malabsorption detected — substrate for opportunistic bacterial fermentation.
- Butyrate-producing bacteria: imbalanced (low) — short-chain fatty acid production compromised.
- Gut barrier protective bacteria: low — protective Akkermansia and Faecalibacterium depleted.
- Anti-inflammatory bacteria: low. Pro-inflammatory bacteria: imbalanced (elevated).
- Pattern: classic Western-diet dysbiosis with autoimmune-promoting microbiome composition.

Allie — Estrobolome & LPS-Producing Bacteria



Firmicutes	Result	-3 -2 -1 0 +1 +2 +3	Reference Interval
Streptococcus salivarius ssp. *thermophilus*	-1		0
Streptococcus spp.	0		0
Veillonella spp.	0		0
Proteobacteria	**Result**	**-3 -2 -1 0 +1 +2 +3**	**Reference Interval**
Proteobacteria	+3		0
Enterobacteriaceae	0		0
Escherichia spp.	+2		0
Acinetobacter junii	0		0
Mycoplasmatota	**Result**	**-3 -2 -1 0 +1 +2 +3**	**Reference Interval**
Metamycoplasma hominis	0		0
Verrucomicrobiota	**Result**	**-3 -2 -1 0 +1 +2 +3**	**Reference Interval**
Akkermansia muciniphila	0		0

- Proteobacteria: +3 (significantly elevated) — the LPS-producing inflammatory phylum.
- Escherichia spp.: +2 — gram-negative bacteria release lipopolysaccharide (LPS), the canonical endotoxin.
- LPS crosses leaky gut barriers → systemic inflammation → metabolic endotoxemia → insulin resistance, weight gain, HPA suppression.
- Akkermansia muciniphila: 0 — the mucin-protective species is GONE. Mucus layer integrity collapsed.
- Estrobolome dysfunction: β-glucuronidase-producing bacteria recycle estrogens back into circulation, driving estrogen excess.
- Mechanism: dysbiosis → LPS leak → adrenal HPA suppression → cortisol dysregulation → sex hormone metabolism disruption → vicious cycle.

Allie — Streptococcus Overgrowth & Oral-Gut Translocation

Pathogenic Bacteria	Result	NG 1+ 2+ 3+ 4+	Reference Interval
Aeromonas spp.	NG		No Growth
Edwardsiella tarda	NG		No Growth
Plesiomonas shigelloides	NG		No Growth
Salmonella group	NG		No Growth
Shigella group	NG		No Growth
Vibrio cholerae	NG		No Growth
Vibrio spp.	NG		No Growth
Yersinia spp.	NG		No Growth
Imbalance Bacteria	**Result**	**NG 1+ 2+ 3+ 4+**	**Reference Interval**
Streptococcus parasanguinis	2+		No Growth
Streptococcus salivarius/vestibularis	1+		No Growth
Yeast	**Result**	**NG 1+ 2+ 3+ 4+**	**Reference Interval**
No yeast isolated	NG		

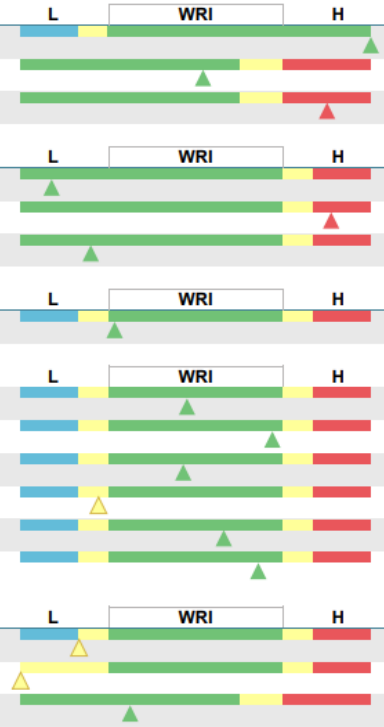
Nematodes - Roundworms	Result
Capillaria hepatica	Not Detected
Capillaria philippinensis	Not Detected
Enterobius vermicularis	Not Detected
Hookworm	Not Detected
Strongyloides stercoralis	Not Detected
Trichuris trichiura	Not Detected

Other Markers	Result	Reference Interval
Yeast	Few	Not Detected – Rare
RBC	Not Detected	Not Detected – Rare
WBC	Not Detected	Not Detected – Rare
Muscle fibers	Not Detected	Not Detected – Rare
Vegetable fibers	Rare	Not Detected – Few
Charcot-Leyden Crystals	Not Detected	Not Detected
Pollen	Not Detected	Not Detected
Macroscopic Appearance	**Result**	**Reference Interval**
Color	Brown	Brown
Consistency	Soft	Soft
Mucus	Negative	Negative

- Streptococcus parasanguinis: 2+ overgrowth (should be 'no growth' in stool culture).
- Streptococcus salivarius/vestibularis: 1+ overgrowth — oral bacteria translocating to gut.
- Stool culture negative for yeast isolation — but microscopy and organic acids will be the better screen.
  - Positive finding on microscopy and organic acids
- Oral-gut bacterial translocation: a marker of compromised upper-GI defenses and weakened gastric acid.
- Streptococcal overgrowth in stool correlates with: bile acid dysregulation, B12 deficiency, increased intestinal permeability.
- Treatment target: antimicrobial herbs (berberine, oregano oil), restore gastric acid, support upper-GI barrier with zinc-carnosine and DGL.

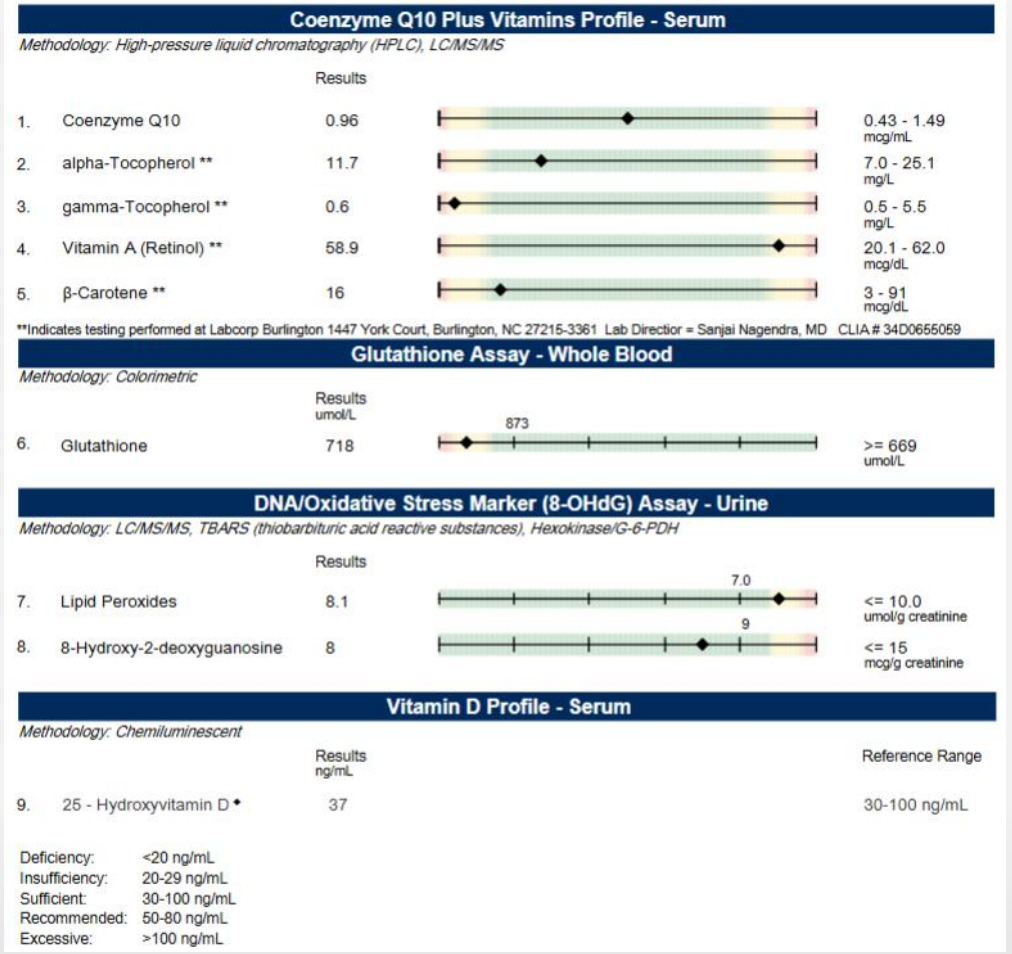
Allie — Lysozyme Elevation & Leaky Gut

Digestion / Absorption	Result	Unit	Reference Interval
Elastase	>500	µg/g	> 200
Fat Stain	Few		None – Moderate
Carbohydrates†	Positive		Negative
Inflammation	**Result**	**Unit**	**Reference Interval**
Lactoferrin	1.3	µg/mL	< 7.3
Lysozyme*	696	ng/mL	≤ 500
Calprotectin	25	µg/g	< 80
Immunology	**Result**	**Unit**	**Reference Interval**
Secretory IgA*	38.9	mg/dL	30 – 275
Short Chain Fatty Acids	**Result**	**Unit**	**Reference Interval**
% Acetate‡	59	%	50 – 72
% Propionate‡	24	%	11 – 25
% Butyrate‡	17	%	11 – 32
% Valerate‡	0.7	%	0.8 – 5.0
Butyrate‡	2.3	mg/mL	0.8 – 4.0
Total SCFA's‡	14	mg/mL	5.0 – 16.0
Intestinal Health Markers	**Result**	**Unit**	**Reference Interval**
pH	5.5		5.8 – 7.0
β-glucuronidase*	<2600	U/h*g	4000 – 9400
Occult Blood	Negative		Negative



- Lysozyme: 696 ng/mL (reference ≤500) — significantly elevated.
- Lysozyme is released from activated neutrophils — direct marker of GI inflammation.
- Elevated lysozyme confirms LEAKY GUT (increased intestinal permeability) — antigens crossing into lamina propria activate immune cells.
- Secretory IgA: 38.9 mg/dL (reference 30-275) — bottom of range. Mucosal immunity collapsed.
- β-glucuronidase: <2600 (LOW) — paradoxically, depleted by inflammation and dysbiosis.
- The autoimmune triad activated: genetic susceptibility + leaky gut + environmental triggers = Graves' disease + metabolic syndrome.

Allie — ION Nutritional Profile (Oct 2024)



Coenzyme Q10 Plus Vitamins Profile - Serum

Methodology: High-pressure liquid chromatography (HPLC), LC/MS/MS

		Results		
1.	Coenzyme Q10	0.96		0.43 - 1.49 mcg/mL
2.	alpha-Tocopherol **	11.7		7.0 - 25.1 mg/L
3.	gamma-Tocopherol **	0.6		0.5 - 5.5 mg/L
4.	Vitamin A (Retinol) **	58.9		20.1 - 62.0 mcg/dL
5.	β-Carotene **	16		3 - 91 mcg/dL

**Indicates testing performed at Labcorp Burlington 1447 York Court, Burlington, NC 27215-3361 Lab Director = Sanjai Nagendra, MD CLIA # 34D0655059

Glutathione Assay - Whole Blood

Methodology: Colorimetric

		Results umol/L	873	
6.	Glutathione	718		>= 669 umol/L

DNA/Oxidative Stress Marker (8-OHdG) Assay - Urine

Methodology: LC/MS/MS, TBARS (thiobarbituric acid reactive substances), Hexokinase/G-6-PDH

		Results	7.0 / 9	
7.	Lipid Peroxides	8.1		<= 10.0 umol/g creatinine
8.	8-Hydroxy-2-deoxyguanosine	8		<= 15 mcg/g creatinine

Vitamin D Profile - Serum

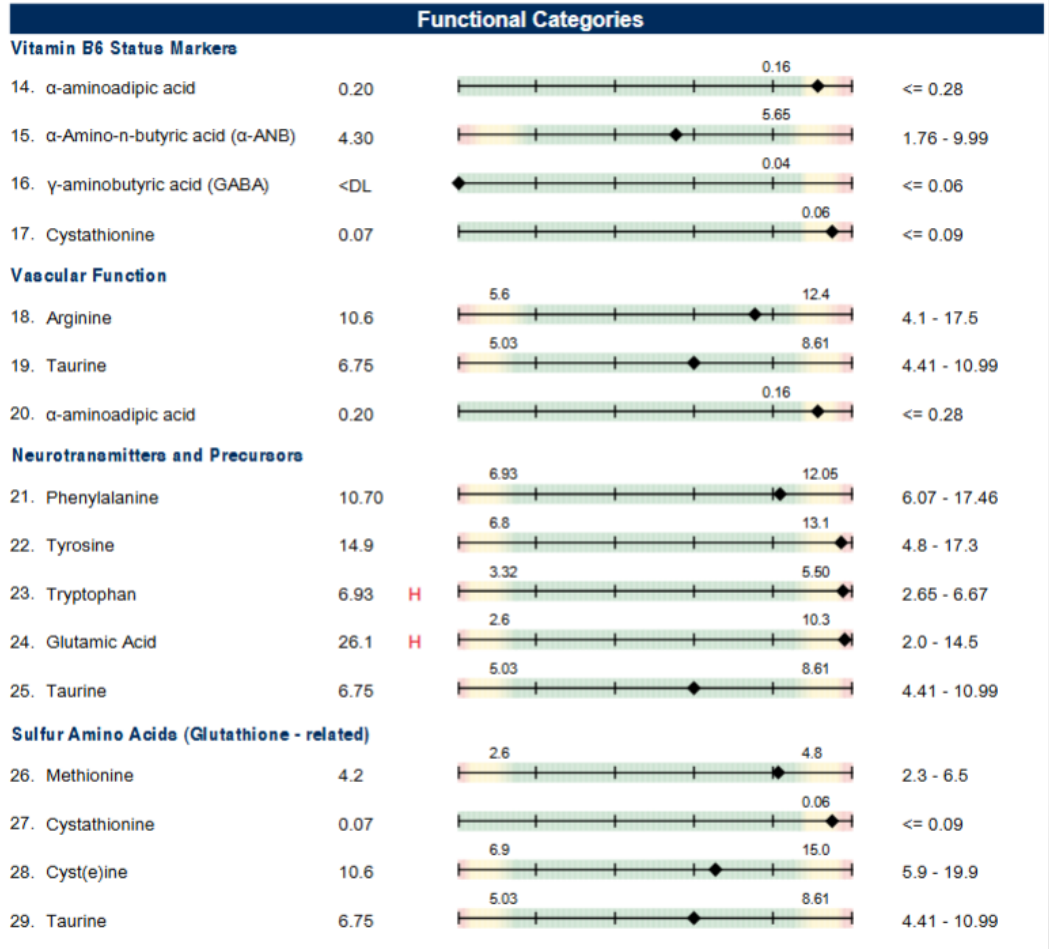
Methodology: Chemiluminescent

		Results ng/mL	Reference Range
9.	25 - Hydroxyvitamin D ♦	37	30-100 ng/mL

Deficiency:	<20 ng/mL
Insufficiency:	20-29 ng/mL
Sufficient:	30-100 ng/mL
Recommended:	50-80 ng/mL
Excessive:	>100 ng/mL

- Comprehensive functional medicine panel: organic acids, amino acids, oxidative stress, fatty acids, vitamins.
- Glutathione: 718 (very low) — antioxidant defense system depleted.
- 8-hydroxy-2'-deoxyguanosine (8-OHdG): elevated — DNA-level oxidative damage.
- Lipid peroxides: high — cell membrane damage from oxidative stress.
- Multiple B-vitamin functional markers low — methylation cofactor depletion.
- Mitochondrial markers (citrate, malate, alpha-ketoglutarate) elevated — TCA cycle dysfunction.

Allie — ION Amino Acids & Methylation Cofactors



Functional Categories				
Vitamin B6 Status Markers				
14. α-aminoadipic acid	0.20		0.16	<= 0.28
15. α-Amino-n-butyric acid (α-ANB)	4.30		5.65	1.76 - 9.99
16. γ-aminobutyric acid (GABA)	<DL		0.04	<= 0.06
17. Cystathionine	0.07		0.06	<= 0.09
Vascular Function				
18. Arginine	10.6		5.6 – 12.4	4.1 - 17.5
19. Taurine	6.75		5.03 – 8.61	4.41 - 10.99
20. α-aminoadipic acid	0.20		0.16	<= 0.28
Neurotransmitters and Precursors				
21. Phenylalanine	10.70		6.93 – 12.05	6.07 - 17.46
22. Tyrosine	14.9		6.8 – 13.1	4.8 - 17.3
23. Tryptophan	6.93	H	3.32 – 5.50	2.65 - 6.67
24. Glutamic Acid	26.1	H	2.6 – 10.3	2.0 - 14.5
25. Taurine	6.75		5.03 – 8.61	4.41 - 10.99
Sulfur Amino Acids (Glutathione - related)				
26. Methionine	4.2		2.6 – 4.8	2.3 - 6.5
27. Cystathionine	0.07		0.06	<= 0.09
28. Cyst(e)ine	10.6		6.9 – 15.0	5.9 - 19.9
29. Taurine	6.75		5.03 – 8.61	4.41 - 10.99

- Tryptophan: elevated — but glycine and serine low, indicating B6 deficiency blocking conversion.
- Cystathionine: elevated — confirms B6 insufficiency in transsulfuration pathway.
- Glutamic acid:glutamine ratio: elevated — neuro-inflammation marker.
- Serotonin metabolite: elevated — poor tryptophan downstream conversion.
- Citramalate: elevated — Candida marker, confirms GI 360 finding.
- Formamidoglutamate: elevated — folate insufficiency.

Allie — Spectracell Micronutrient (Oct 2024)

- Intracellular micronutrient analysis — measures functional, not just serum, status.
- Vitamin A: deficient; Vitamin E: low; Vitamin D: needs higher dosing.
- B5 (pantothenate): low — adrenal cortex synthesis cofactor.
- Glutamine: very low — gut barrier repair amino acid depleted.
- Choline: low — methylation and liver detox impairment.
- Manganese, calcium utilization, total antioxidant function: borderline dysfunctional.

Allie — Methylation Profile (Oct 2024)

Methylation Profile; plasma

PRIMARY & INTERMEDIATE METABOLITES

	RESULT/UNIT	REFERENCE INTERVAL
Methionine	2.4 µmol/dL	1.6 - 3.6
Cysteine	34 µmol/dL	20 - 38
S-adenosylmethionine (SAM)	120 nmol/L	86 - 145
S-adenosylhomocysteine (SAH)	18.9 nmol/L	10 - 22
Homocysteine	7.4 µmol/L	< 11
Cystathionine	0.03 µmol/dL	< 0.05

PERCENTILE: 2.5th, 16th, 50th, 84th, 97.5th; 68th, 95th

METHYLATION INDEX

	RESULT	REFERENCE INTERVAL
SAM : SAH	6.3	> 4

PERCENTILE: 68th, 95th

- S-adenosylmethionine (SAMe): 120 — universal methyl donor.
- Homocysteine: 7.4 — better than blood reading, but transsulfuration capacity limited.
- Cysteine: 34 — substrate for glutathione synthesis.
- Combined picture: methylation working but inefficient, transsulfuration restricted.
- Critical because COMT-dependent estrogen detoxification requires methylation.
- Treatment: methylated B12, methylfolate, B6 (P5P), TMG, magnesium, choline.

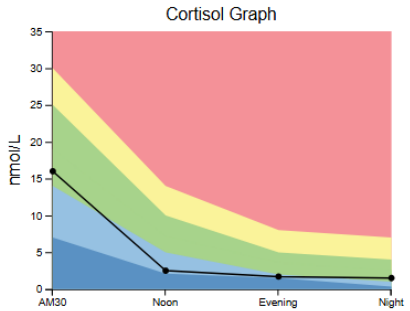
Allie — Salivary Hormones (Dec 2024)

Analyte	Result	Unit	L	WRI	H	Reference Interval	Supplementation Range**
Estradiol (E2)	3.9	pg/mL		◆		0.6 – 4.5	1.0 – 6.0
Progesterone (Pg)	237	pg/mL		◆		127 – 446	400 – 4000
Pg/E2 Ratio†	60.8		↓			≥ 200	≥ 200
Testosterone	235	pg/mL			↑	6 – 49	25 – 60
DHEA*	144	pg/mL		◆		106 – 300	

- DD reference ranges reflect population norms; functional medicine targets for a perimenopausal female are tighter. Green-diamond WRI markers sit within DD reference but well above optimal functional targets — that gap is the entire teaching point.
- Testosterone: 235 (target 6-10 for a 48 y/o female) — 23-fold elevation.
- Estradiol: 3.9 (target ~1.1) — 3.5x elevation from exogenous cream + adipose stores.
- Progesterone: 237 — significantly elevated from oral micronized progesterone.
- DHEA: 144 — suboptimal for absorption pattern.
- Salivary measurement captures the free, biologically active fraction — the actual tissue burden.
- Serum testing on this same patient would have read 'normal' — a critical monitoring failure.

Allie — Salivary Cortisol Curve (Dec 2024)

Analyte	Result	Unit	L	WRI	H	Optimal Range	Reference Interval
Cortisol AM30	16	nmol/L		◆		14.0 – 25.0	7.0 – 30.0
Cortisol Noon	2.5	nmol/L	◆			5.0 – 10.0	2.1 – 14.0
Cortisol Evening	1.7	nmol/L	◆			2.0 – 5.0	1.5 – 8.0
Cortisol Night	1.5	nmol/L		◆		1.0 – 4.0	0.33 – 7.0
DHEA*	144	pg/mL		◆			106 – 300



Adrenal Phase: 1

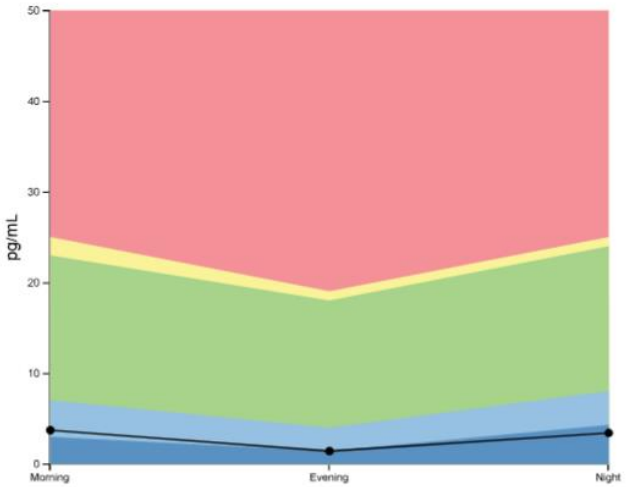
Hormone Comments

- AM cortisol level appears adequate, although the suboptimal diurnal cortisol pattern is suggestive of early (Phase 1) HPA axis (adrenal gland) dysfunction.

- Morning cortisol: 16 — within optimal 14-25 range.
- Noon: 2.5 — below optimal (5-10), significant drop from morning peak.
- Evening: 1.7 — below optimal (2-5), flat slope continues.
- Bedtime: 1.5 — within reference, but the entire curve is flat with low total output rather than a healthy diurnal slope.
- Pattern: classic Stage 2-3 HPA dysfunction — flattened diurnal slope, low total output across afternoon/evening.
- Note: DD's algorithmic comment reads 'Phase 1' — functional medicine staging applies tighter criteria, placing this at Stage 2-3.
- Blunted CAR + flat slope predicts treatment-resistant depression and metabolic decline.

Allie — Salivary Melatonin (Dec 2024)

Analyte	Result	Unit	L	WRI	H	Reference Interval
Melatonin Morning*	3.7	pg/mL				3.0 – 25
Melatonin Evening*	<1.4	pg/mL				1.4 – 19
Melatonin Night*	3.4	pg/mL				4.3 – 25



Melatonin Comments

Melatonin levels follow a diurnal rhythm in response to the light-dark cycle, with highest levels produced at night during times of darkness. Melatonin and cortisol levels have an inverse relationship. Whereas optimal cortisol levels are highest 30 minutes after waking with a gradual decline throughout the waking day and continued decline to lower night-time levels, melatonin levels are lower during the daytime and gradually rise later in the evening when light is dim, beginning approximately 2 hours before bed time. Disruptions in expected melatonin and/or cortisol pattern(s) may result in sleep disturbances.
Exposure to light may suppress melatonin levels. Nuts, fruits, fruit juice, wine, rice, and coffee are known to contain melatonin, and may increase levels. Additionally, anti-anxiety and anti-depressant medications categorized as SSRI's and SNRI's (selective serotonin and/or norepinephrine reuptake inhibitors) may increase melatonin, as can commonly used herbs such as St. John's Wort and Feverfew.

- Pineal melatonin: evening (<1.4) and night (3.4) BELOW reference; morning (3.7) at the bottom of reference range.
- Pattern: suppressed nocturnal melatonin production — the oncoprotective signal is absent when it matters most.
- Loss of nocturnal melatonin disrupts: sleep architecture, free radical scavenging, oncoprotective signaling.
- Melatonin is a quantitative breast cancer risk biomarker — IARC classifies shift work (low melatonin) as a probable carcinogen.
- Mechanism: chronically elevated evening cortisol suppresses pineal melatonin secretion.
- Compounded by: blue light, late screens, late caffeine, anticipatory stress.
- Treatment target: melatonin restoration via SR formulation + circadian hygiene + cortisol normalization.

Allie — Clinical Diagnoses at Workup

- Metabolic syndrome with progression toward Type 2 diabetes mellitus (E11.65)
- Insulin resistance with hyperinsulinemia (E88.81)
- Autoimmune thyroid disease — Graves' disease (E05.0) — on methimazole
- Polycystic ovarian syndrome-like presentation (E28.2) — hyperandrogenism, anovulation
- Adrenal insufficiency / HPA dysfunction (E27.40) — Stage 2-3 burnout pattern
- Leaky gut syndrome with severe dysbiosis (K63.89) and SIgA deficiency
- Hormone receptor resistance from supraphysiologic HRT (Z79.890)
- Systemic inflammation (CRP 7.8); nutrient deficiencies; methylation dysfunction; mitochondrial dysfunction

Allie — Treatment Regimen: Foundational Phase

- Anti-inflammatory diet: eliminate gluten, dairy, sugar, beef, pork, IgG-reactive foods. 90-day minimum.
- Gut 4R protocol — Saccharomyces boulardii, broad-spectrum probiotic, L-glutamine (gut barrier repair), butyric acid + calmag (enterocyte fuel).
- Methylation cofactors: methyl-B12, methylfolate, B6 (P5P), trimethylglycine (TMG), magnesium glycinate, choline.
- Antioxidant support: liposomal glutathione, N-acetylcysteine 1200-1800 mg/day, alpha-lipoic acid 600 mg BID, CoQ10 200 mg.
- Mitochondrial support: acetyl-L-carnitine, B-complex, ribose, CoQ10, magnesium.
- Begin HRT taper — reduce testosterone first, gradual estradiol/progesterone wind-down over 4-6 months.

Allie — Treatment Regimen: Adrenal & Sleep Support

- Sleep Tides (Biotics Research) — a 3-phase support formula for adrenal/sleep/cortisol:
- Phase 1 (early evening): adaptogens (ashwagandha, rhodiola, holy basil) to calm HPA axis activation before bed.
- Phase 2 (bedtime): GABA, L-theanine, passionflower, magnolia — parasympathetic activation, sleep induction.
- Phase 3 (overnight): low-dose sustained melatonin + glycine — maintains sleep depth, supports neurogenesis.
- Mechanism: addresses elevated nighttime cortisol AND low melatonin simultaneously — the dual failure pattern.
- Adjuncts: phosphatidylserine 300 mg at bedtime (cortisol blunting), DHEA pulse-dosing AM, pregnenolone 25-50 mg AM for upstream substrate.

Allie — Estrogen Detox & Metabolic Support

- Estrogen metabolism: DIM (diindolylmethane) 100-200 mg/day, calcium-D-glucarate (β-glucuronidase inhibition), sulforaphane (NRF2 activation).
- Phase II conjugation: methylated B-complex (already covered), sulfation cofactors (MSM, taurine), glycine.
- Insulin sensitization: berberine 500 mg TID with meals, chromium picolinate, alpha-lipoic acid (dual antioxidant + insulin role).
- Liver support: NAC, milk thistle (silymarin), choline, betaine — for NAFLD reversal.
- Cardiovascular protection: omega-3 fatty acids (2-4 g EPA+DHA), grape seed extract, hawthorn, taurine.
- Thyroid support adjunctive to methimazole: low-dose iodine, selenium, zinc, L-tyrosine.

Thyroid Pharmacology — Why Porcine NP Often Isn't Enough

- Most thyroid patients in functional medicine arrive on porcine NP (Armour, NP Thyroid, Nature-Throid).
- Porcine thyroid delivers T4 and T3 in a fixed 4:1 ratio (~38 mcg T4 + 9 mcg T3 per grain).
- Human physiology runs ~12:1 T4:T3 — the body converts T4→T3 on demand under tissue-level regulation.
- Patients on porcine-only often get chronically overdosed on T3: palpitations, anxiety, insomnia, paradoxical weight gain via cortisol/insulin dysregulation, accelerated bone turnover, elevated AFib risk.
- My approach: combine low-dose levothyroxine + small dose porcine NP so the delivered T4:T3 ratio approaches the human 12:1 while still feeding active T3 conversion through the NP. Straight levo misses poor converters; straight porcine T3-overdoses; the blend covers both.
- Allie's case sits in the inverse mirror — Graves' overproduction on methimazole, not replacement therapy. The same ratio principle governs every dosing decision once a patient overshoots the other way, as she did in May 2025.

Allie — May 2025 Follow-up: Inflammation

LipoProtein Markers

Test Name	In Control	Moderate	High Risk	In Control Range	Moderate Range	High Risk Range	Previous
Lp(a)	<7			≤29		≥30	

Inflammation

Test Name	In Control	Moderate	High Risk	In Control Range	Moderate Range	High Risk Range	Previous
Homocysteine		10		≤9	10-14	≥15	
hs-CRP			5.2	≤0.9	1-3	≥3.1	5.9
ox-LDL*	56.5			≤99.1		≥99.2	

- Lp(a): <7 (in control) — cardiovascular risk marker stable.
- Homocysteine: 10 (moderate range, target <9) — methylation support beginning to work.
- hs-CRP: 5.2 (DOWN from 7.8 at baseline) — measurable but incomplete inflammatory reduction.
- ox-LDL: 56.5 (in control) — oxidized LDL within range.
- Pattern: directional improvement but inflammation still elevated. Treatment intensity needs to continue.
- Clinical decision: increase antioxidant load, NAC, omega-3, address persistent gut/oral dysbiosis.

Allie — May 2025 Follow-up: Glycemic Control

Glycemic Control

Test Name	In Control	Moderate	High Risk	In Control Range	Moderate Range	High Risk Range	Previous
Hemoglobin A1c	5.4			≤5.6	5.7-6.4	≥6.5	5.8

Beta Cell Function

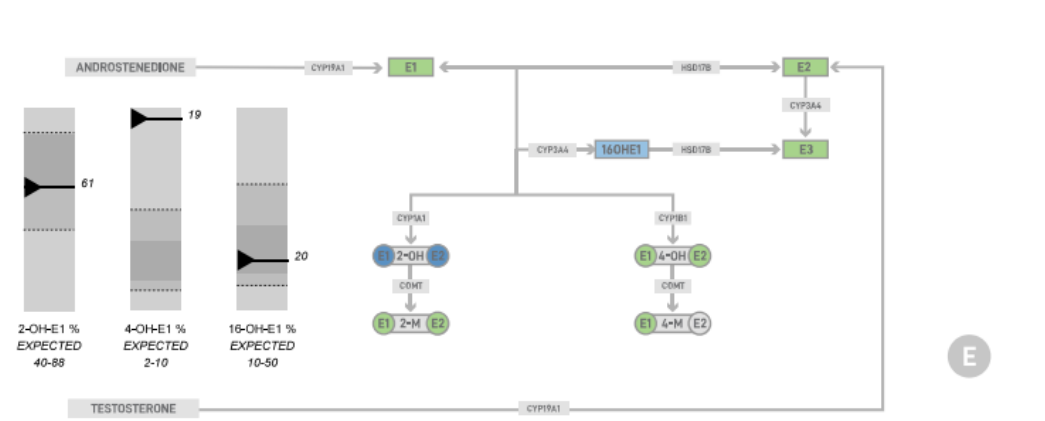
Test Name	In Control	Moderate	High Risk	In Control Range	Moderate Range	High Risk Range	Previous
Insulin			25.4	2.6-24.9		≤2.5 & ≥25	23.5

- Hemoglobin A1c: 5.4 (DOWN from 6.2 at baseline, target ≤5.6) — back in normal range. Significant win.
- Insulin: 25.4 (HIGH, target 2.6-24.9) — still elevated, indicating ongoing insulin resistance.
- Interpretation: short-term glucose control improved with diet, but pancreatic beta-cell stress persists.
- Mechanism: A1c reflects 90-day average glucose; insulin reflects current beta-cell workload. Both must normalize for true remission.
- Treatment intensification: berberine, alpha-lipoic acid, chromium, continued carbohydrate restriction.
- C-peptide testing ordered for next round to assess true endogenous insulin production.

Allie — HUMAP Overview (Oct 2025)

- Doctors Data HUMAP — 24-hour urinary hormone metabolite profile, the deepest hormone test available.
- Single dashboard reveals: estrogen production + Phase I/II metabolism, full cortisol rhythm with cortisone clearance, androgen pathway, methylation status, oxidative DNA damage.
- Color coding: blue (below reference), light-blue (WRI low), green (optimal), yellow (WRI high), red (above reference).
- Three findings demand immediate clinical attention: 4-OH-E1 dominance, methylation insufficiency on the 4-OH pathway, DHEA-S depletion.
- Serum testing on this same patient would have shown ZERO of these patterns — clinically silent until disease appears.
- This is the panel that exposes the metabolic risk picture that drives breast and endometrial pathology.

Allie — HUMAP Estrogen 4-OH Finding



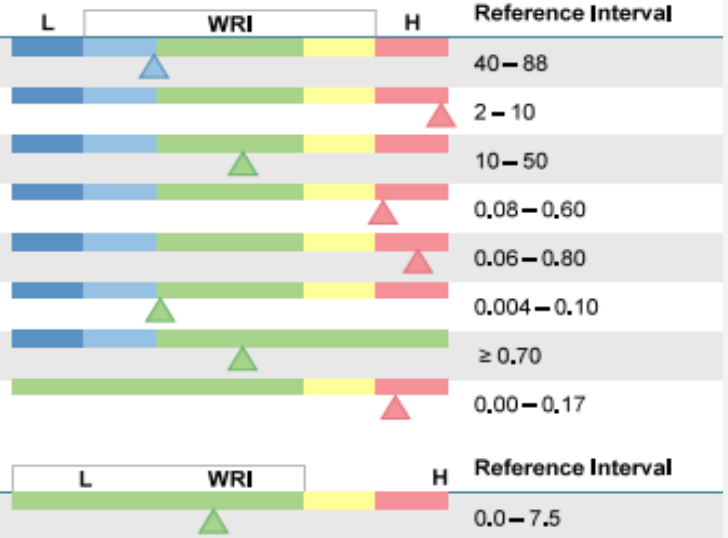
Estrogens		Result	Unit	L	WRI	H	Reference Interval
Estrone‡	(E1)	14	ng/mg Creat/Day				3.8 – 22
2-Hydroxyestrone‡	(2-OH-E1)	5.0	ng/mg Creat/Day				13 – 34
4-Hydroxyestrone‡	(4-OH-E1)	1.5	ng/mg Creat/Day				0.0 – 2.9
16α-Hydroxyestrone‡	(16-OH-E1)	1.7	ng/mg Creat/Day				1.4 – 15
2-Methoxyestrone‡	(2-M-E1)	3.2	ng/mg Creat/Day				1.0 – 7.10
4-Methoxyestrone‡	(4-M-E1)	0.014	ng/mg Creat/Day				0.005 – 0.060
Estradiol‡	(E2)	3.7	ng/mg Creat/Day				1.0 – 9.0
2-Hydroxyestradiol‡	(2-OH-E2)	0.24	ng/mg Creat/Day				0.80 – 3.9
4-Hydroxyestradiol‡	(4-OH-E2)	0.63	ng/mg Creat/Day				0.0 – 1.2
2-Methoxyestradiol‡	(2-M-E2)	0.36	ng/mg Creat/Day				0.06 – 0.70
Estriol‡	(E3)	7.2	ng/mg Creat/Day				2.8 – 23

- 4-Hydroxyestrone (4-OH-E1): 19% of total estrogen metabolites — nearly 2x the upper reference (2-10%).
- The 4-OH pathway is the GENOTOXIC pathway — these catechol estrogens oxidize to quinones that form DNA adducts.
- 2-OH-E1: 61% — protective pathway is intact but being out-competed by 4-OH shunting.
- 16-OH-E1: 20% — proliferative pathway within range.
- Estradiol level 'normal' on serum — but the METABOLIC FATE of estrogen is the breast/endometrial cancer risk driver.
- Family history of breast cancer + 4-OH dominance + low methylation = immediate intervention required.

Allie — HUMAP Ratios, Methylation & 8-OHdG

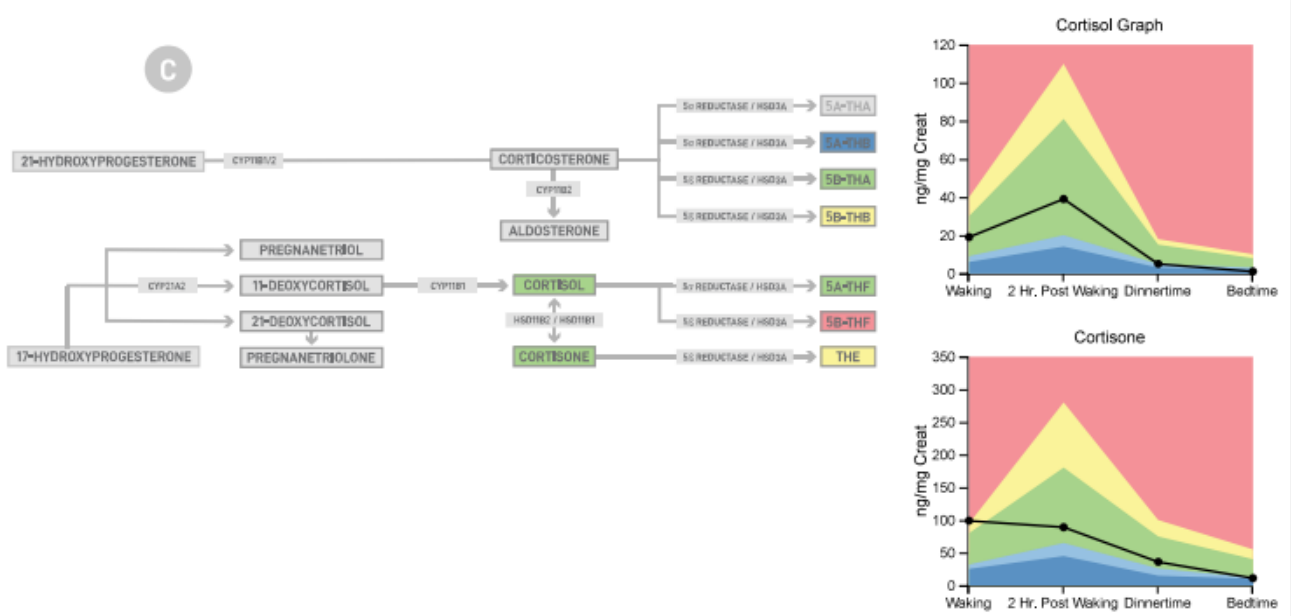
Ratios and Calculations		Result	Unit	Reference Interval
2-OH-E1 %‡	(2-OH-E1 %)	61	%	40 – 88
4-OH-E1 %‡	(4-OH-E1 %)	19	%	2 – 10
16-OH-E1 %‡	(16-OH-E1 %)	20	%	10 – 50
2-M-E1:2-OH-E1‡	(COMT/Methylation activity)	0.62		0.08 – 0.60
2-M-E2:2-OH-E2‡	(COMT/Methylation activity)	1.5		0.06 – 0.80
4-M-E1:4-OH-E1‡	(COMT/Methylation activity)	0.0083		0.004 – 0.10
2-OH-E1:16-OH-E1 ‡		3.0		≥ 0.70
4-OH-E1:2-OH-E1‡		0.31		0.00 – 0.17

Oxidative Stress Metabolite		Result	Unit	Reference Interval
8-hydroxy-2'-deoxyguanosine‡	(8-OHdG)	5.2	ng/mg Creat/Day	0.0 – 7.5



- 4-M-E1:4-OH-E1 methylation ratio: 0.0083 — bottom of reference range (0.004-0.10).
- Means: COMT can NOT methylate the dangerous 4-OH metabolites as fast as they're being produced.
- 4-OH-E1:2-OH-E1 ratio: 0.31 — well above upper limit (≤0.17). Quantitative confirmation of pathological shunting.
- 2-M-E2:2-OH-E2 ratio: 1.5 — protective pathway methylation is excellent. Issue is specifically 4-OH methylation.
- 8-Hydroxy-2'-deoxyguanosine (8-OHdG): 5.2 (ref 0.0-7.5) — upper third of range but MUCH IMPROVED
- Direct evidence of DNA-level oxidative damage occurring in real time from quinone formation.

Allie — HUMAP Cortisol & 11β-HSD2 Activity



Free Cortisol and Cortisone		Result	Unit	L WRI H	Reference Interval
Cortisol Waking‡		19	ng/mg Creat		6 – 40
Cortisol Waking+2hrs‡		39	ng/mg Creat		14 – 110
Cortisol Dinnertime‡		5	ng/mg Creat		3 – 18
Cortisol Bedtime‡		1	ng/mg Creat		2 – 10
Cortisol/day‡	(F)	21	ng/mg Creat/Day		9 – 35
Cortisone Waking‡		99	ng/mg Creat		25 – 95
Cortisone Waking+2hrs‡		89	ng/mg Creat		45 – 280
Cortisone Dinnertime‡		36	ng/mg Creat		15 – 100
Cortisone Bedtime‡		11	ng/mg Creat		10 – 55
Cortisone/day‡	(E)	58	ng/mg Creat/Day		30 – 95
Creatinine Waking		53.0	mg/dL		30 – 225

- Cortisol Waking: 19 ng/mg Cr — normal range.
- Cortisol Waking +2 hrs (CAR): 39 — robust 2x rise, indicating intact HPA reactivity.
- Cortisol Dinnertime: 5; Bedtime: 1 — appropriate diurnal decline.
- Cortisol/Cortisone ratio (11β-HSD2 activity): 0.36 (reference 0.18-0.60) — normal tissue conversion.
- Cortisone curve mirrors cortisol — clearance pathway functioning.
- Among all the abnormal HUMAP findings, the cortisol RHYTHM is the only reassuring one — recovery is possible.

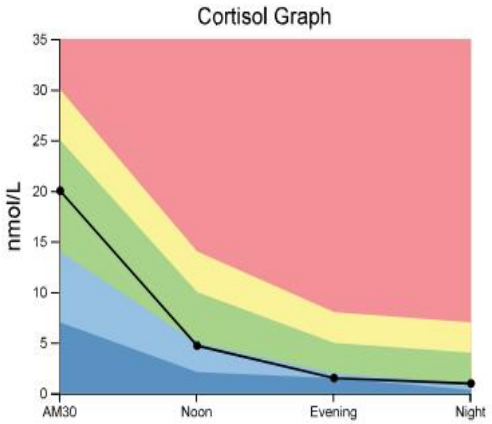
Allie — Follow-up CHP+ Hormones (Mar 2026)

Analyte	Result	Unit	L	WRI	H	Reference Interval	Supplementation Range**
Estrone (E1)*	22.0	pg/mL		◆		< 45	
Estradiol (E2)	4.2	pg/mL	3.9	◆		0.6 – 4.5	1.0 – 6.0
Estriol (E3)*	54.1	pg/mL		◆		7.5 – 66	45 – 680
EQ (E3 / (E1 + E2)) Ratio	2.1			◆		≥ 1.0	
Progesterone (Pg)	582	pg/mL	237		↑	127 – 446	400 – 4000
Pg/E2 Ratio†	139		60.8 ↓			≥ 200	≥ 200
Testosterone	73.0	pg/mL	235		↑	3.0 – 45	20 – 55
DHEA*	283	pg/mL	144	◆		106 – 300	

- Testosterone: 1.6 ng/mg Creat/Day — NORMAL (was 235 in Dec 2024). 99% reduction without exogenous androgen.
- Estradiol metabolites: trending toward normal balance — 4-OH dominance resolving.
- Progesterone: lower than peak — lipophilic depot still slowly releasing from adipose stores.
- DHEA-S: climbing from 7 toward 180+ range — adrenal reserve recovering.
- Hormone profile demonstrates: the body CAN re-regulate when exogenous load is removed and substrate is restored.
- Body composition improved: 22 lbs weight loss; HRT-driven adipose burden reducing.

Allie — Follow-up Cortisol Curve (Mar 2026)

Analyte	Result	Unit	L	WRI	H	Optimal Range	Reference Interval
Cortisol AM30	20	nmol/L	16	◆		14.0 – 25.0	7.0 – 30.0
Cortisol Noon	4.7	nmol/L	2.5	◆		5.0 – 10.0	2.1 – 14.0
Cortisol Evening	1.5	nmol/L	1.7	◆		2.0 – 5.0	1.5 – 8.0
Cortisol Night	0.97	nmol/L	1.5	◆		1.0 – 4.0	0.33 – 7.0
DHEA*	283	pg/mL	144	◆			106 – 300



Hormone Comments

- AM cortisol level appears adequate, although the suboptimal diurnal cortisol pattern is suggestive of early (Phase 1) HPA axis (adrenal gland) dysfunction.
- DHEA level may be adequate depending upon clinical presentation. Note: Ensure adherence to proper dosage interval.

Adrenal Phase: 1

- Morning cortisol improved from 16 → 20 ng/mg Cr.
- Noon cortisol DOUBLED from 2.5 → 4.7 — significant adrenal recovery.
- Diurnal slope: nighttime cortisol now WNL - appropriate decline restored.
- Attributed to: pregnenolone substrate replacement, Sleep Tides 3-phase support, adaptogenic herbs (ashwaganda), removal of inflammatory triggers.
- Adrenal recovery confirmed objectively — the foundational system rebuild is working.

Allie — Follow-up Inflammation (Mar 2026)

▲	C-Reactive Protein, Cardiac [01] 9.2	4.90	High	5.0	mg/L	0.00-3.00
				Relative Risk for Future Cardiovascular Event Low <1.00 Average 1.00 - 3.00 High >3.00		
	Homocyst(e)ine [01]	8.7			umol/L	0.0-14.5
	TSH [01]	1.520			uIU/mL	0.450-4.500
	Thyroxine (T4) [01]	6.8			ug/dL	4.5-12.0
	T3 Uptake [01]	26			%	24-39
	Free Thyroxine Index	1.8				1.2-4.9
	Triiodothyronine (T3) [01]	104			ng/dL	71-180
	Triiodothyronine (T3), Free [01]	3.2			pg/mL	2.0-4.4
	. [01]					
	CBC, Platelet Ct, and Diff [01]					
	WBC [01]	8.5			x10E3/uL	3.4-10.8
	RBC [01]	4.66			x10E6/uL	3.77-5.28
	Hemoglobin [01]	14.8			g/dL	11.1-15.9
	Hematocrit [01]	44.2			%	34.0-46.6
	MCV [01]	95			fL	79-97
	MCH [01]	31.8			pg	26.6-33.0
	MCHC [01]	33.5			g/dL	31.5-35.7
	RDW [01]	12.7			%	11.7-15.4

- C-Reactive Protein, Cardiac: 4.90 mg/L (HIGH, ref <3.00) — improved from peak 9.2 in Sept 2025.
- Trajectory: 7.8 (baseline) → 5.0 (May 2025) → 5.9 → 9.2 (Sept 2025) → 4.90 (Mar 2026).
- Inflammation reduced ~47% from Sept 2025 peak, still above target — work continues.
- Homocysteine: 8.7 — moderate range, B-vitamin support reducing methylation burden.
- Lesson: inflammation trajectories are NON-LINEAR. Initial improvement, transient worsening, then steady recovery.

Allie — March 2026 Follow-up: Insulin Still Elevated

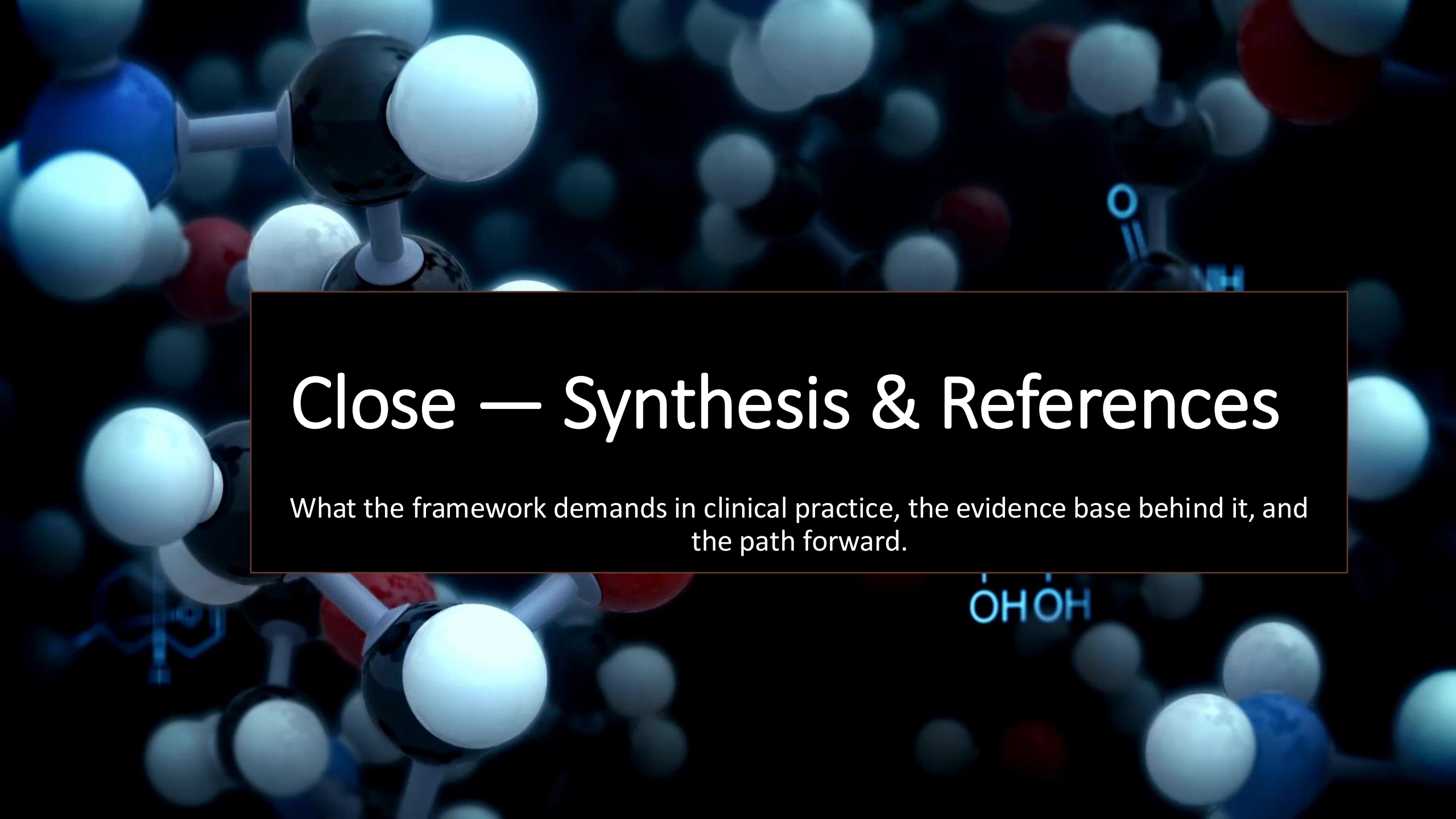
C-Peptide, Serum

Test	Current Result and Flag	Previous Result and Date	Units	Reference Interval
▲ C-Peptide, Serum [01]	5.5 High		ng/mL	1.1-4.4
C-Peptide reference interval is for fasting patients.				

- C-Peptide, Serum: 5.5 ng/mL (HIGH, ref 1.1-4.4) — pancreatic beta-cells still working overtime.
- Insulin, fasting: 30.4 µIU/mL (HIGH, ref 2.6-24.9) — elevated from 23.5 previously.
- Despite A1c improvement, the underlying insulin resistance has NOT fully resolved.
- Mechanism: persistent inflammation + retained adipose hormone burden + cortisol-driven gluconeogenesis = ongoing insulin demand.
- Critical teaching: an 'improved A1c' can mask ongoing hyperinsulinemia and beta-cell stress.
- Treatment intensification: continued insulin sensitization, weight loss focus, address fatty liver, melatonin (anti-NAFLD).

Allie — Outcomes & Teaching Synthesis

- 18-month functional medicine arc: complete hormone discontinuation, full gut/methylation/adrenal rebuild, lifestyle restructuring.
- All major lab markers normalized or trending toward normal — without lifelong HRT, without chronic medications adding up.
- Body composition: 22+ lbs weight loss; insulin sensitivity recovered; thyroid medication reduced from 40 mg → 10 mg/day.
- Critical teaching points: serum monitoring missed everything important; saliva + HUMAP exposed the actual metabolic and risk picture.
- The 'menopausal symptoms' she reported when discontinuing HRT were actually her body's adjustment to receding supraphysiologic exposure — not true deficiency.
- Patients can recover. The functional medicine framework — gut, methylation, mitochondria, adrenals, inflammation, then hormones — is what made it possible.



Close — Synthesis & References

What the framework demands in clinical practice, the evidence base behind it, and the path forward.

Clinical Decision Framework

- Use the right test for the right question — production → blood; metabolism → urine; tissue exposure → saliva. Each answers a fundamentally different question.
- Always assess HPA before HRT — the cortisol curve and CAR diagnose the substrate every other hormone depends on. Skip this and protocols fail.
- For parenteral HRT (pellets, troches, sublingual, creams, gels, IM injections): serum is blind. Saliva is the only valid medium for monitoring titration and avoiding overdose.
- When breast cancer risk is in play: add HUMAP for methylation and metabolite ratios — quantifiable risk markers and direct intervention targets.
- Demand methodologically rigorous evidence: retrospective marketing data is not prospective clinical evidence. Methodology matters.
- Combine media — saliva + HUMAP delivers the body burden picture serum cannot provide, and gives you both the production-side and elimination-side of estrogen biology.

References (1/2)

- Mendel CM. The free hormone hypothesis: a physiologically based mathematical model. Endocr Rev 1989;10(3):232-274. PMID: 2673754
- Pardridge WM. Transport of protein-bound hormones into tissues in vivo. Endocr Rev 1981;2(1):103-123. PMID: 7028469
- Riad-Fahmy D, et al. Steroids in saliva for assessing endocrine function. Endocr Rev 1982;3(4):367-395. PMID: 6217969
- Vining RF, McGinley RA, et al. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. Ann Clin Biochem 1983;20(Pt 6):329-335. PMID: 6316831
- Aardal E, Holm AC. Cortisol in saliva: reference ranges and relation to cortisol in serum. Eur J Clin Chem Clin Biochem 1995;33(12):927-932. PMID: 8845424
- O'Leary P, et al. Salivary, but not serum or urinary levels of progesterone are elevated after topical application of progesterone cream. Clin Endocrinol (Oxf) 2000;53(5):615-620. PMID: 11106923
- Stanczyk FZ, et al. Percutaneous administration of progesterone: blood levels and endometrial protection. Menopause 2005;12(2):232-237. PMID: 15772572
- Du JY, et al. Percutaneous progesterone delivery via cream or gel application in postmenopausal women. Menopause 2013;20(11):1169-1175. PMID: 23652031
- Lewis JG. Steroid analysis in saliva: an overview. Clin Biochem Rev 2006;27(3):139-146. PMID: 17268582
- Wood P. Salivary steroid assays: research or routine? Ann Clin Biochem 2009;46(Pt 3):183-196. PMID: 19176642
- Bradlow HL, et al. 2-hydroxyestrone: the 'good' estrogen. J Endocrinol 1996;150 Suppl:S259-265. PMID: 8943806
- Eliassen AH, et al. Urinary estrogens and estrogen metabolites and subsequent risk of breast cancer among premenopausal women. Cancer Res 2012;72(3):696-706. PMID: 22144471

References (2/2)

- Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. N Engl J Med 2006;354(3):270-282. PMID: 16421368
- Pruessner JC, et al. Free cortisol levels after awakening: a reliable biological marker. Life Sci 1997;61(26):2539-2549. PMID: 9416776
- Clow A, et al. The cortisol awakening response: more than a measure of HPA axis function. Neurosci Biobehav Rev 2010;35(1):97-103. PMID: 20026350
- Fries E, Hesse J, Hellhammer J, Hellhammer DH. A new view on hypocortisolism. Psychoneuroendocrinology 2005;30(10):1010-1016. PMID: 15950390
- Plottel CS, Blaser MJ. Microbiome and malignancy. Cell Host Microbe 2011;10(4):324-335. PMID: 22018233
- Kwa M, et al. The intestinal microbiome and estrogen receptor-positive female breast cancer. J Natl Cancer Inst 2016;108(8):djw029. PMID: 27107051
- Baker JM, et al. Estrogen-gut microbiome axis: physiological and clinical implications. Maturitas 2017;103:45-53. PMID: 28778332
- Glaser RL, Dimitrakakis C. Reduced breast cancer incidence in women treated with subcutaneous testosterone, or testosterone with anastrozole. Maturitas 2013;76(4):342-349. PMID: 24028858
- Rossouw JE, et al. (WHI). Risks and benefits of estrogen plus progestin in healthy postmenopausal women. JAMA 2002;288(3):321-333. PMID: 12117397
- Garland CF, et al. Vitamin D and prevention of breast cancer: pooled analysis. J Steroid Biochem Mol Biol 2007;103(3-5):708-711. PMID: 17368188
- Holick MF. Cancer, sunlight and vitamin D. J Clin Transl Endocrinol 2014;1(4):179-186. PMID: 29159099
- Hill SM, et al. Melatonin: an inhibitor of breast cancer. Endocr Relat Cancer 2015;22(3):R183-R204. PMID: 25876649

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Questions? Thank You.

- Brandon M. Lundell, DC, APC, DABCI, FMCP, Dipl. Ac., NE, DAAVC
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- Educational Partner — Biotics Research Corporation
- assistant@drbrandonlundell.com | www.nutritionalpathology.com

- "Good practitioners know WHAT to do. GREAT practitioners know WHY."™
- Sincere thanks to Doctors Data for the invitation, and to you for your time and engagement.

Questions?

•Are there ways to monitor thyroid function without blood testing? I have a peds client with elevated TSH, and needle phobia
•Best way for monitoring for someone who had hormone positive breast cancer in the past who is currently on HRT?
•Can you truly impact hormone dysregulation without HRT?
•Do you hormone test all of your patients as a baseline?
•How and when to test hormones alongside HRT ovaginal oestrogen + oral progesterone (standard bioidentical protocol in UK and EU)
•How long to suspend topical hormone treatment before repeat testing of saliva?
•How to optimize Testosterone in senior athletes without TRT
•I am getting mixed messages. I took Dr Rachel Rubin's hormone class and she says that serum hormone testing is the most accurate
•I'd love some reference ranges for serum hormone levels
•If a woman has had breast cancer, is HRT forever contraindicated?
•If someone applies estrogen in the morning and progesterone cream at night is there an appropriate time to collect the saliva?
•Please go over progesterone dosages to balance different dosages of estrogen
•salivia testing for thyroid panel
•The better test to monitor female fertility? thanks.
•What to do for low testosterone
•Would you ever or do you ever test serum cortisol? Is there any value to using blood testing for cortisol?