Sleep and Cortisol:
Beyond the Diurnal Rhythm

Presented by: **Allison Smith,** *ND*

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DUTCH Clinical Education Lead

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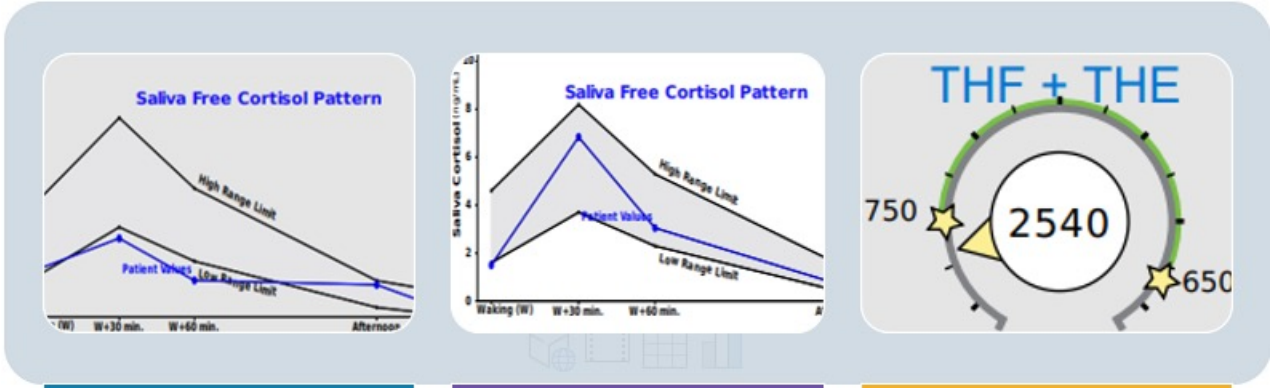
Today's Agenda

- Introduce Free Cortisol diurnal rhythm as unique identifier of circadian problems
- Cortisol Awakening Response as unique identifier of stress resilience and HCT (hippocampal-cortical-thalamic) coordination with HPA (hypothalamic-pituitary-adrenal) axis
- Cortisol and Cortisone Metabolites *levels and distribution* as evidence of the types of stressors leading to cortisol dysfunction and help tailor approach to SLEEP complaints



Today's Agenda

- Review basic sleep physiology
- Hormone influences as the sleep cycle repeats
- Risks and conditions associated with chronic sleep problems
- Nutritional and lifestyle interventions that address cortisol and improve sleep

A Healthcare Gap?

"Chronic insomnia is highly prevalent and affects approximately 30% of the general population. Insomnia impairs cognitive and physical functioning and is associated with a wide range of impaired daytime functions across a number of emotional, social, and physical domains."

Roth, T. Insomnia: Definition, Prevalence, Etiology, and Consequences. Jrnl Clin Sleep Med; 2019.

"The odds of using medications for sleep disturbance decreased 31% between 2013 and 2018 (odds ratio = 0.69, 95% confidence interval = 0.51-0.93, P = .015). This trend was driven by declines in use of [FDA]-approved medications for sleep disturbance, especially for medium- and long-term duration of use. Notably, among those age 80+ years, we observed an 86% decline (odds ratio = 0.14, 95% confidence interval = 0.05-0.36, P < .001) in use of [FDA]-approved sleep medications over the study period."

Kaufmann CN, et al. Declining trend in use of medications for sleep disturbance in the United States from 2013 to 2018. J Clin Sleep Med. 2022.

Phases of the Sleep Cycle

Hippocampus
Thalamus
Cortex

N1 – Transition

N2 – Light sleep (50% of night)

N3 – Deep sleep (Slow wave sleep SWS)

REM – Dream sleep

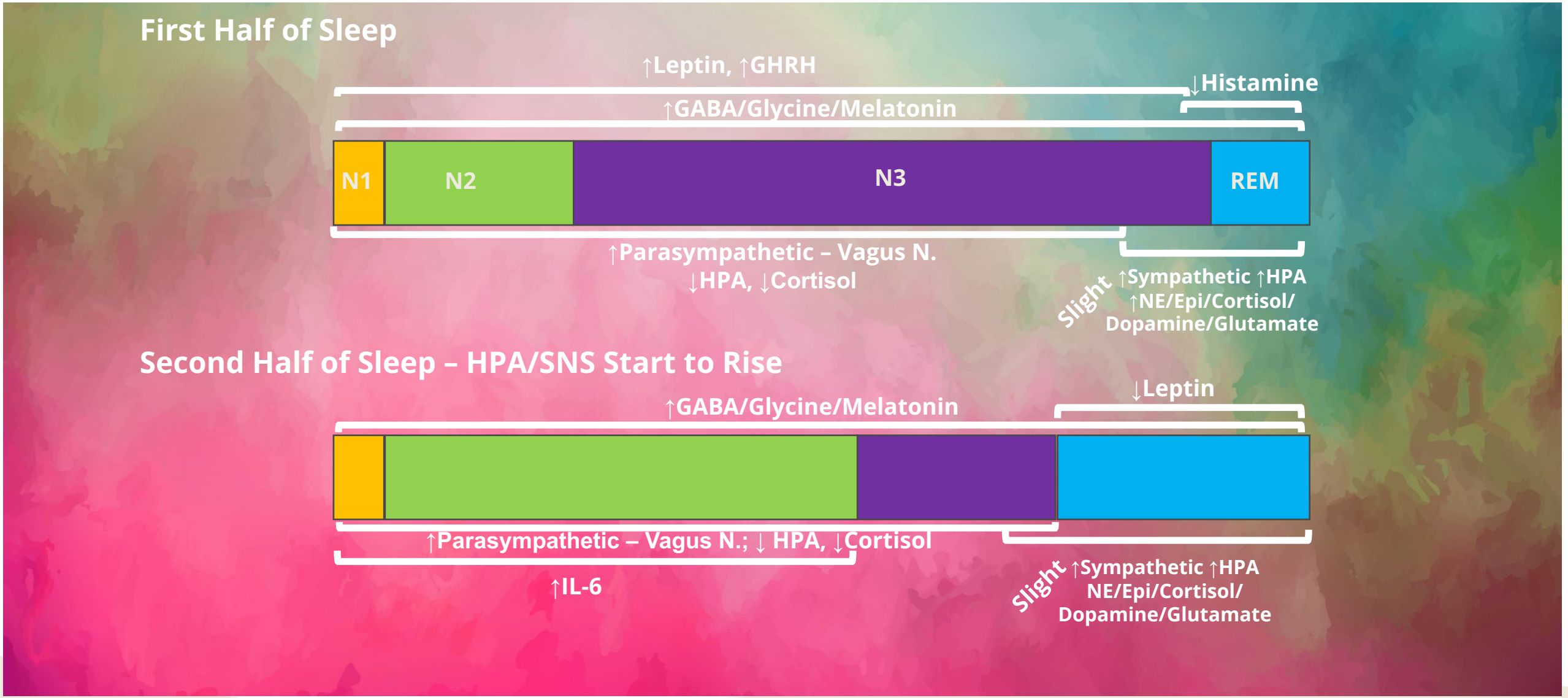
- Basic Neuroendocrine Contributors to Deep Sleep:
  - Excitatory Suppression:
    - Cortisol
    - Norepinephrine/Epinephrine
  - Inhibitory Upregulation
    - Melatonin production
    - GABA/Glycine/Acetylcholine activity
- 4-6 cycles of N2-REM per night
- 1st half of night
  - N3 or SWS is long and strong
  - Hippocampal spindles
  - Memory consolidation, immune
  - Cortisol is LOW → NADIR
- 2nd half of night
  - N2 Light sleep lengthens, N3 shortens
  - REM time ↑ as Cortisol rises from NADIR → ↑AM
  - LAST Sleep cycle REM phase super important for feeling rested

Hormone (and other factor) Cycles and Sleep

- UPSWINGS at Night
  - Pineal-produced melatonin (dim light + circadian influences)
  - GABA/Glycine system
  - TSH (Thyroid stimulating hormone)
  - GHRH (Growth hormone releasing hormone blocks HPA axis)
  - TNFα (Tumor necrosis factor)
  - Leptin (peak at early-midsleep, blocks orexin, then drops again by waking)
  - Ghrelin (increases early in night)
  - DHA (docosahexaeneoic acid)
  - PC (Phosphatidylcholine)
  - IL-6 (Interleukin-6 rises to peak 5am)

- DOWNSWINGS at Night
  - Adrenal-produced cortisol
  - SNS (NE/EPI) activity; PNS (ACh) takes over
  - Glutamate activity
  - Histamine
  - Adiponectin
  - Orexin-A (suppressed by leptin rise)
  - Active Ghrelin (drops after initial rise - nadir @ 8am)
  - Insulin

Positive Influences on Sleep Architecture



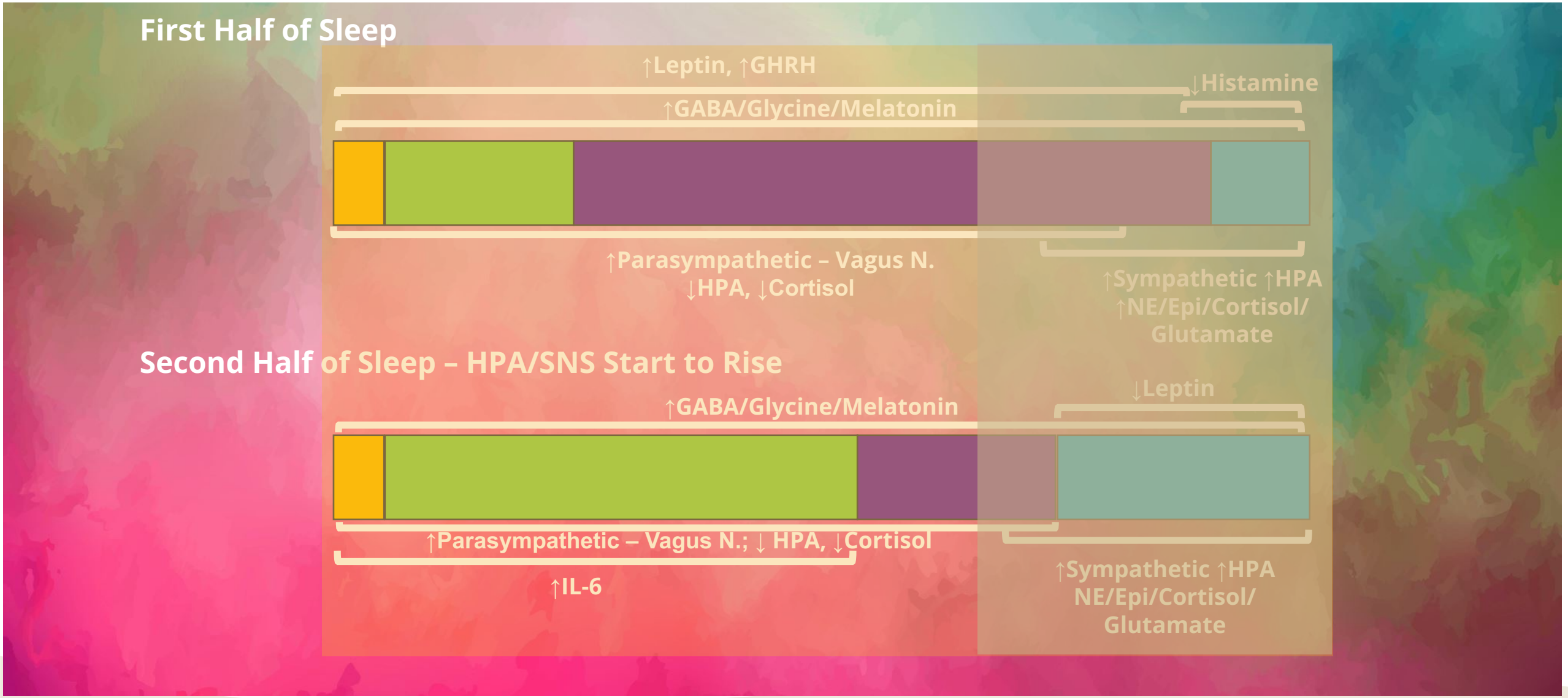
What this means for cortisol and sleep……

- If cortisol is high at night:
  - Lengthened N1-N2 light sleep phase
  - Shortened N3 deep sleep phase
  - Overall shortened (1st cycle is longer but progressively and paradoxically shorter thereafter) and denser REM phase (more active movements)
  - ***Disturbed sleep continuity***
    - May feel like: difficulty falling asleep + frequent waking after sleep onset

- If cortisol is LOW at night:
  - Shortened N2 light sleep phase
  - Lengthened N3 deep sleep phase
  - Shortened REM phases
  - ***Disturbed sleep continuity***
    - May feel like: frequent early morning wakings + shorter sleep duration

Garcia-Borreguero D, et al. Glucocorticoid replacement is permissive for rapid eye movement sleep and sleep consolidation in patients with adrenal insufficiency. J Clin Endocrinol Metab. 2000.

Positive Influences on Sleep Architecture



Why do we use cortisol to assess sleep?

Cortisol synchronizes peripheral clock gene expression

EVERY morning with the sunrise

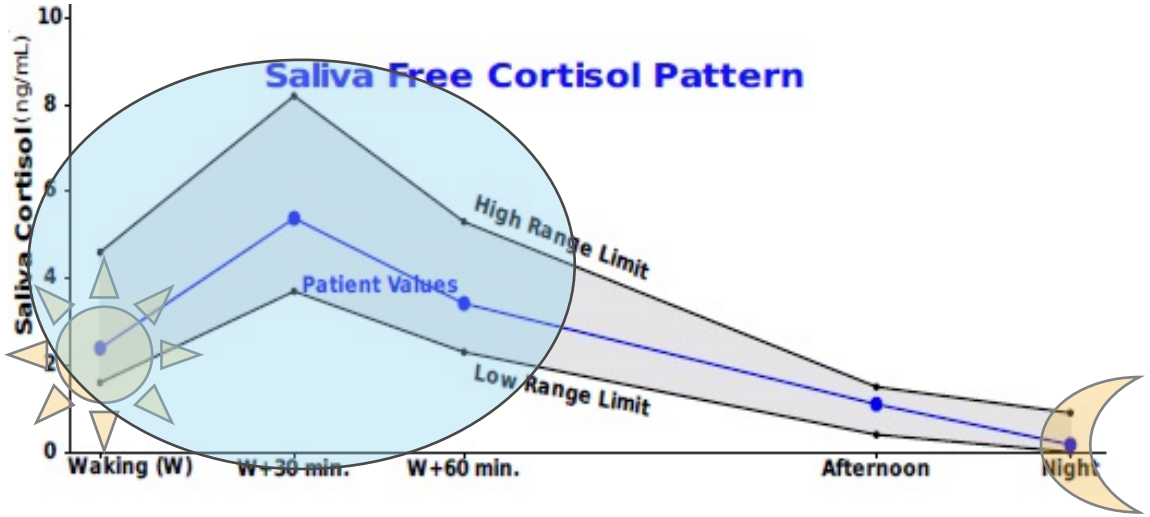
Cortisol = DAYTIME CONDUCTOR

Cortisol zeniths with the rising sun and nadirs in the dark of night

Its diurnal rhythm entrains and synchronizes the peripheral cellular clock genes → homeostasis

- Even small decreases in amplitude of the cortisol rhythm can result in desynchronization, downstream hormone imbalance, and disease

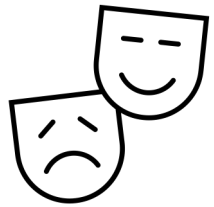
Mavroudis PD, et al. Entrainment of peripheral clock genes by cortisol. Physiol Genomics. 2012;44(11):607-621.

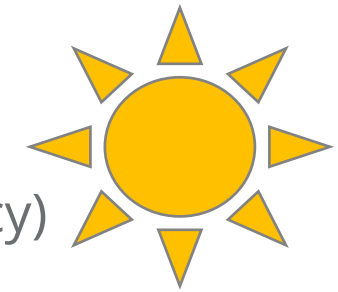


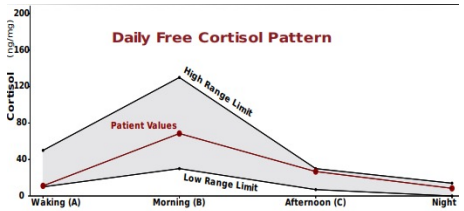

Cortisol interacts with the DNA in
target tissues to control gene expression
to maintain HOMEOSTASIS
when we're under stress

Cortisol Responds to STRESS

- **Upregulation**
  - Glucose generation (GNG, glycogenolysis)
  - Lipolysis (subQ brown fat only)
    - Dependent on insulin/epi
  - White fat redistribution
  - Protein catabolism
  - Glucagon (tissue self sufficiency)
  - Activation of Norepi → Epinephrine (Adrenaline)

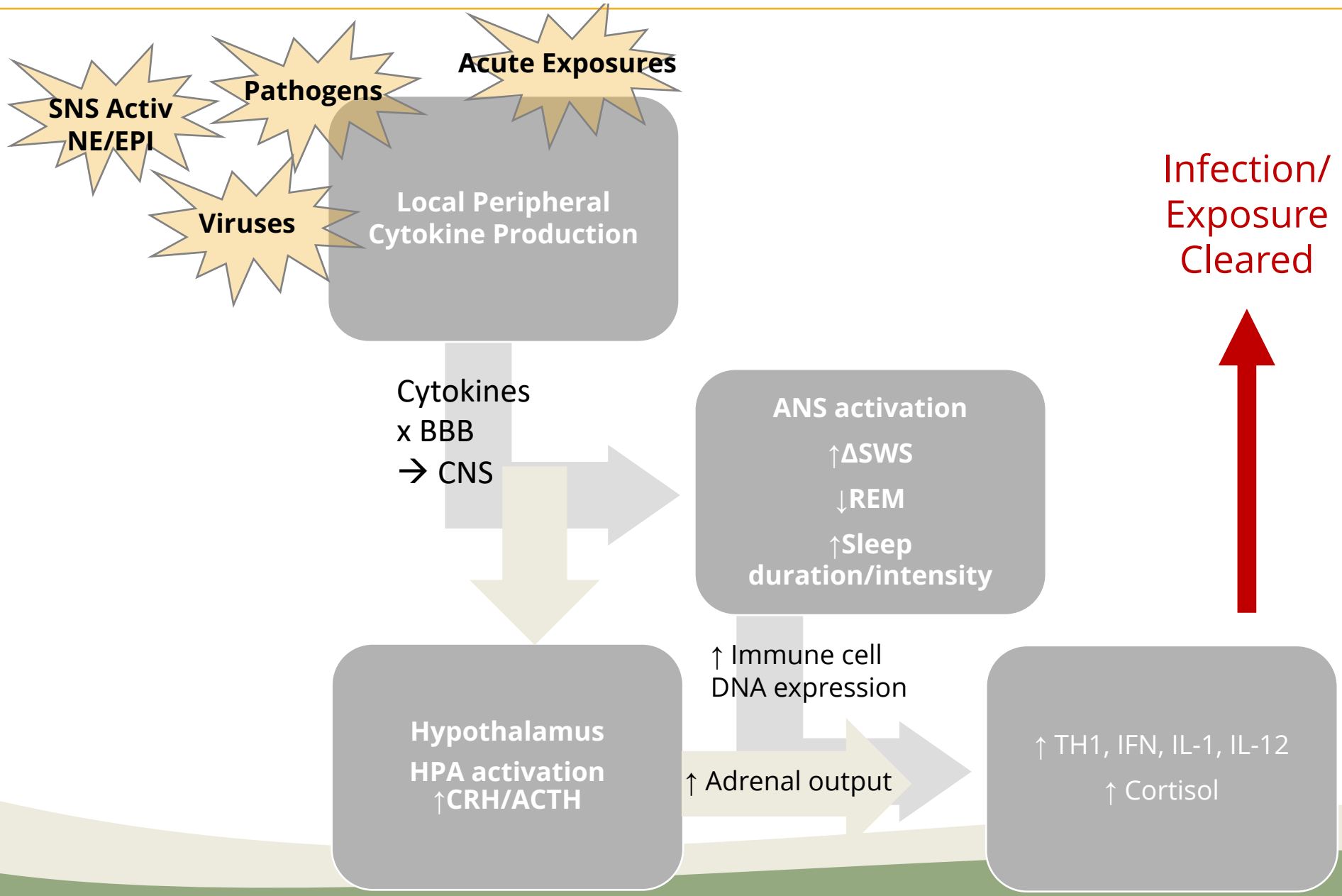




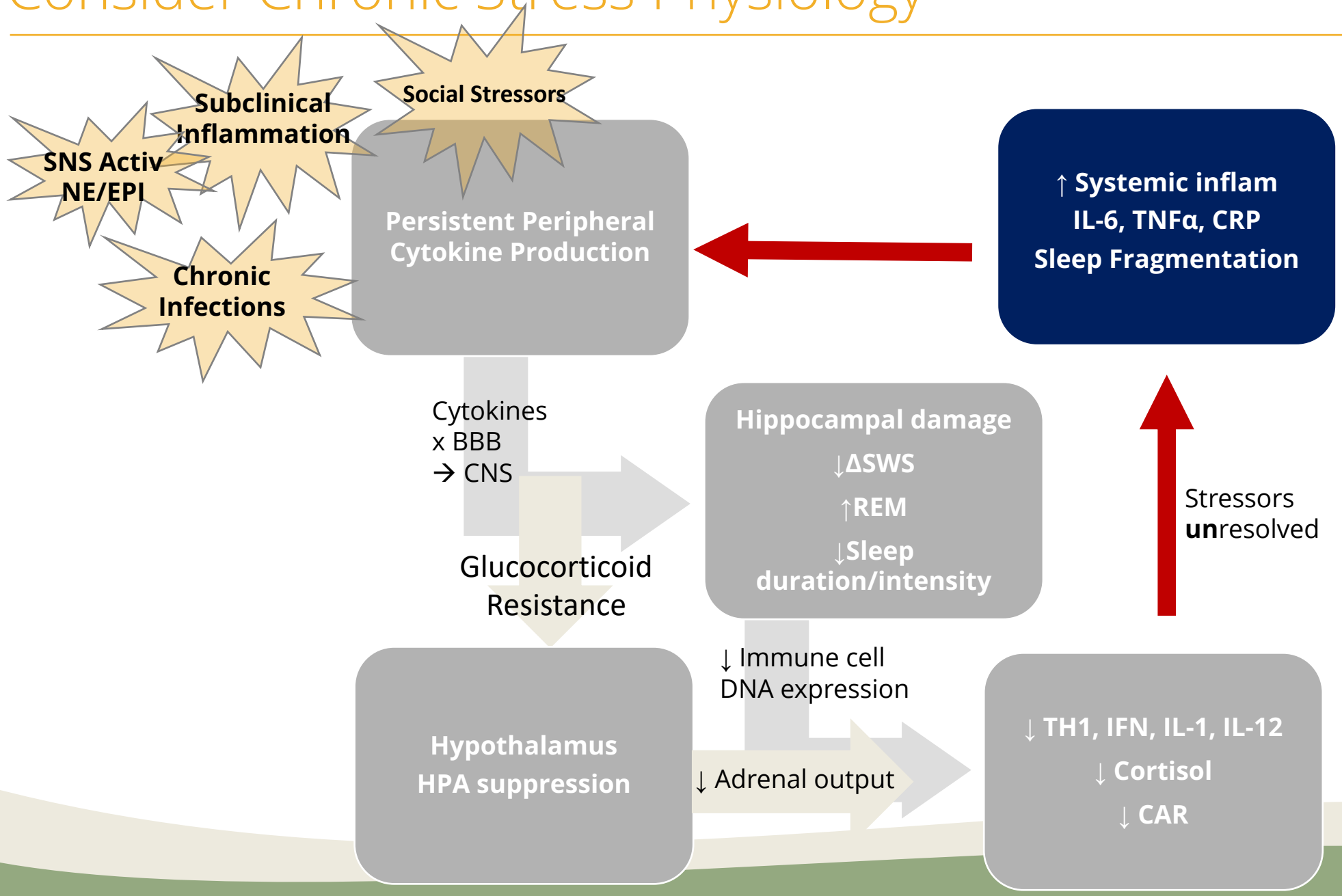


- **Downregulation**
  - Proinflammatory cytokines + chemokines (IL-6, TNFα, IL-1β, CCL2, 3)
  - Immune activity
    - Suppress B cells, T cells
    - ↓ Movement of Neutrophils
  - Insulin sensitivity
  - fT4 → fT3, TSH
  - Nrf2-mediated gene exp
  - H_2O_2 detox

Recall Acute Stress Physiology



Consider Chronic Stress Physiology



The Flat-Line Curve's Link to Chronic Stress

Why isn't the negative feedback loop informing the brain that ACTH stimulation is needed?

Chronic Stressors → HPA Axis Dysfunction
Chronic Stressors → Hippocampal Volume Decrease

How?

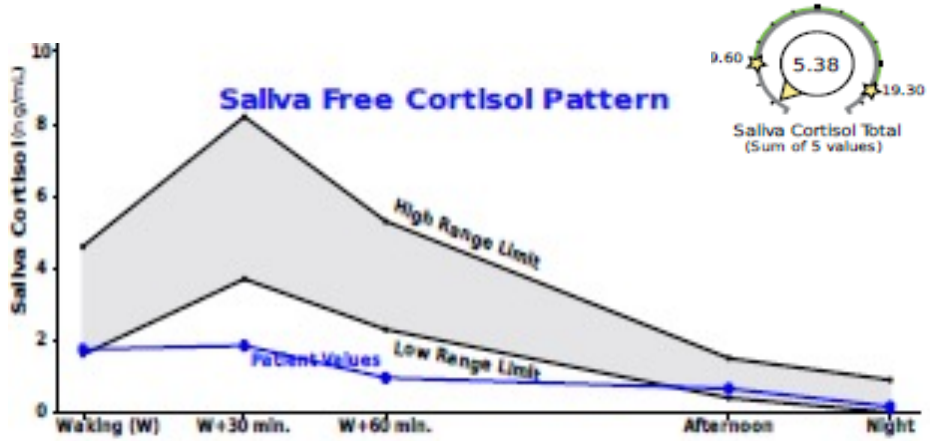
Glucocorticoid Resistance

Hypothalamic sensitivity ↓

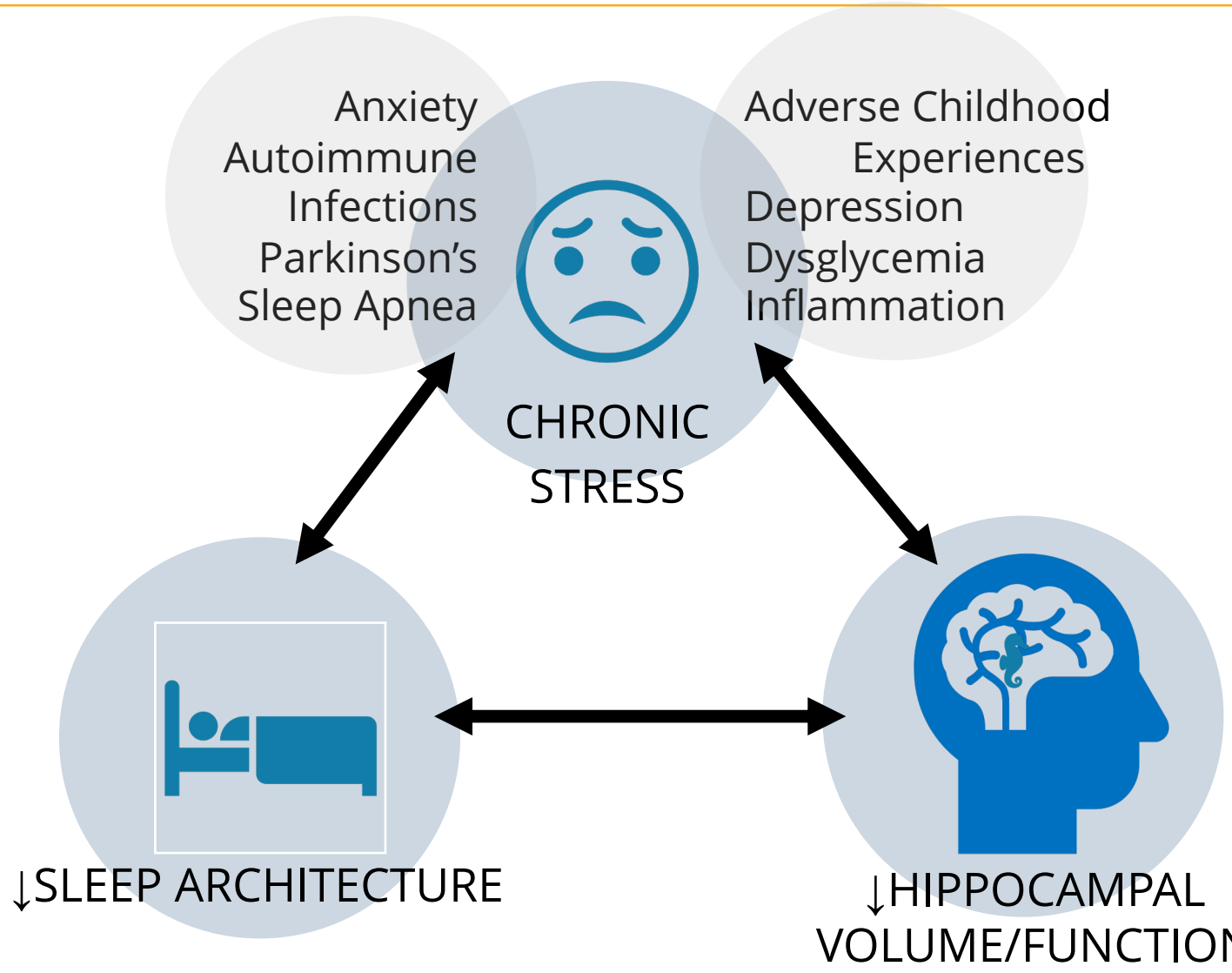
- Cytokines ↑ SAPK/JNK gene expression and stress-activated protein kinases block GC binding to central GC receptors

Peripheral sensitivity ↓

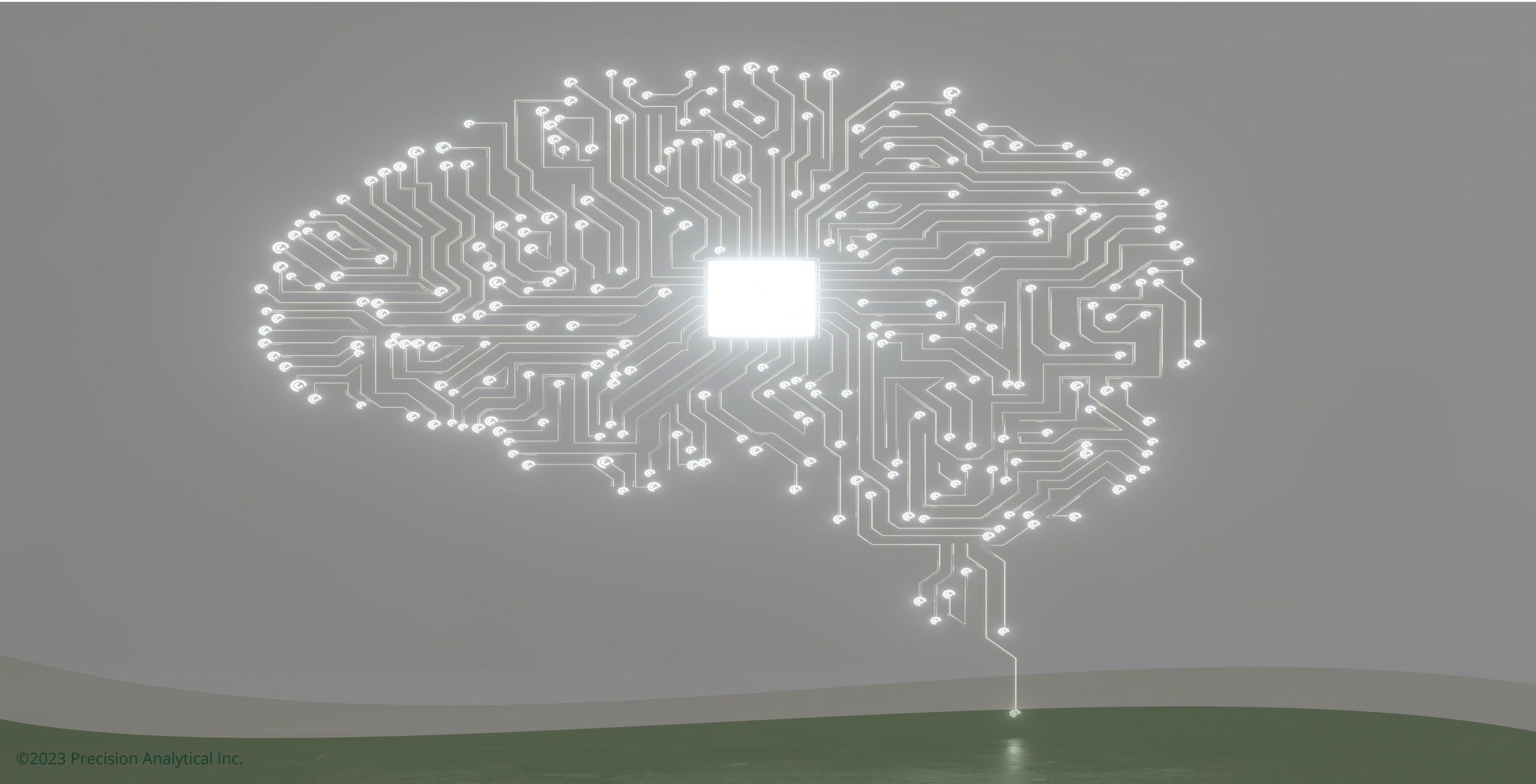
- Cytokines slowly ↑ GRβ expression over GRα in target tissues leading to peripheral GC resistance over time



Sleep, Stress, and the Brain



Sleep, Stress, and the Brain

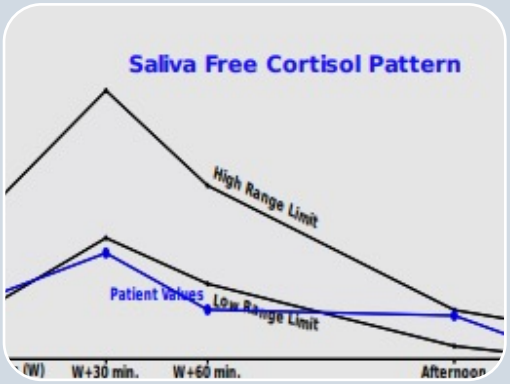


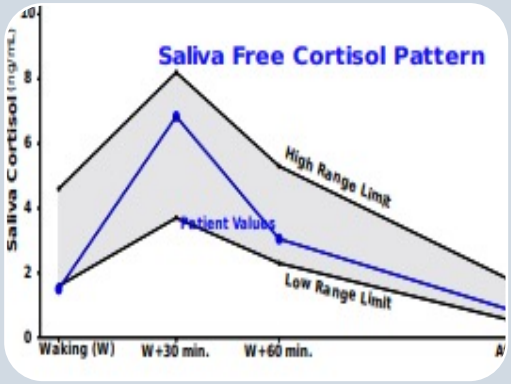
Conditions Associated with Chronic Sleep Loss Etiology

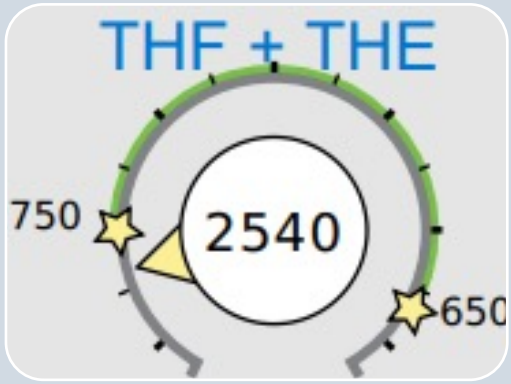
- Aging (accelerated)
- Anxiety
- Cancer Outcomes
- Cardio- and Cerebrovascular Diseases
- Cognitive Decline
- Depression
- Diabetes (T2DM)
- Fatigue (Chronic)
- Inflammation (General)
- Mast Cell Activation – Allergic responses
- Metabolic Syndrome

How do we use cortisol to assess sleep?

3 Ways of Assessing Cortisol Activity





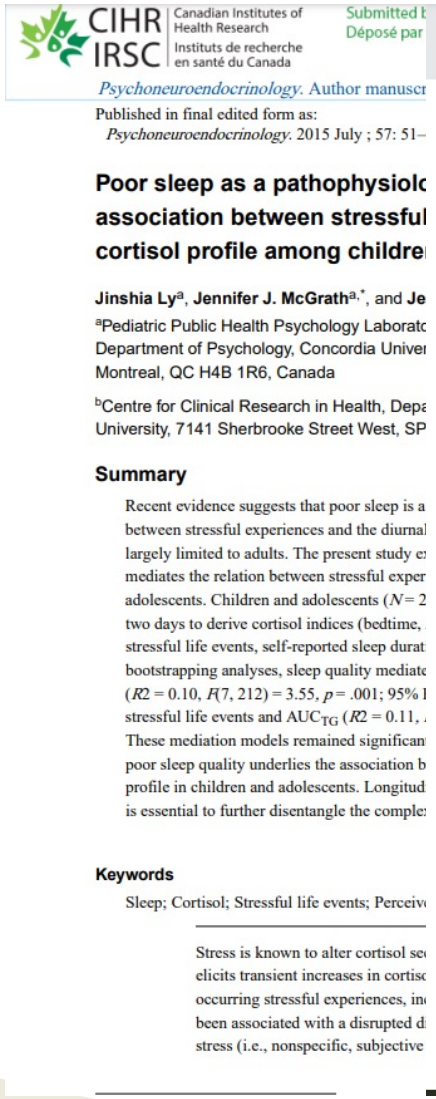


Background Diurnal Pattern

HPA Response to Stress (CAR)

Tissue Metabolism

Diurnal Cortisol Pattern Routinely Studied Relating to Sleep



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Poor sleep as a pathophysiolc association between stressful cortisol profile among childre

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Summary

Recent evidence suggests that poor sleep is a
between stressful experiences and the diurnal
largely limited to adults. The present study e
mediates the relation between stressful exper
adolescents. Children and adolescents ($N = 2$
two days to derive cortisol indices (bedtime,
stressful life events, self-reported sleep durat
bootstrapping analyses, sleep quality mediate
($R2 = 0.10$, $F(7, 212) = 3.55$, $p = .001$; 95% I
stressful life events and AUC_{TG} ($R2 = 0.11$,
These mediation models remained significan
poor sleep quality underlies the association b
profile in children and adolescents. Longitud
is essential to further disentangle the comple

Keywords

Sleep; Cortisol; Stressful life events; Perceiv

Stress is known to alter cortisol se
elicits transient increases in cortis
occurring stressful experiences, in
been associated with a disrupted d
stress (i.e., nonspecific, subjective

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Insomnia Severity is Associate Cortisol and Psychological He

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Sleep Sci 2023;16:92–96.

Abstract

Studies suggest associations betwe
diurnal rhythm. The evidence abo
cortisol is mixed. Chronic insom
disorders. The aim of this study w
and objective sleep pattern with m
instruments used were the Inson
Depression Inventory (BDI), and P
analyzed by chemiluminescence. T
ISI with morning cortisol level ($r =$
POMS-tension anxiety ($r = 0.39$, $p =$
POMS-fatigue ($r = 0.46$, $p < 0.01$;
tively correlated with POMS-tens
severity was associated with highe
Sleep stage N2 was associated wit
tension-anxiety and fatigue.

Keywords
- sleep initiation
- sleep disorders
- sleep maintenance
- depression
- anxiety

Introduction

Insomnia is a prevalent disorder worldwide. It is characterized by difficulty initiating sleep, and/or maintaining sleep, and/or early-morning awakening with inability to return to sleep. Having significant distress or impairment in social, occupational, educational, academic, behavior or other important areas of functioning is also a component of the definition of insomnia. Moreover, sleep difficulty should occur at least 3 times/week, for at least 3 months, despite adequate opportunity for sleep, and not be explained by

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Review article

Interactions between sleep, stress, a From physiological to pathological c

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Obesity

ABSTRACT

Poor sleep quality due to sleep dis
society. Underlying mechanisms s
sleep and metabolism through hyp
deprivation and sleep disorders ar
leading to neuroendocrine dysreg
insulin and decrease adiponectin
relationship between sleep, stress,
conditions, highlighting effective t

1. Introduction

Sleep and stress interact in a bidirectional fashion, sharing multiple pathways that affect the central nervous system (CNS) and metabolism, and may constitute underlying mechanisms responsible in part for the increasing prevalence of metabolic disorders such as obesity and diabetes [1]. Hormones like melatonin and others from the hypothalamic–pituitary–adrenal (HPA) axis modulate the sleep–wake cycle, while its dysfunction can disrupt sleep. In turn, sleep loss influence the HPA axis, leading to hyperactivation [2]. In the first part of this paper, we focus on the definitions of sleep and the HPA axis, and the relationship between sleep and

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Association of Sleep Duration and Quality With Alterations in the Hypothalamic-Pituitary Adrenocortical Axis: The Multi-Ethnic Study of Atherosclerosis (MESA)

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Context: Short sleep duration and poor sleep quality are associated with cardiovascular outcomes. One mechanism proposed to explain this association is altered diurnal cortisol secretion.

Objective: The objective of the study was to examine the associations of sleep duration and sleep quality with diurnal salivary cortisol levels.

Design: This was a cross-sectional analysis using data from examination 5 (2010–2012) of the Multi-Ethnic Study of Atherosclerosis. Actigraphy-based measures of sleep duration and efficiency were collected over 7 days, and salivary cortisol samples were collected over 2 days from participants aged 54–93 years (n = 600 with analyzable data).

Results: Shorter average sleep duration (<6 h/night) was associated with less pronounced late decline in cortisol [2.2% difference in slope; 95% confidence interval (CI) 0.8–3.7; $P \leq .01$] and less pronounced wake-to-bed slope (2.2% difference; 95% CI 1.0–3.4; $P \leq .001$) compared with longer sleep duration (≥6 h/night). Lower sleep efficiency (<85%) was associated with less pronounced early decline in cortisol (29.0% difference in slope; 95% CI 4.1–59.7; $P < .05$) compared with higher sleep efficiency (≥85%). Subjects reporting insomnia had a flatter cortisol awakening response (−16.1% difference in slope; 95% CI −34.6 to −0.1; $P < .05$) compared with those not reporting insomnia.

Conclusions: Shorter sleep duration, lower sleep efficiency, and insomnia are associated with alterations in diurnal cortisol levels consistent with changes in hypothalamic-pituitary-adrenal regulation. (*J Clin Endocrinol Metab* 100: 3149–3158, 2015)

The impact of sleep duration and sleep quality on health is widely recognized. Short sleep duration and poor sleep quality have been linked to coronary heart disease (1), cardiovascular disease (CVD) (2), and CVD risk factors (3). Short sleep duration has also been linked to higher risk of subclinical CVD (4). Insomnia is also associated with a higher risk of coronary heart disease (5) and CVD mortality (6).

One potential mechanistic link between sleep and health outcomes involves alteration of the function of the hypothalamic-pituitary-adrenal (HPA) axis with consequences for cortisol levels over the day. Diurnal cortisol

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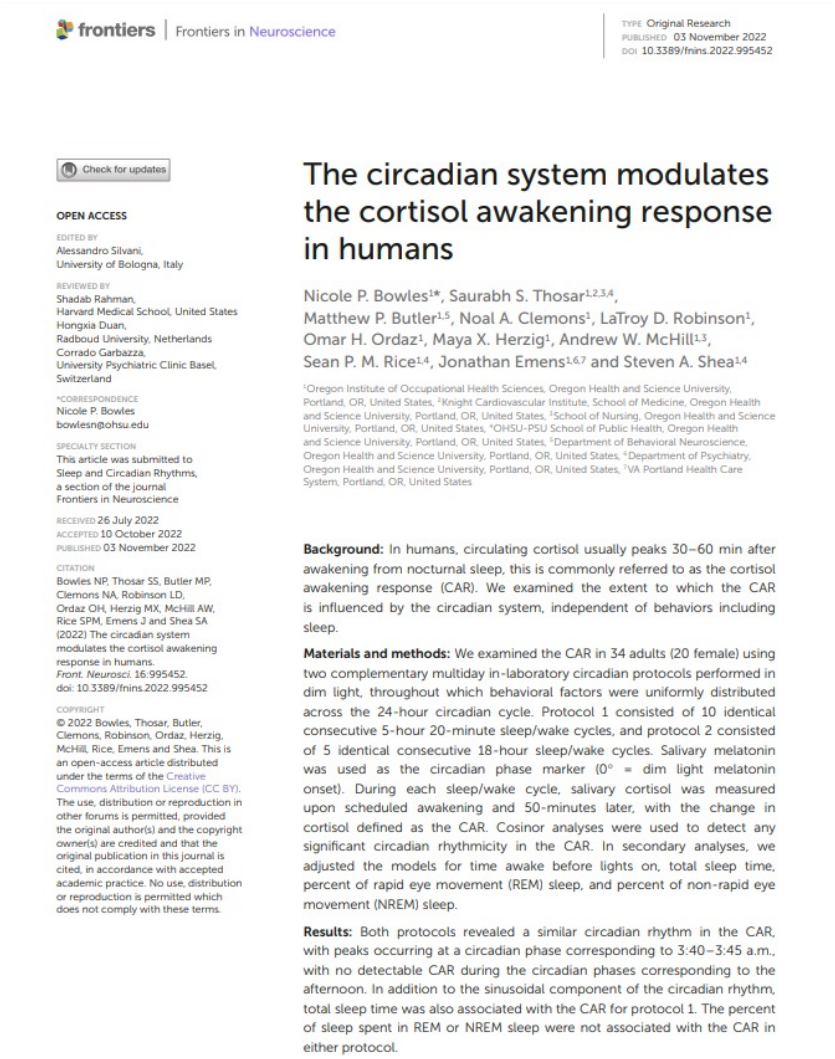
Abbreviations: AUC, area under the curve; CAR, cortisol awakening response; CES-D, Center for Epidemiology Studies-Depression; CI, confidence interval; CVD, cardiovascular disease; HPA, hypothalamic-pituitary-adrenal; MESA, Multi-Ethnic Study of Atherosclero-

The CAR : HPA-Axis Generated, Circadian-Regulated



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The circadian system modulates the cortisol awakening response in humans

Nicole P. Bowles[1]*, Saurabh S. Thosar[1,2,3,4], Matthew P. Butler[1,5], Noal A. Clemons[1], LaTroy D. Robinson[1], Omar H. Ordaz[1], Maya X. Herzig[1], Andrew W. McHill[1,3], Sean P. M. Rice[1,4], Jonathan Emens[1,6,7] and Steven A. Shea[1,4]

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Background: In humans, circulating cortisol usually peaks 30–60 min after awakening from nocturnal sleep, this is commonly referred to as the cortisol awakening response (CAR). We examined the extent to which the CAR is influenced by the circadian system, independent of behaviors including sleep.

Materials and methods: We examined the CAR in 34 adults (20 female) using two complementary multiday in-laboratory circadian protocols performed in dim light, throughout which behavioral factors were uniformly distributed across the 24-hour circadian cycle. Protocol 1 consisted of 10 identical consecutive 5-hour 20-minute sleep/wake cycles, and protocol 2 consisted of 5 identical consecutive 18-hour sleep/wake cycles. Salivary melatonin was used as the circadian phase marker (0° = dim light melatonin onset). During each sleep/wake cycle, salivary cortisol was measured upon scheduled awakening and 50-minutes later, with the change in cortisol defined as the CAR. Cosinor analyses were used to detect any significant circadian rhythmicity in the CAR. In secondary analyses, we adjusted the models for time awake before lights on, total sleep time, percent of rapid eye movement (REM) sleep, and percent of non-rapid eye movement (NREM) sleep.

Results: Both protocols revealed a similar circadian rhythm in the CAR, with peaks occurring at a circadian phase corresponding to 3:40–3:45 a.m., with no detectable CAR during the circadian phases corresponding to the afternoon. In addition to the sinusoidal component of the circadian rhythm, total sleep time was also associated with the CAR for protocol 1. The percent of sleep spent in REM or NREM sleep were not associated with the CAR in either protocol.

High vs. Low CAR

- Elevated and/or Prolonged
  - HPA axis hyperreactivity
    - ↑Sympathetic tone
  - Common conditions:
    - Anticipatory stress, Early-stage Anxiety, Depression, PTSD, Insomnia, Essential Fatty Acid deficiencies

ACUTE

ALARM

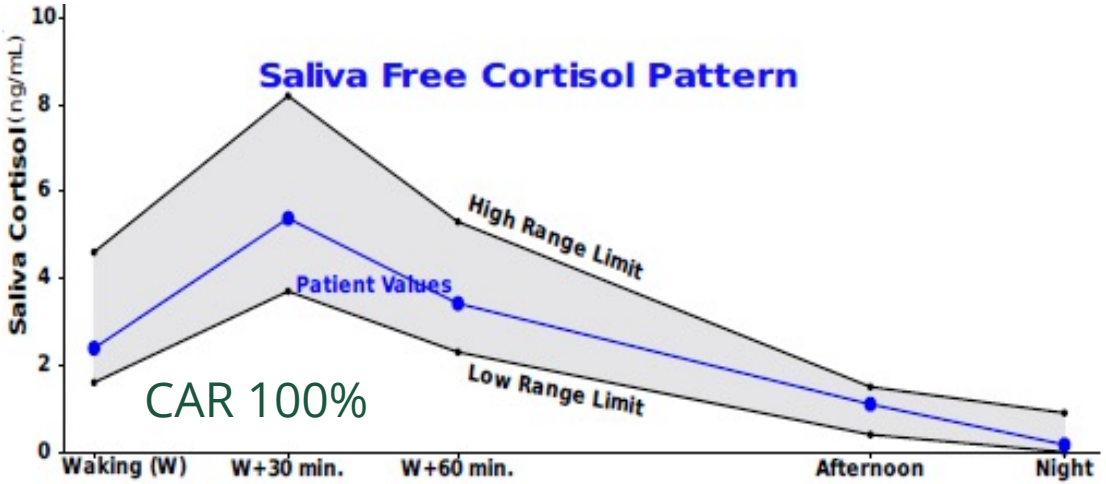
- Blunted or Absent
  - HPA axis hyporeactivity
    - ↓ Hippocampal volume
    - HPA axis dysfunction
  - Common conditions:
    - Adverse Childhood Experiences, Autoimmune, Later-stage Anxiety, Depression, PTSD, Insomnia, Sleep Apnea; Psychological burnout

CHRONIC

GC Resistant

Bowles NP, et al. The circadian system modulates the cortisol awakening response in humans. Front Neurosci. 2022;16:995452.

The Normal CAR Rise = 50 – 160% within 30 minutes



Correct Collection of the CAR is Imperative

Otherwise, they're just extra cortisol points on the diurnal curve.

Important to collect IMMEDIATELY at waking:

Delaying the Waking collection more than 15 minutes can result in UNDERESTIMATION of the CAR and a STEEPER diurnal slope.

COLLECTION RULES

Caffeine, alcohol, and strenuous exercise may affect results. Avoid morning food and drink until after collection #3. Do not brush your teeth until after collections #3 and #5. **Do not floss** on the day of collection or until **ALL** samples are collected. Consult your provider if you have questions.

WHEN TO COLLECT

While adhering to your most common wake/sleep schedule, collect as close as possible to the below timeline.

Upon Waking
Collect saliva **immediately** upon waking, complete within 5 minutes, and set a timer for 30 minutes for Sample #2.

#1|Waking Sample

30 Minutes After Waking
Collect saliva, then set timer for 30 minutes for Sample #3.

#2|+30 Minute Sample

60 Minutes After Waking
Collect saliva.

#3|+60 Minute Sample

Before Your Evening Meal, or Approximately 4-5PM
Collect saliva. Rinse your mouth with water 10 minutes prior to collecting.

#4|4PM-5PM Sample

At Bedtime, But Not Later Than Midnight
Collect saliva. Rinse your mouth with water 10 minutes prior to collecting.

#5|Bedtime Sample

Extra Cortisol Collection
If you struggle with insomnia collect during the night, otherwise at noon. See below.

#6|Extra Sample (Optional)

(OPTIONAL) EXTRA CORTISOL COLLECTION

If you struggle with staying asleep during the night, this sample can be used overnight. Keep the collection tube close to your bed and collect at the time of your sleep disturbance (minimize light exposure). You may complete the label after rising for the day. Otherwise, you may collect this sample at noon or as instructed by your provider.

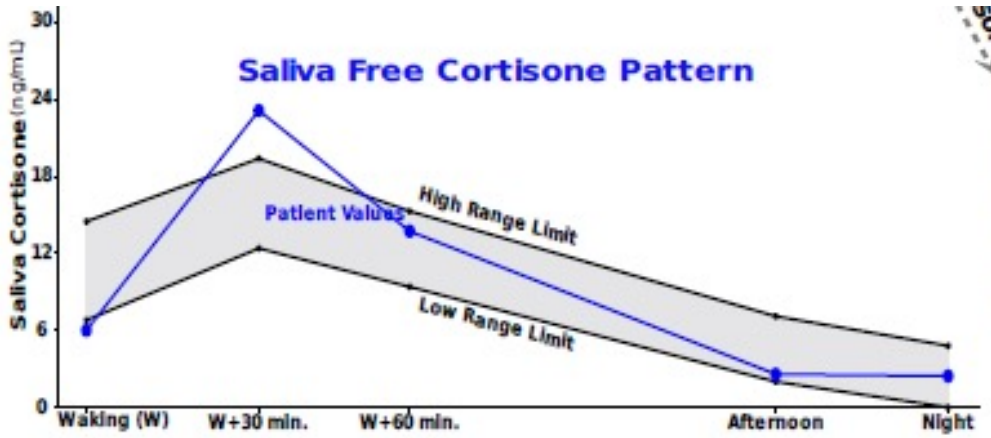
3 Main Abnormal CAR Findings Suggesting Low Stress Resilience

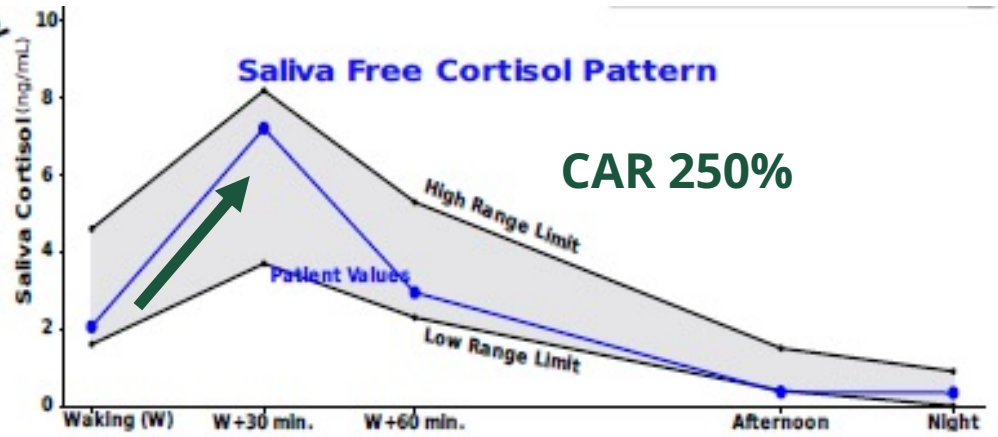
High

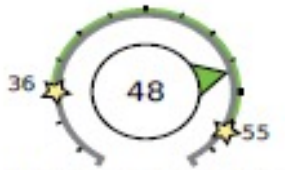
Low

Prolonged

High CAR – Severe anxiety, moderate fatigue, sleep onset probs





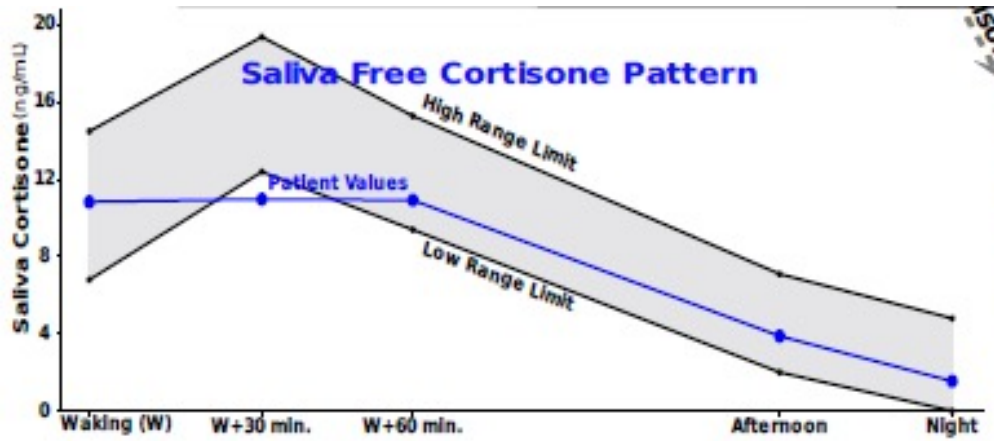


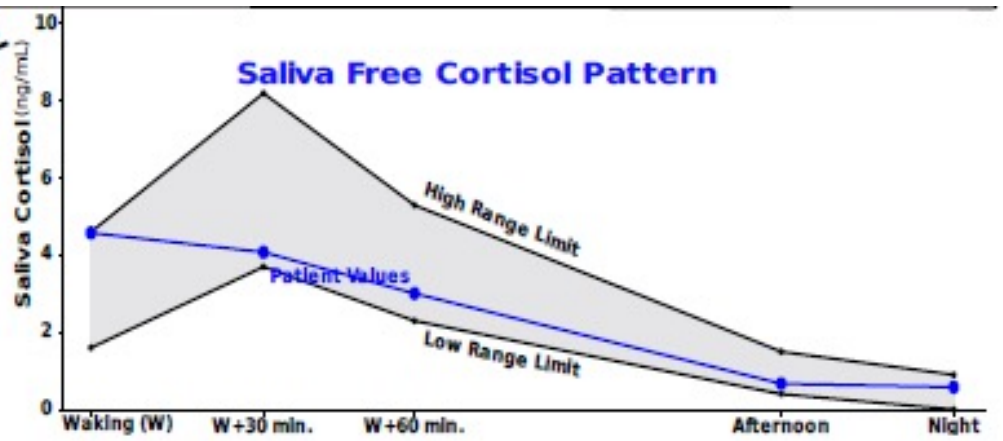
Saliva Cortisone Total
(Sum of 5 values)

Saliva Cortisol Total
(Sum of 5 values)

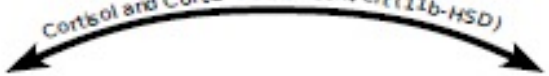
The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. This patient shows a waking cortisol of 2.06 and an increase to 7.2 after 30.0 minutes. This is an increase of 5.2ng/mL or 250%. Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common with samples collected 30 minutes after waking. These guidelines are considered research only.
This patient shows a salivary cortisol of 2.95 measured 60 minutes after waking. This is an increase of 0.89ng/mL or 43.2% compared to the waking sampe. To date, data suggests that expected results may be 0-70%, and this guideline is considered for research only.

Low/Blunted CAR – Severe chronic insomnia, morning fatigue





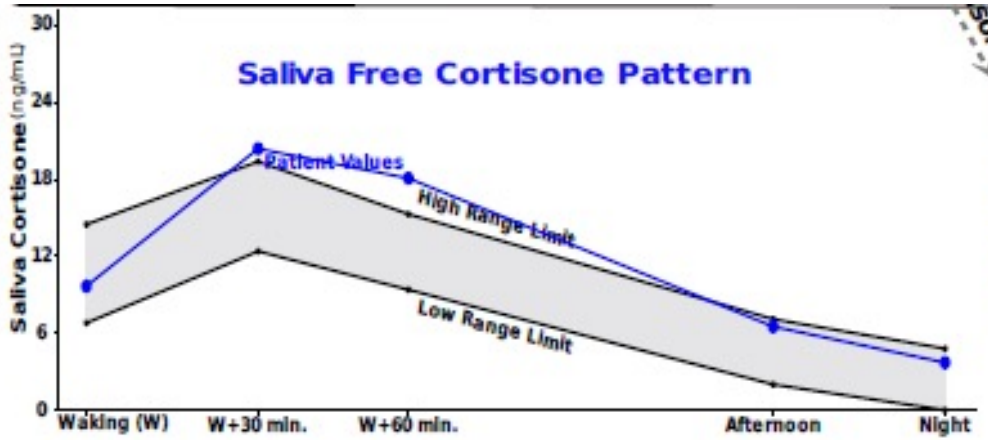
Saliva Cortisone Total
(Sum of 5 values)

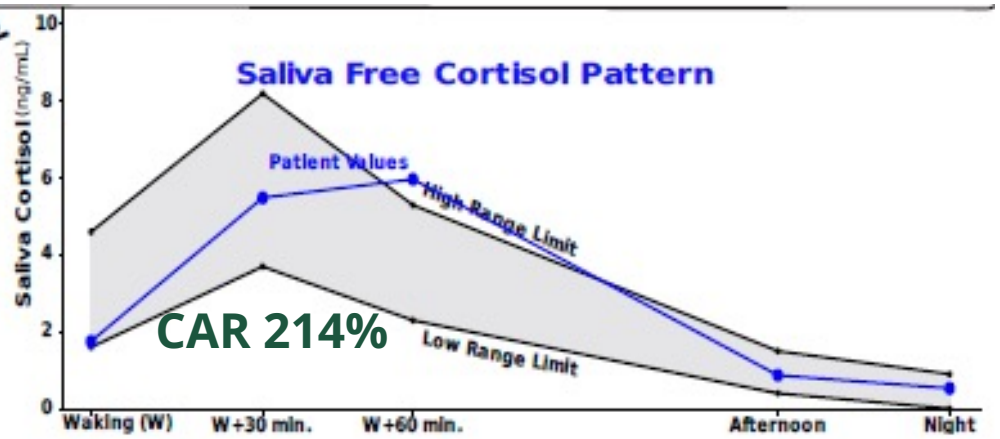


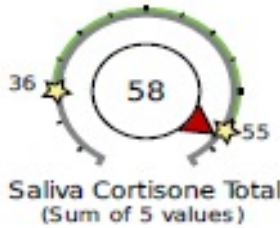
Saliva Cortisol Total
(Sum of 5 values)

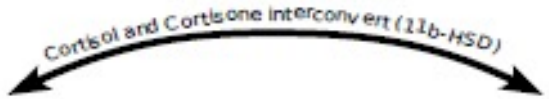
The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. **This patient shows a waking cortisol of 4.58 and was actually lower at 4.08 after 25.0 minutes. This implies potential dysfunction in the HPA-axis or possibly improper collection.** Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common. These guidelines are considered research only.
This patient shows a salivary cortisol of 3.01 measured 60 minutes after waking. Generally this result is a little higher than the waking sample but is not in this case. To date, data suggests that expected results may be 0-70% higher, and this guideline is considered for research only.

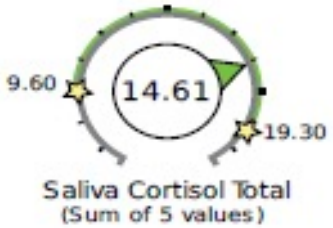
Prolonged (and High) – Severe anxiety, severe fatigue, frequent waking











The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. **This patient shows a waking cortisol of 1.75 and an increase to 5.5 after 30.0 minutes. This is an increase of 3.74ng/mL or 214%.** Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common with samples collected 30 minutes after waking. These guidelines are considered research only.
This patient shows a salivary cortisol of 6.0 measured 60 minutes after waking. This is an increase of 4.22ng/mL or 241% compared to the waking sampe. To date, data suggests that expected results may be 0-70%, and this guideline is considered for research only.

What does the CAR tell us that diurnal rhythm alone doesn't?

An assessment of the cortisol stress response DIRECTLY without an ACTH stimulation test	• A more specific marker of brain-adrenal communication than the diurnal curve of free cortisol
When **high**: Over-active stress response – TOUCHY!	• Omega-3 fatty acid (DHA) deficiency, Acute insomnia, Oxidative stress, Inflammation, Serotonin issues, High SNS activity
When **low**: Poor HPA communication and hippocampal damage	• Chronic pain, Chronic stress, Chronic insomnia, High BMI and WC, Insulin resistance, Psychological burnout, PTSD, SAD, Sleep apnea

Timing Treatments to Improve the CAR

In general, to affect the CAR in the short term, treatment is best applied:

In the morning immediately upon waking

At night before bed (or sleep supports during the night)

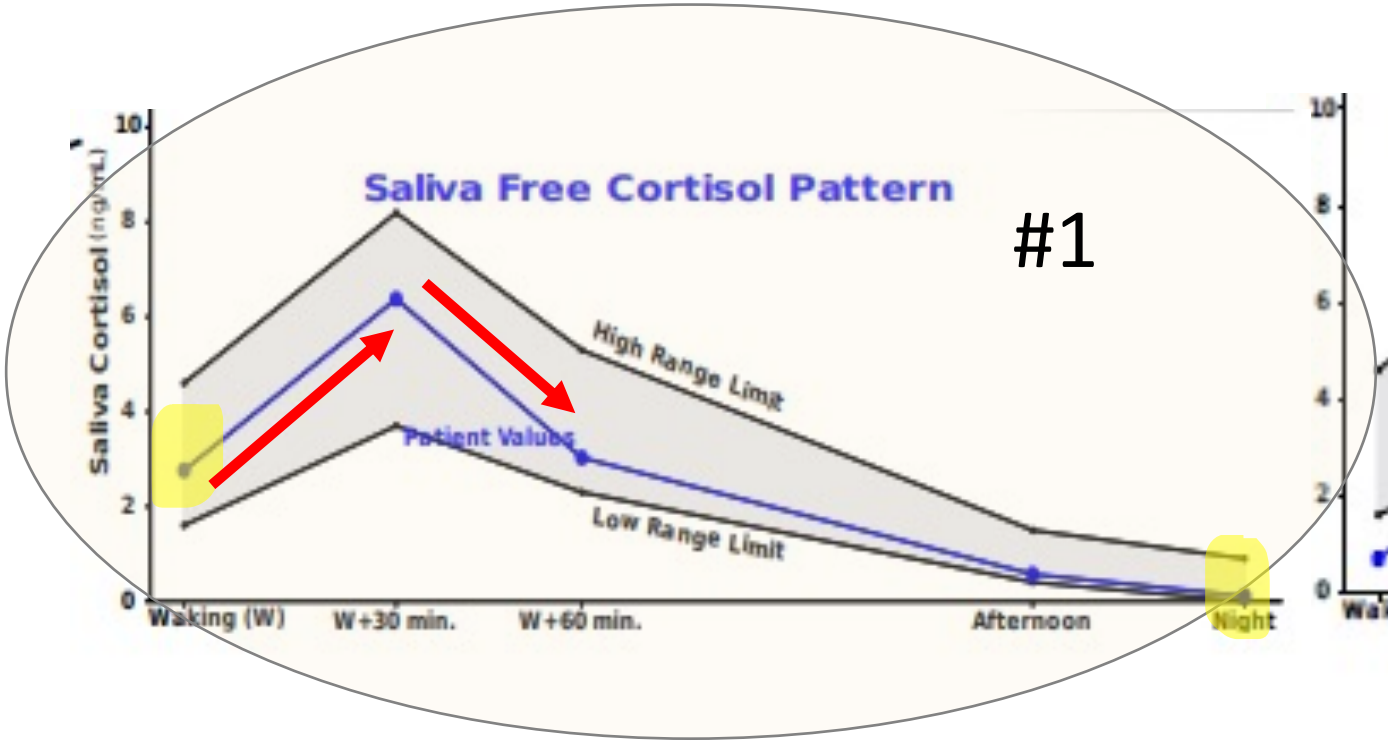
To affect the CAR in the long term, treatment is best applied:

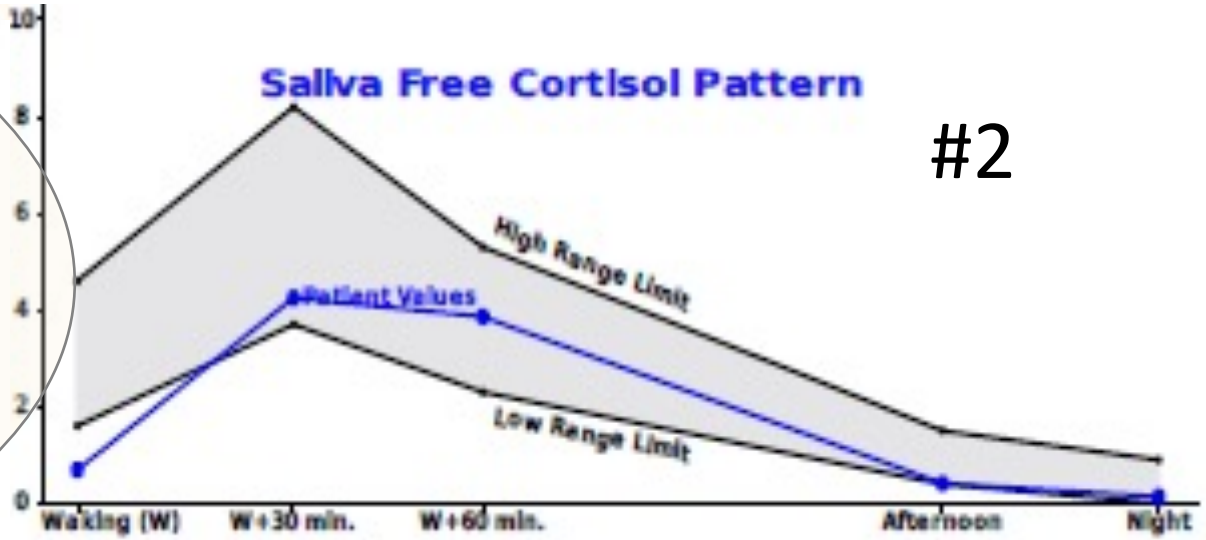
As regular and daily application of lifestyle supports and treatment of any known underlying condition influencing the CAR

Diurnal and CAR Irregularities

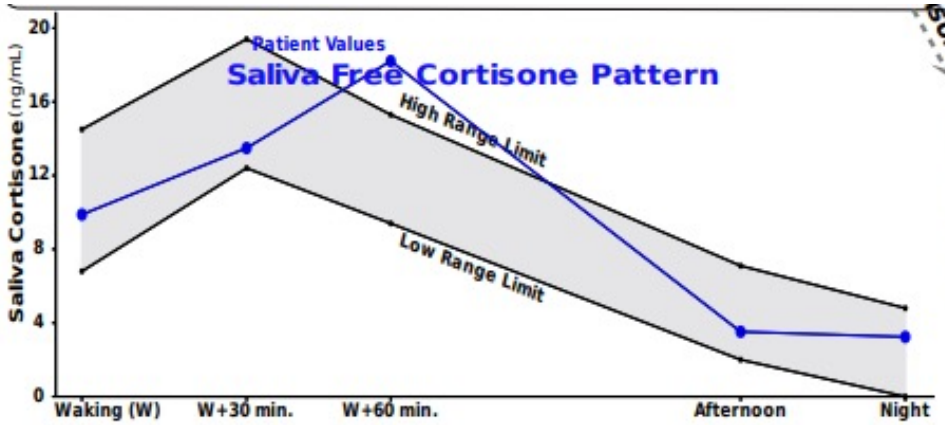
Free Cortisols

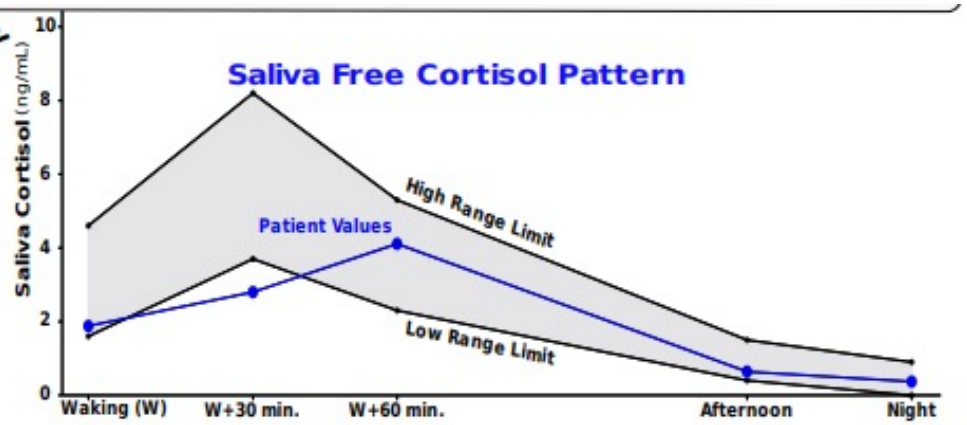
Pop Quiz – Which cortisol curve is more resilient?

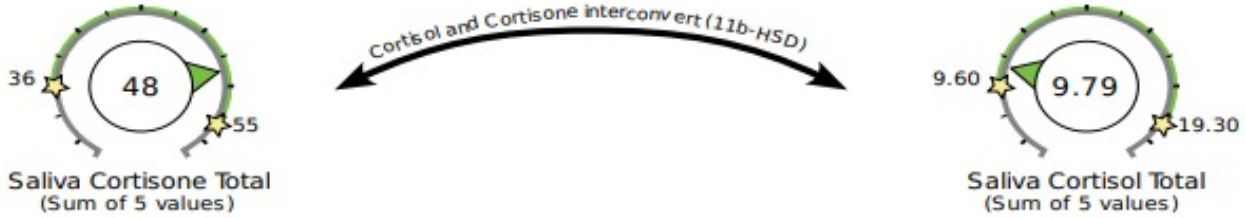




Don’t Forget to Assess Middle-of-the-Night Cortisol Levels







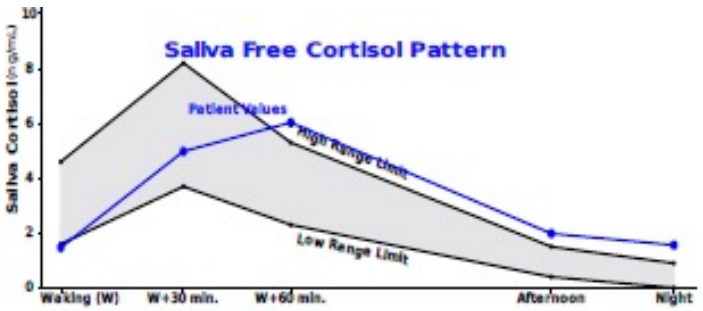
- The patient submitted an Insomnia salivary sample. The cortisol result for this sample was 1.30ng/mL (expected range 0-0.9) The cortisone result for this sample was 7.7 ng/mL (expected range 0-4.8)

The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. **This patient shows a waking cortisol of 1.88 and an increase to 2.80 after 30.0 minutes. This is an increase of 0.92ng/mL or 48.9%.** Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common with samples collected 30 minutes after waking. These guidelines are considered research only.
This patient shows a salivary cortisol of 4.11 measured 60 minutes after waking. This is an increase of 2.23ng/mL or 119% compared to the waking sampe. To date, data suggests that expected results may be 0-70%, and this guideline is considered for research only.

Flipped Curve

Saliva

Low morning, high night



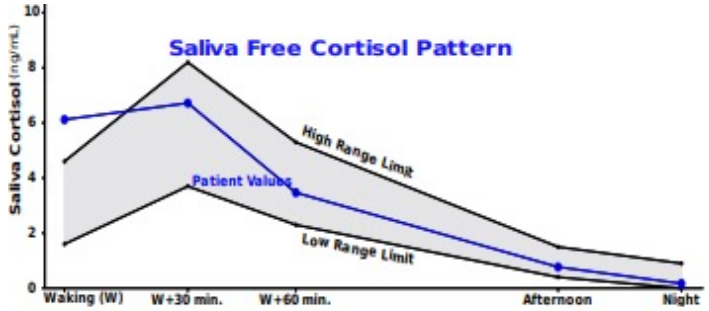
Elevated (233% rise), Prolonged CAR:

- Anticipatory stress
- Anxiety
- Depression
- Delayed sleep onset
- Inflammation
- Insomnia (Acute)

Dysglycemic Pattern

Saliva

Waking cortisol is high, CAR is low



CAR is blunted (8.3%), Drops beyond waking:

- Dysglycemia
- Sleep Apnea
- Insomnia (Chronic) – frequent waking, early waking

Chronic Poor Sleep Quality

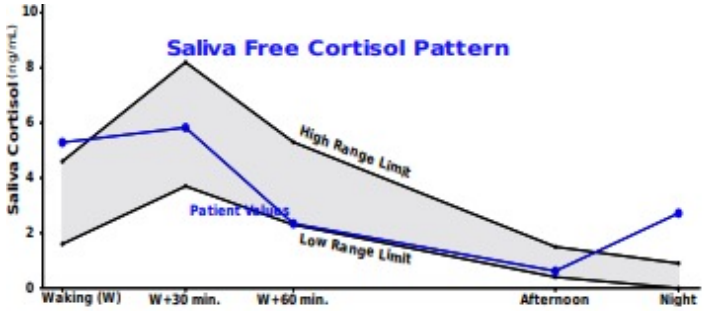
Saliva

Night C ↑ (sleep onset)

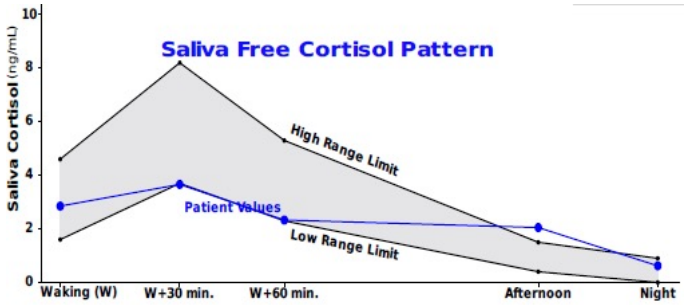
AM Cortisol ↓, Afternoon C ↑ (low AUC)

CAR is blunted (<50% rise)

CAR <10%



CAR 16%

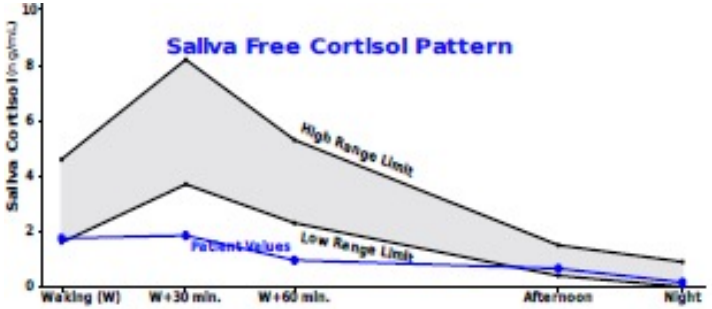


Flat-Line Pattern

Saliva

Flat cortisol pattern

CAR is blunted



*Associated with

HPA Axis dysfunction

Sleep Problems

Fatigue

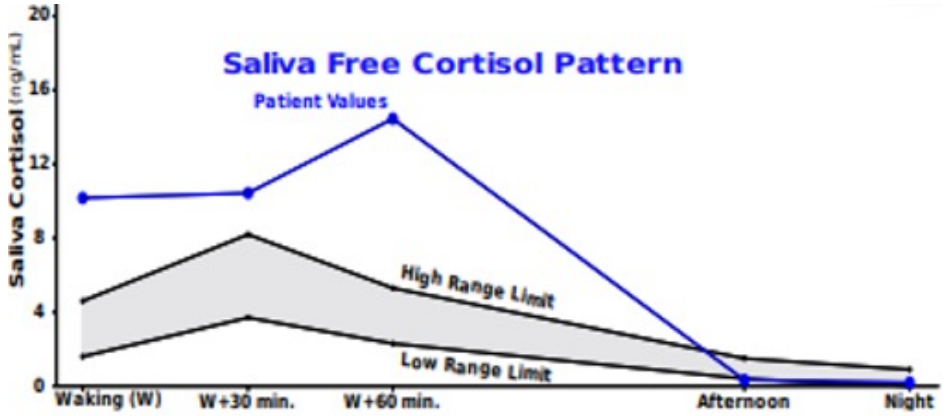
Medications

Acute Immune and Acute Pain

Saliva

Waking cortisol is very high

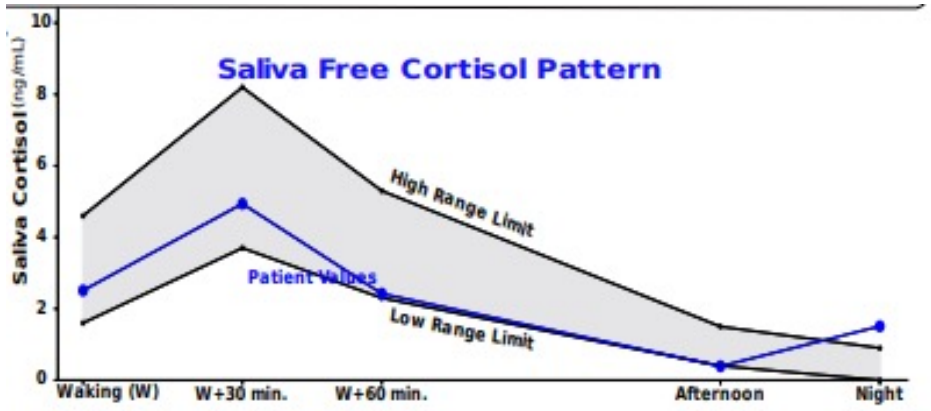
CAR is sluggish and prolonged



Acute viral illness day of testing

Waking cortisol and CAR normal

Low afternoon, high bedtime



Headache - onset 6pm between afternoon and night samples

The Flat-Line Curve's Link to Chronic Stress

Why isn't the negative feedback loop informing the brain that ACTH stimulation is needed?

Chronic Stressors → HPA Axis Dysfunction

How?

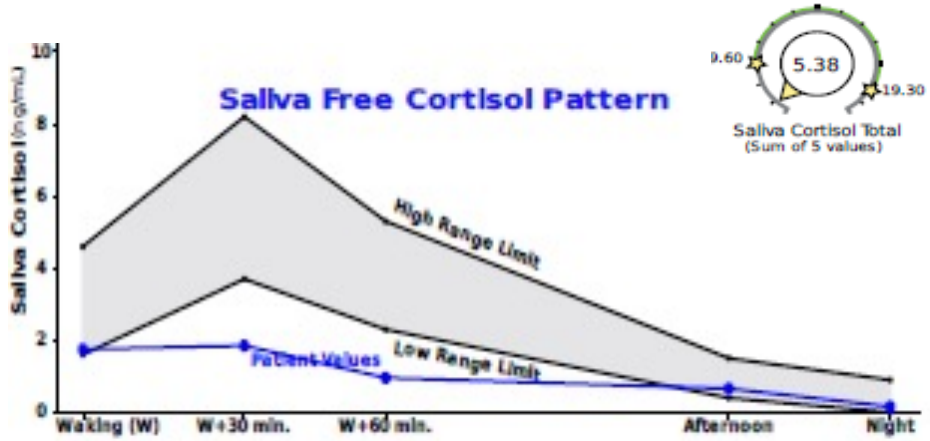
Glucocorticoid Resistance

Hypothalamic sensitivity ↓

- Cytokines ↑ SAPK/JNK gene expression and stress-activated protein kinases block GC binding to central GC receptors

Peripheral sensitivity ↓

- Cytokines slowly ↑ GRβ expression over GRα in target tissues leading to peripheral GC resistance over time



Examples of Therapeutic Considerations - Diurnal Irregularities

- Morning Light Therapy
- Sleep Hygiene Practices; napping ok if sleep problems are sporadic but minimize if chronic
- Regular Aerobic Exercise
- Insulin/Glucose-Regulating Dietary Plan
- Manage Chronic Infections
  - Dental abscess, Periodontitis, Intracellular pathogens
- Stress Modulating Practices
  - Journaling, Meditation, Breathing, Mindfulness, HRV-Biofeedback, Emotional Freedom Technique, Music Therapy, Dance, Being Outside…
- Probiotics
  - Bifidobacterium longum 1714, Lactobacillus plantarum PS128, and more – choose your favorites
- Herbal Adaptogens (modulate cortisol rhythm) and:
  - Promote GABA system: Ashwagandha, Relora/Magnolia, Bacopa, L-theanine, Lemon Balm, Holy Basil, Jujube, Skullcap
  - Stimulate Sympathetic NS: Rhodiola, Schisandra, Ginsengs
  - Immunomodulate: Rehmannia, Cordyceps, Shiitake/Maitake/Reishi, Licorice extract
  - Androgen supportive: Ashwagandha, Shatavari, Maca, Epimedium, Tribulus, Fenugreek
  - Nourish/Repair Hippocampus: Ginkgo, Bacopa, Reishi, Lion's Mane, Curcumin, Eleuthrococcus, Rhodiola, Schisandra
- Nutritional Adaptogens
  - Phosphatidylcholine, DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), Pantothenic Acid, Zinc, Magnesium….

Cortisol vs. Cortisone

When Does Evidence of Cortisone Formation Matter?

What's Cortisone? Inactivated Cortisol

Cortisol

Binds GC receptors and response elements all over the body, turning on genes that control, set, and maintain HOMEOSTATIC activities

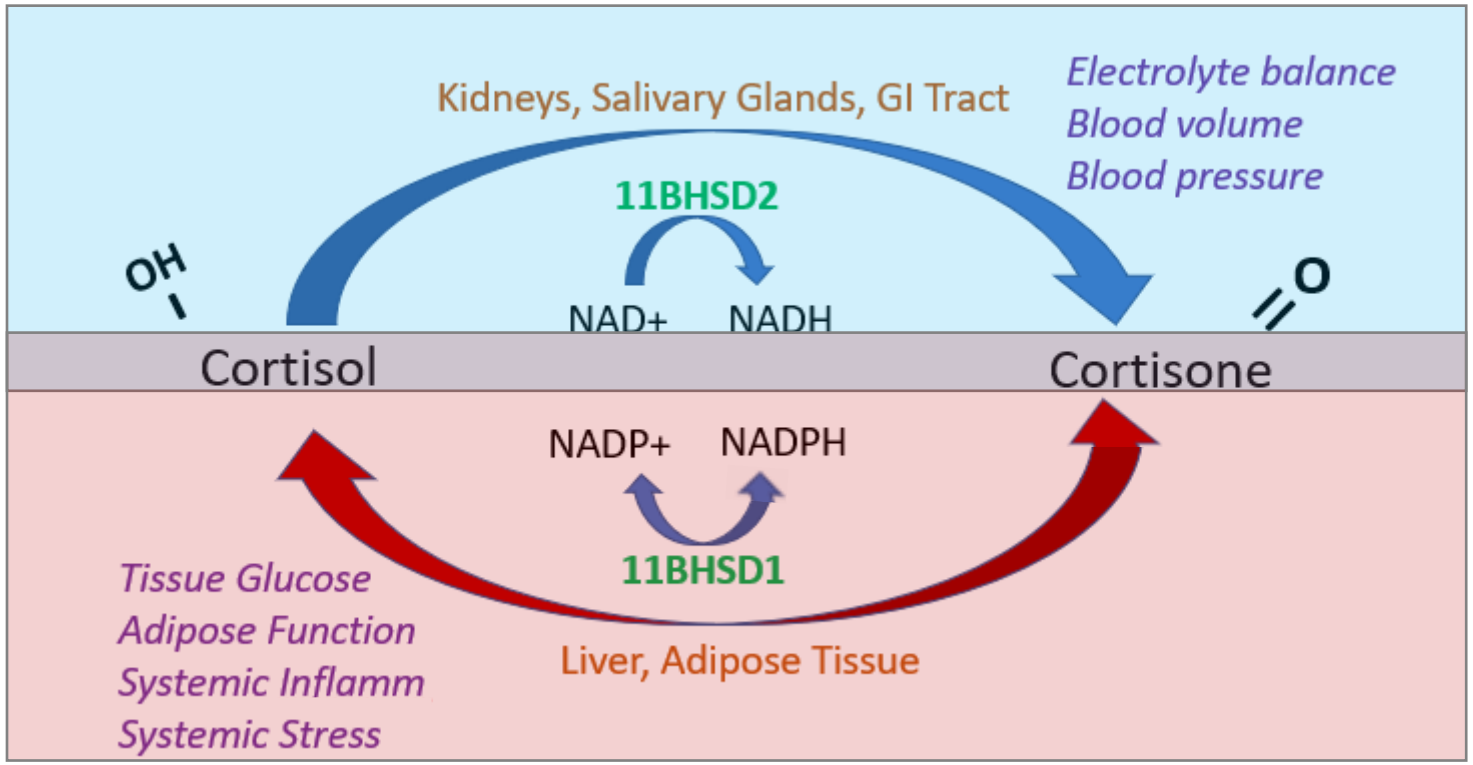
Cortisone

- OXIDIZED form of cortisol - INACTIVE

Cortisol

- REDUCED form of cortisone - ACTIVE

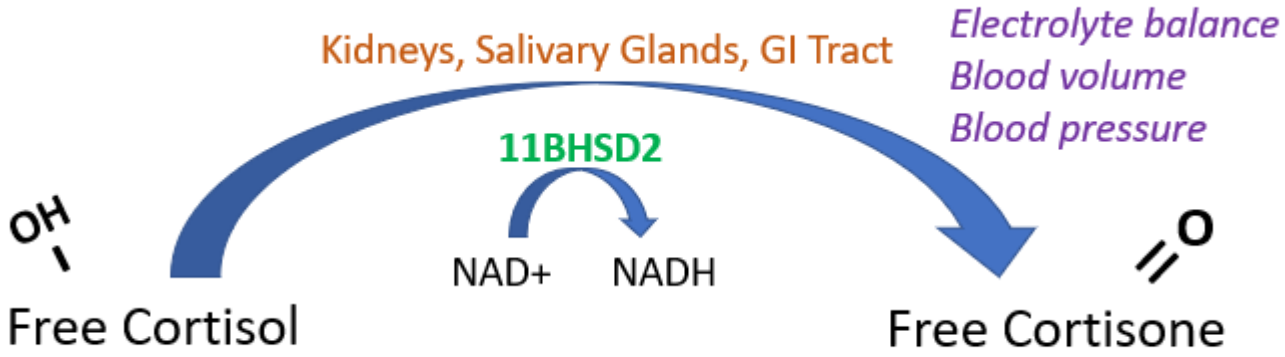
11β–Hydroxysteroid Dehydrogenase (11βHSD)

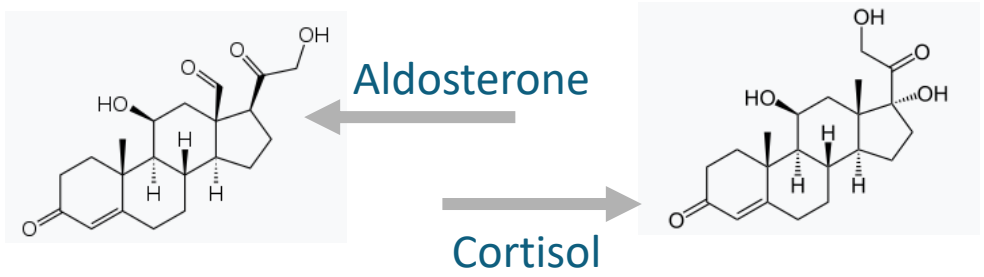


11BHSD2 Forms CORTISONE

Cortisone is formed from cortisol locally within tissues

- Kidney (urine), Salivary gland (saliva), GI (stool) – anywhere electrolyte balance is controlled (11BHSD2 enzyme oxidizes only)
  - These tissues are **mineralocorticoid receptor-rich**
  - MRs may be bound by cortisol but NOT CORTISONE (protects blood pressure)
  - 11BHSD2 activity in the kidney helps ensure that only aldosterone can bind MRs



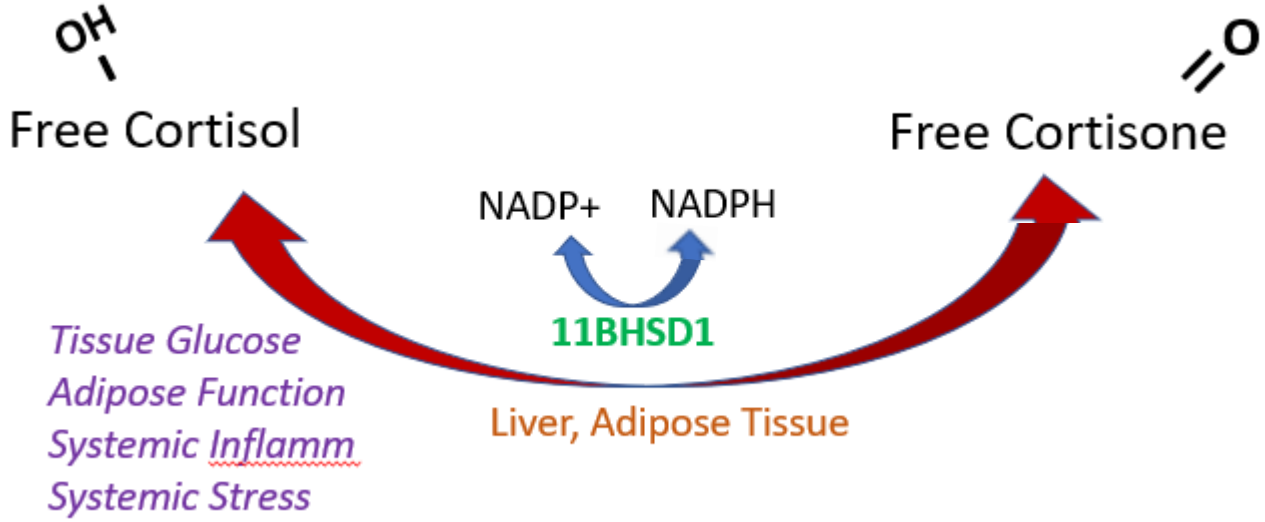


Structures:
https://en.wikipedia.org/wiki/Cortisol
https://en.wikipedia.org/wiki/Aldosterone

11BHSD1 Forms CORTISOL and CORTISONE

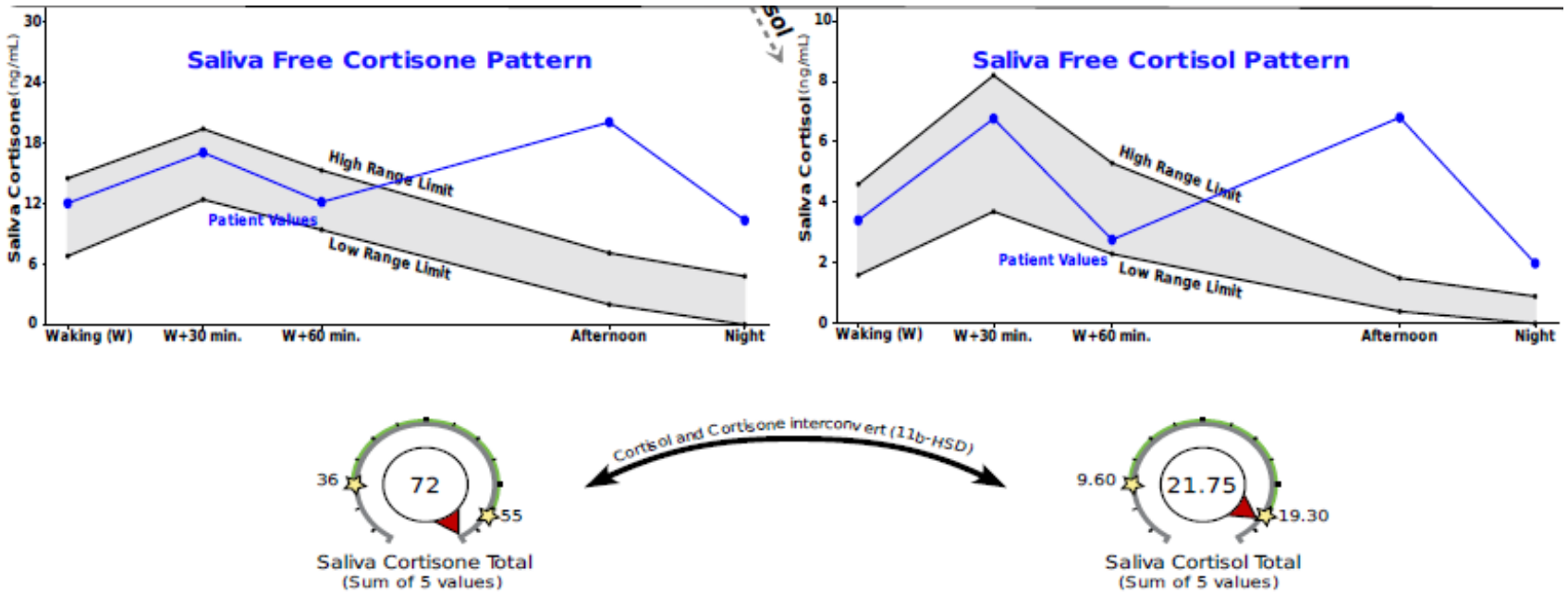
Cortisone is formed from cortisol locally within tissues

- Oxidation to Cortisone can happen in the liver and adipose tissues
- Reduction back to Cortisol can ALSO happen in the liver and adipose tissues
  - 11BHSD1 enzyme actually works both ways – it can both reduce and oxidize to generate cortisol OR cortisone depending on needs and NADP/NADPH availability



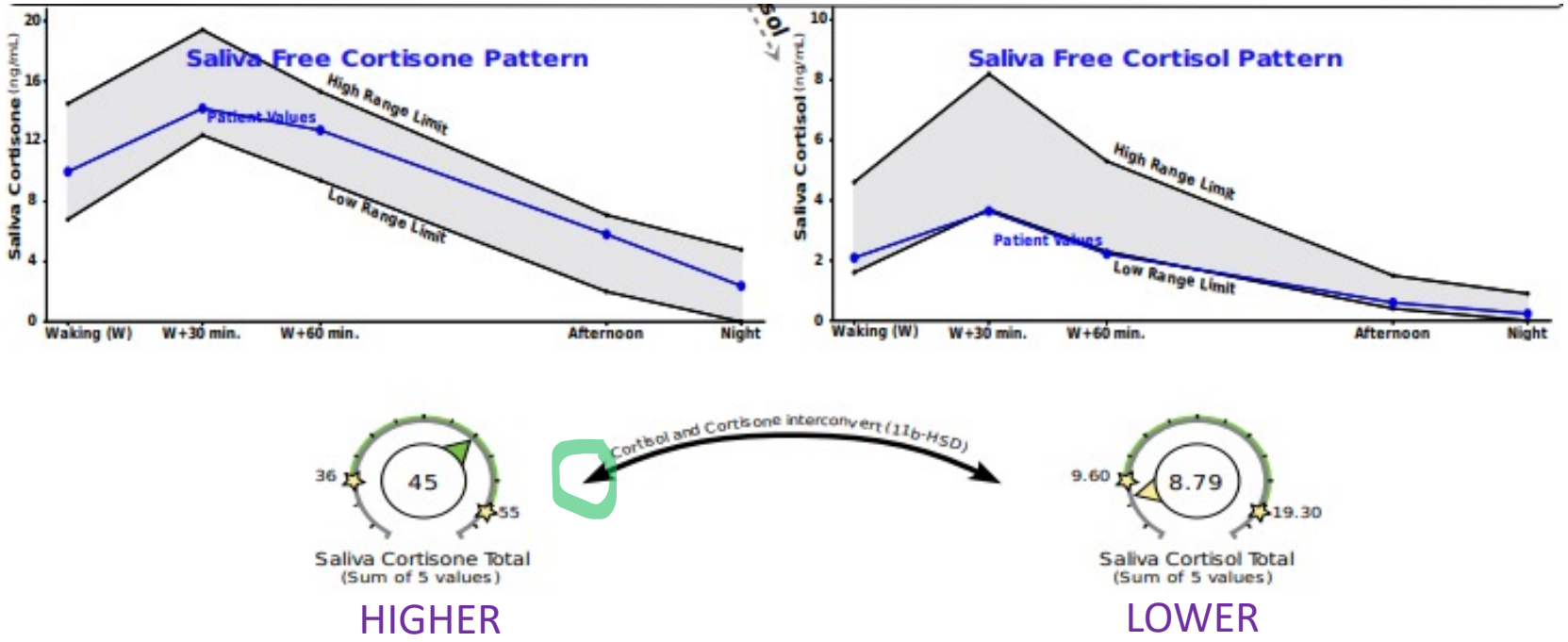
Cortisone is Constant in Serum

- Cortisone in serum does NOT have a diurnal pattern
  - BUT because of cortisol's diurnal rhythm and local 11BHSD2 activity within the salivary glands and kidney, Cortisone in saliva and urine has a diurnal pattern that reflects Cortisol's



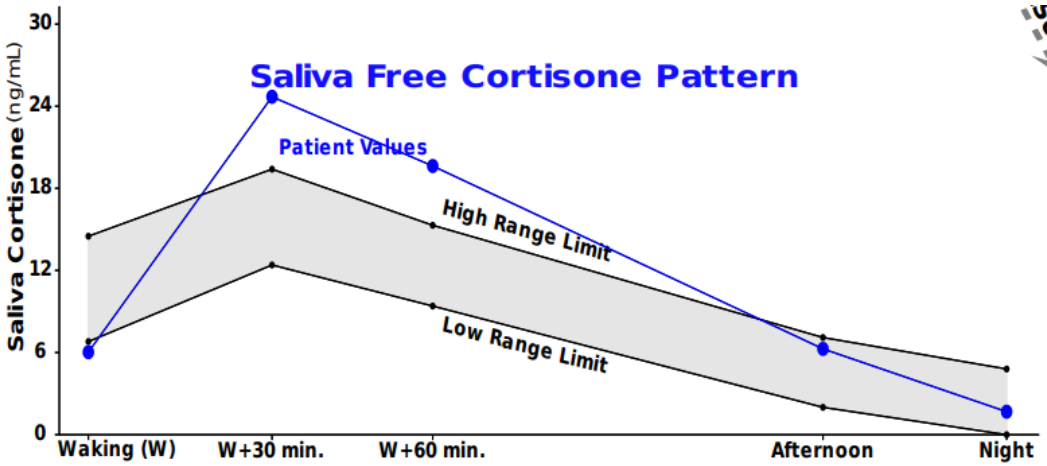
Patients with HIGH 11BHSD2 Activity

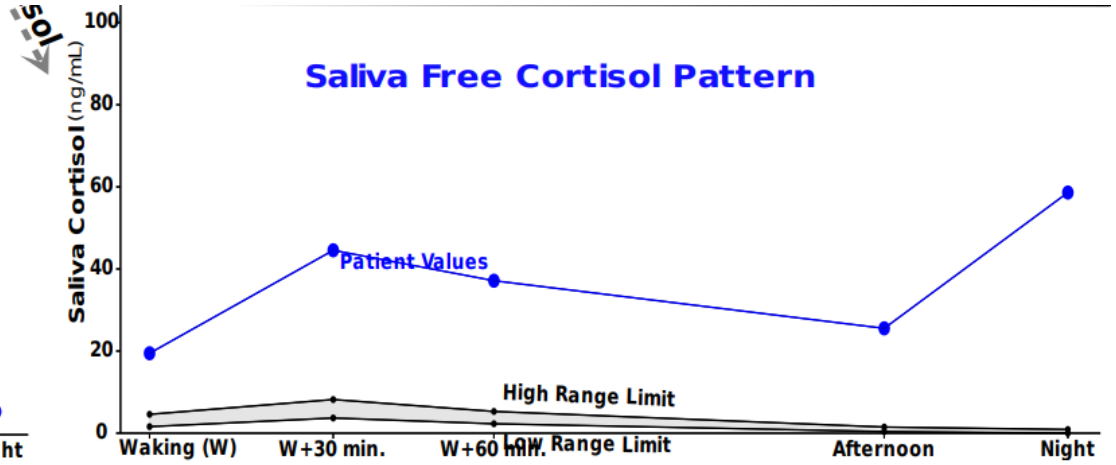
- Free Cortisol LOW and flat compared to Free Cortisone
- MUST consider free cortisone pattern as a surrogate for cortisol's
- Causes of high 11BHSD2: Blood pressure meds, obesity, electrolyte imbalance

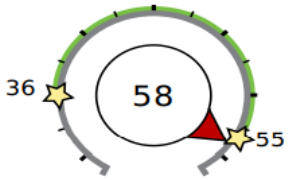


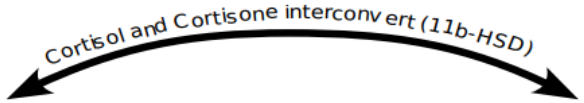
Perogamvros I, et al. Salivary cortisone is a potential biomarker for serum free cortisol. J Clin Endocrinol Metab. 2010;95(11):4951-4958.

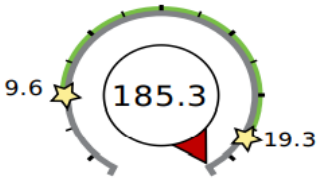
Patients using Topical Hydrocortisone





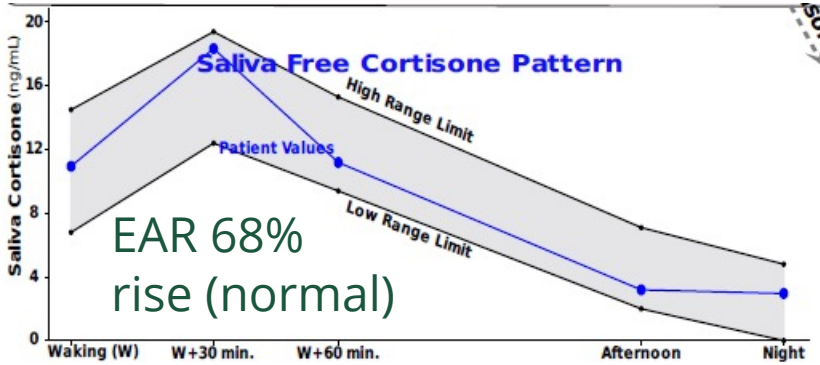


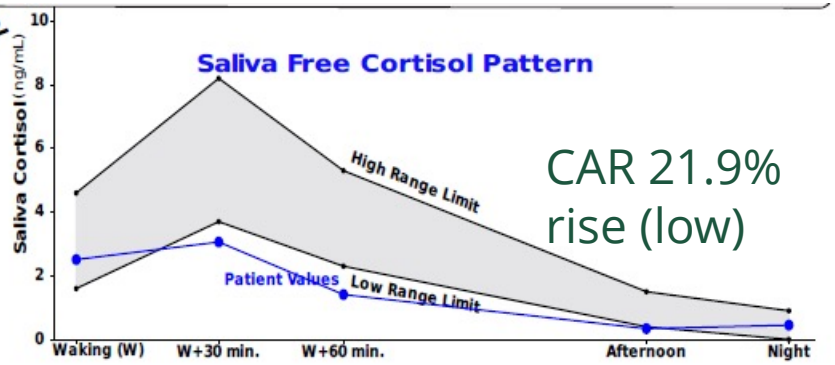




Perogamvros I, et al. Salivary cortisone is a potential biomarker for serum free cortisol. J Clin Endocrinol Metab. 2010;95(11):4951-4958.

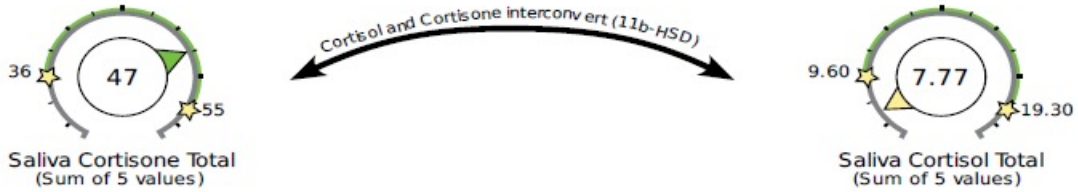
Assess the EAR with the CAR when 11BHSD2 is High?





Calculate the EAR!

(W+30 – W)/W x 100



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This patient shows a salivary cortisol of 1.41 measured 60 minutes after waking. Generally this result is a little higher than the waking sample but is not in this case. To date, data suggests that expected results may be 0-70% higher, and this guideline is considered for research only.

New Study!

We may be able to use the EAR as a surrogate for the CAR when 11BHSD2 is high!

Anderson T, et al. The association between the cortisol and cortisone awakening responses. Psychoneuroendocrinology. 2023.

Cortisol Metabolism Irregularities

Free Corts VS Metabolized Corts

Tetrahydrocortisol + Tetrahydrocortisone

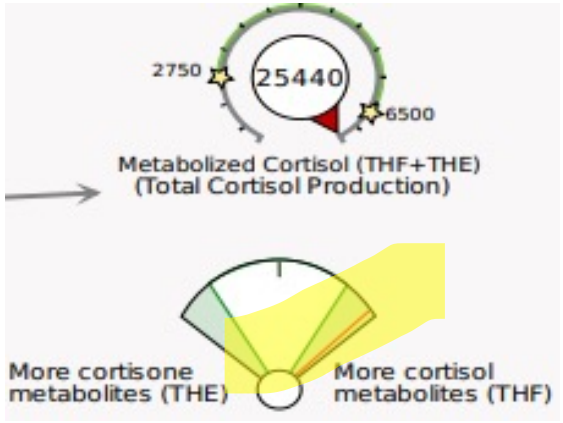
THF

THE

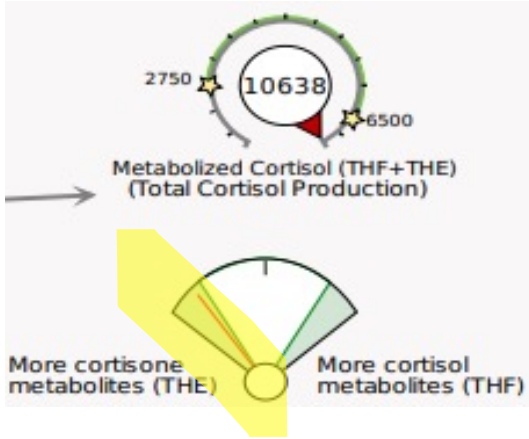
Formed Where	How	Why
• Liver • Adipose • Directly from uptake of circulating cortisol and cortisone	• 11βHSD1 Cortisol ⟷ Cortisone • 5α-Reductase • 5β-Reductase	• Cortisol's total production + clearance x 24 hours • Normal vs Hypo vs Hypermetabolism

Cortisol Metabolism SHIFTS

Acute Stress



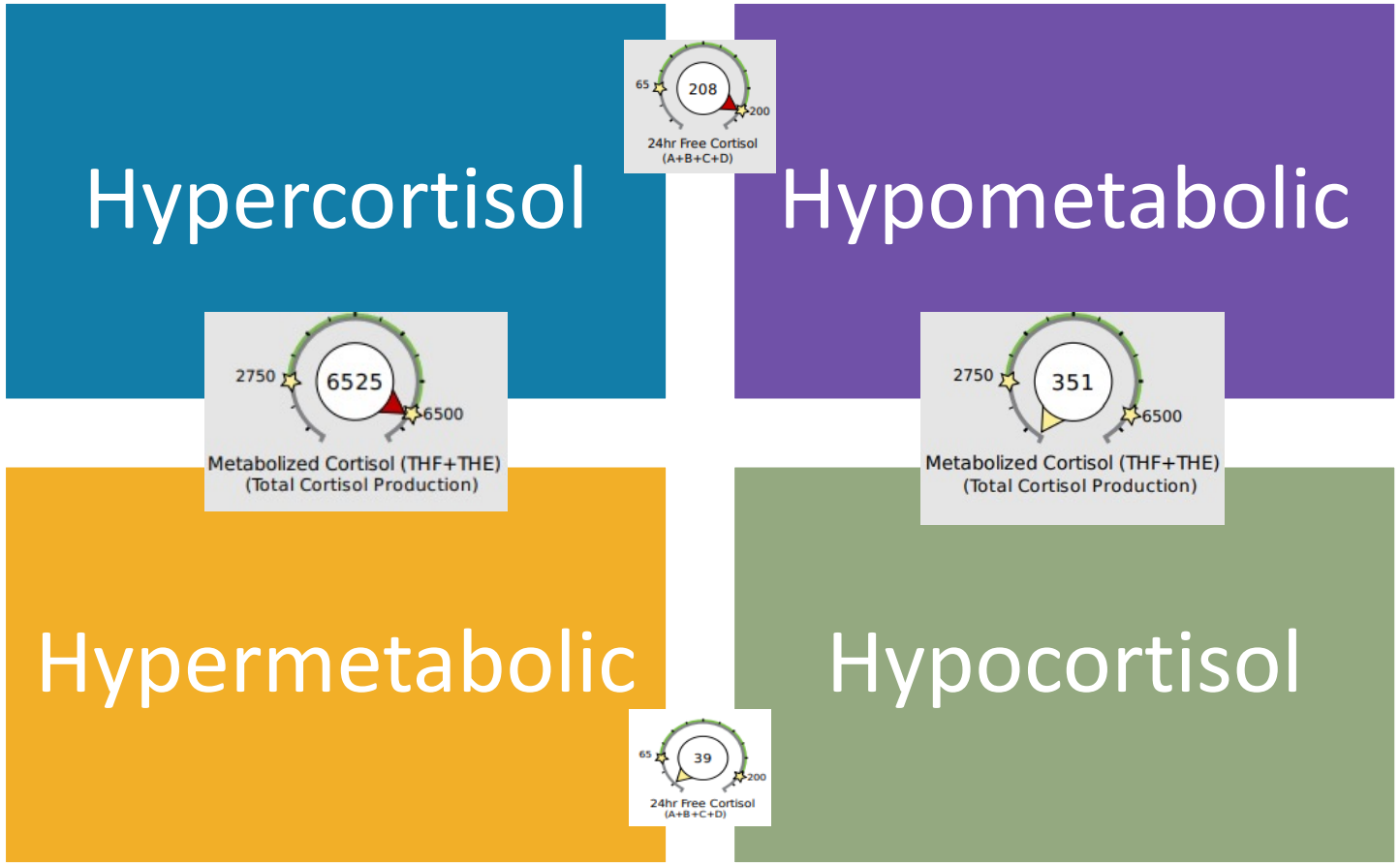
Chronic Stress*



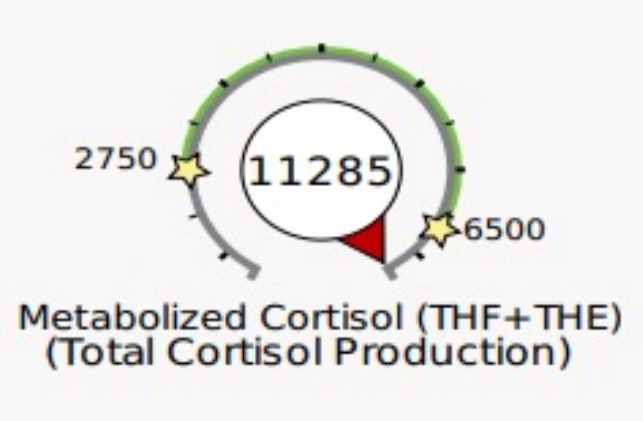
*Or hypermetabolism in HYPERTHYROIDISM

Chapman K, et al. 11beta-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. Physiol Rev. 2013;93(3):1139-1206.

4 Cortisol Metabolic Patterns



High Metabolized Cortisol



High Metabolized Cortisol Considerations

Can Indicate:

1. High Cortisol *Production*
   (Free cortisol is low, normal or high)

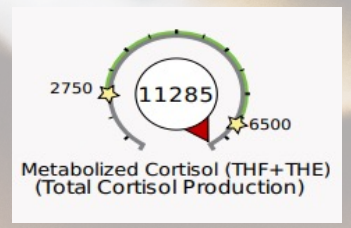
and/or

2. High Cortisol *Clearance*
   (Free cortisol contrasts low)
   High 11βHSD1 Activity

Top Considerations:

- Inflammation
- Insulin Resistance
- Infection
- Obesity
- Fatty Liver
- Elevated Leptin
- Sleep Apnea
- Hyperthyroid

High Metabolized Cortisol = High 11βHSD1 Activity in Obesity



- 11βHSD1 enzyme present tissues (and others) in the LIVER, ADIPOSE, breast, and ovarian
- Requires NADP+ (generated by HMP aka PP shunt)
- Synthesizes CORTISOL from cortisone in the presence of NADP+
  - And can return cortisol → cortisone in the presence of NADPH
- Involved in "Extra-Adrenal" Cortisol generation
  - Cortisol then acts locally and metabolizes to THF or converts back to cortisone and metabolizes to THE (via 5α- and 5β-Reductase)
- Associated with insulin resistance and adipose tissue dysfunction
- Waist circumference is a significant predictor of 11BHSD1 activity/high cortisol and cortisone metabolites

Engeli S, et al. Regulation of 11beta-HSD genes in human adipose tissue: influence of central obesity and weight loss. Obes Res. 2004;12(1):9-17.
Simonyte K, et al. Obesity is accompanied by disturbances in peripheral glucocorticoid metabolism and changes in FA recycling. Obesity (Silver Spring). 2009.

What Increases 11BHSD1 Expression/Activity?

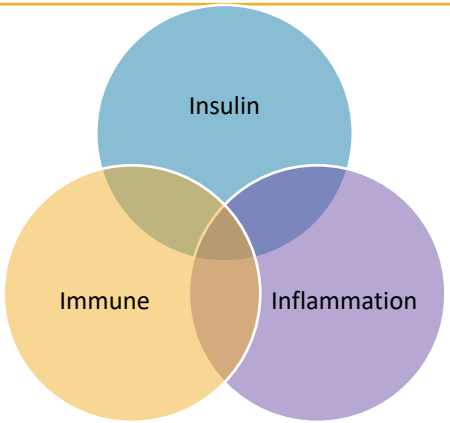
Poss Modifiable Factors
Inflammation
Insulin Resistance
Oxidative Stress
Central Adiposity
Injury
Infections

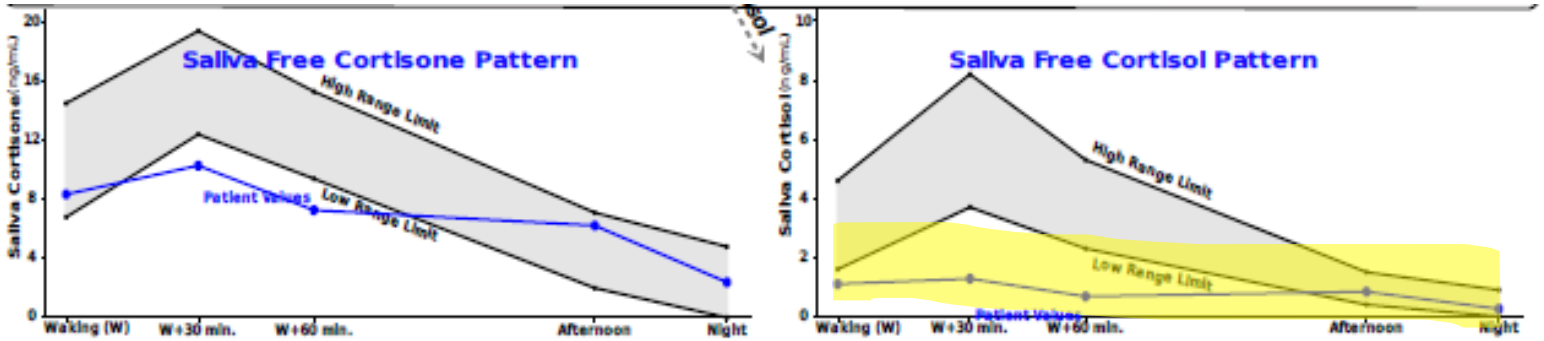
Genetics/Epigenetics
rs846910
rs1206634
rs4844880
rs3753519
Maternal factors during gestation: prenatal GCs, malnutrition

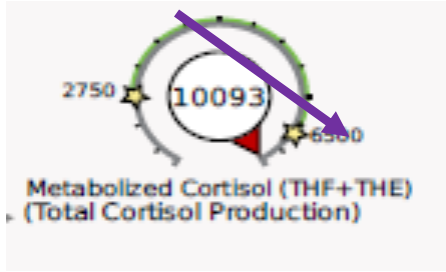
Gambineri A, et al. A combination of polymorphisms in HSD11B1 associates with in vivo 11βHSD1 activity and metabolic syndrome in women with and without PCOS. Eur J Endocrinol. 2011.

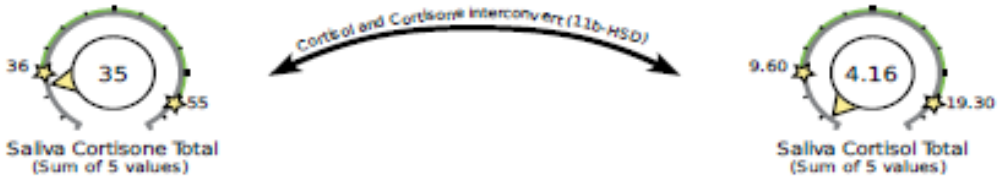
Hypermetabolism Assoc with Obesity

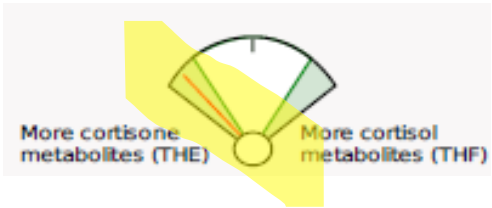
- Example: 28yoF, BMI 42
- Shift to free cortisone
- High metabolized cortisol, shift to THE







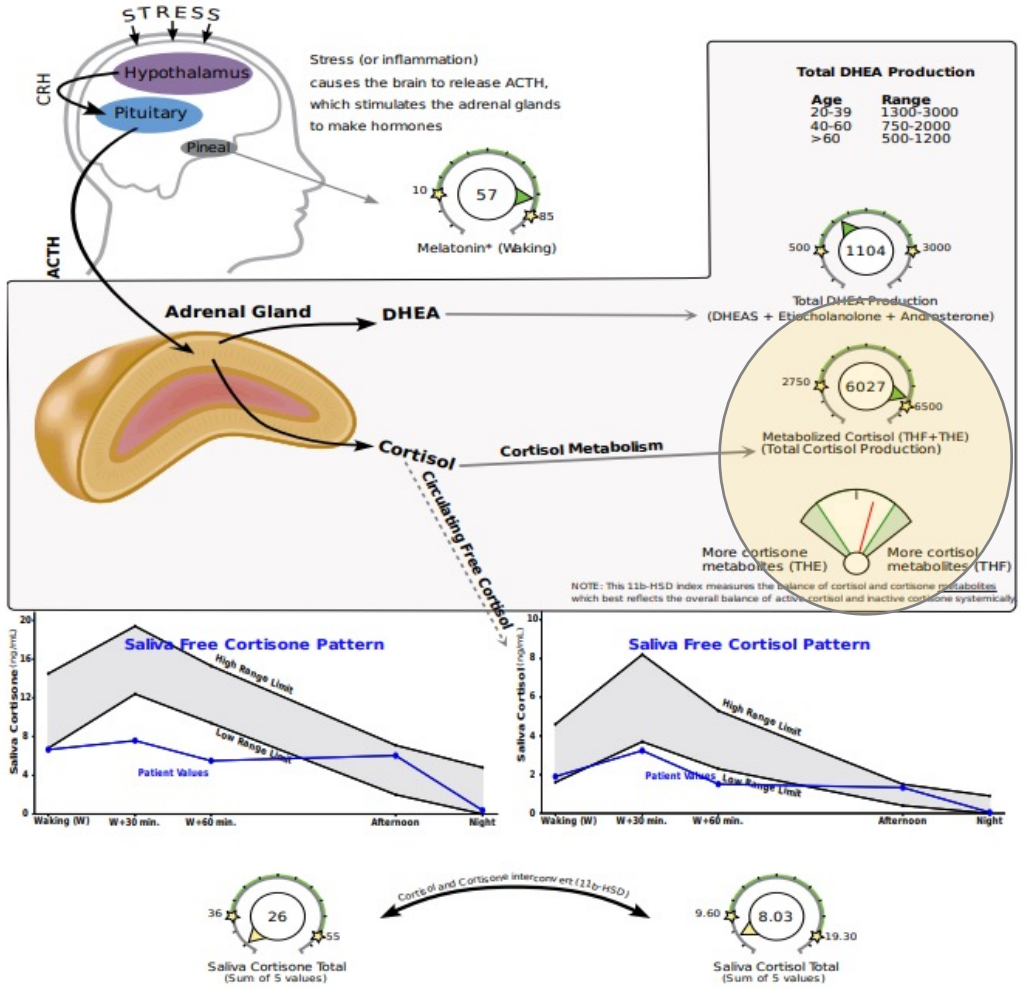




27 yo Female with irregular cycles and severe PMS

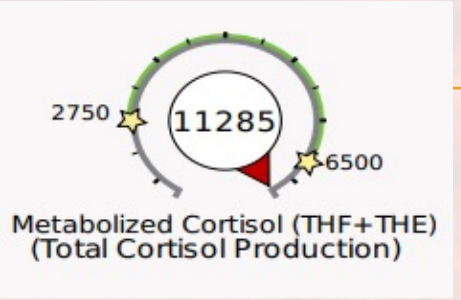
- Impressions:

- HPA axis dysfunction unlikely
  - CAR % rise is normal
- Hypermetabolic Cortisol Pattern
  - Cortisol is being taken up at the liver/adipose and cleared quickly
  - Consider insulin resistance, inflammation, and immune activation
  - Treat the cause



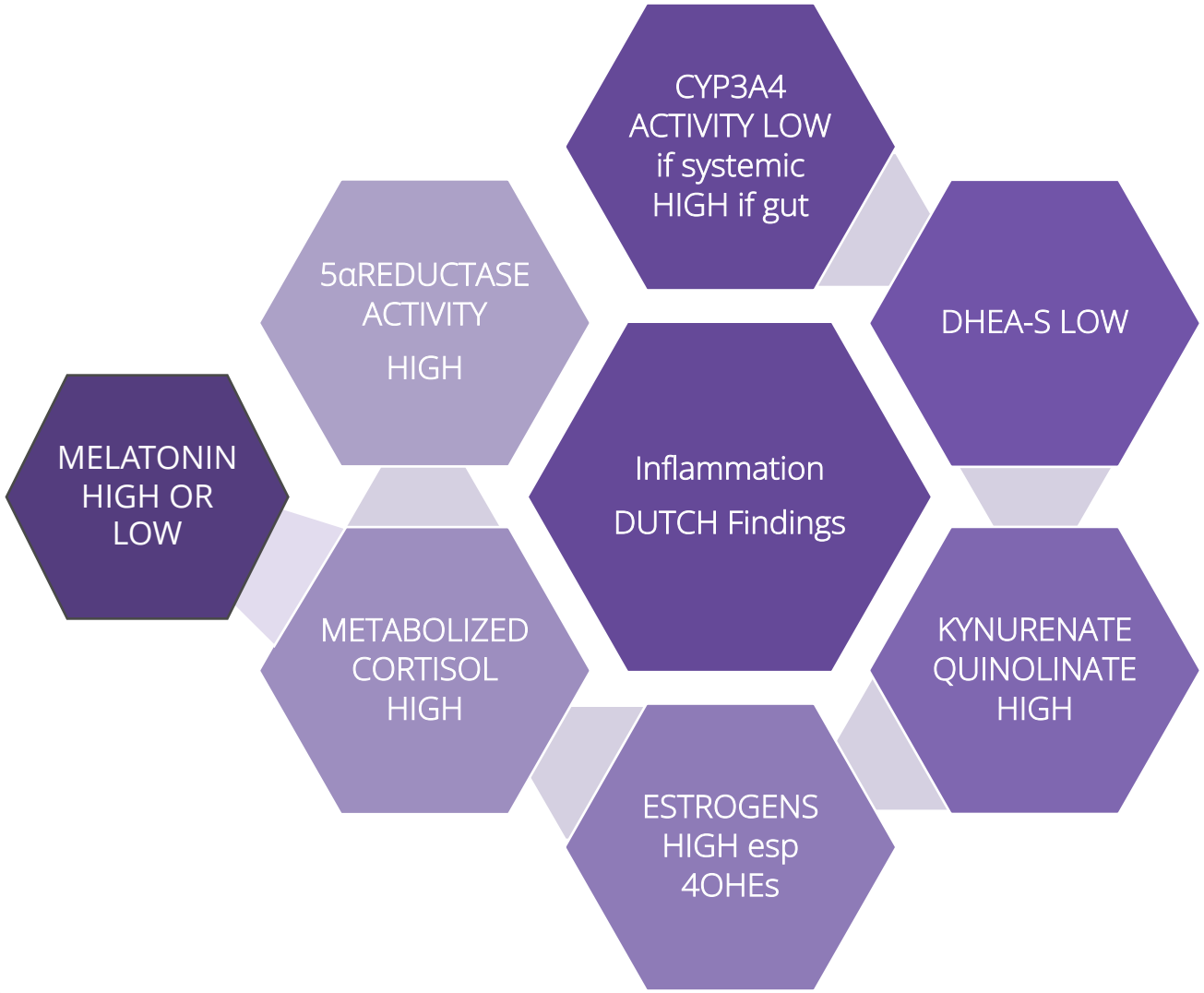
The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. **This patient shows a waking cortisol of 1.90 and an increase to 3.24 after 30.0 minutes. This is an increase of 1.34ng/mL or 70.5%.** Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common with samples collected 30 minutes after waking. These guidelines are considered research only.
This patient shows a salivary cortisol of 1.51 measured 60 minutes after waking. Generally this result is a little higher than the waking sample but is not in this case. To date, data suggests that expected results may be 0-70% higher, and this guideline is considered for research only.

Treatment Goals – Solve the Tissue Issue



- Sleep issues in this group may be due to:
  - Inflammation, Oxidative Stress, Insulin Resistance, or -
- Assess for sleep apnea
  - Wearables can help, sleep studies are getting less invasive
- Rule out hyperthyroid and autoimmune thyroiditis
- Assess for metabolic syndrome (leptin resistant/cortisone dominant)
  - Especially if weight loss not forthcoming with best efforts → PreDM, T2DM
  - **Direct 11BHSD1 inhibiting herbs:**
    - **Bitter Melon, Cinnamon, Curcumin, EGCG**

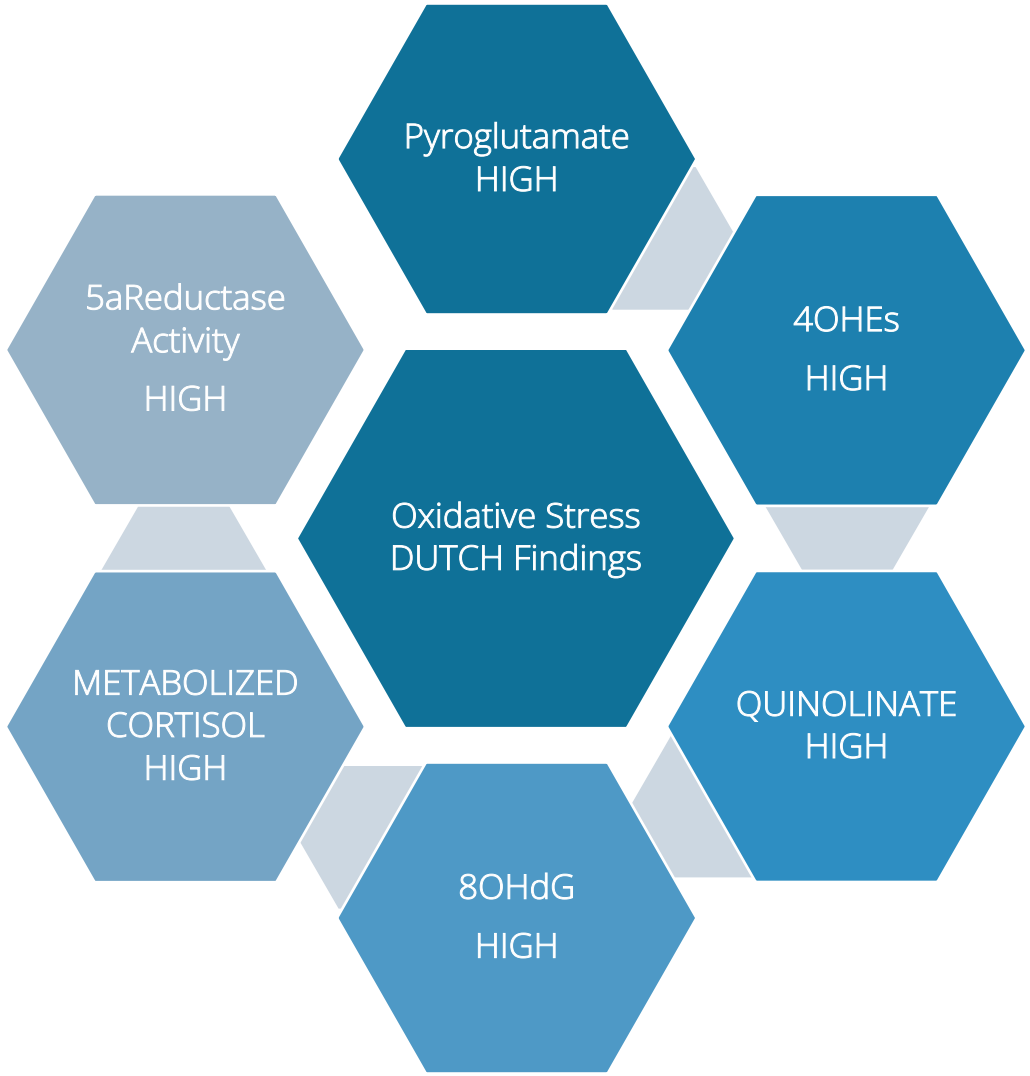
DUTCH Indicators of Chronic Inflammation



Anti-Inflammatory Supports

- Boswellia
- Curcumin
- EGCG (Green Tea Extract)
- Enzyme Therapy
  - Bromelain, Lumbrokinase, Nattokinase, Pancreatic Enzymes, Wobenzym
- Garlic
- Ginger
- Maca
- Medicinal Mushrooms
  - Lion's Mane, Maitake, Reishi, Shiitake
- Melatonin
- MSM
- Myoinositol
- Oils/Fatty Acids
  - EPA, DHA, SPMs, Borage, Evening Primrose
- Probiotics
- Rosemary
- Scutellaria baicalensis
- Quercetin
- Resveratrol
- Vitamins A, D, E

DUTCH Indicators of Oxidative Stress



Antioxidant Supports

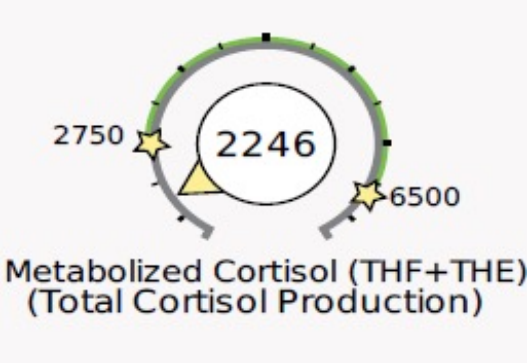
- R-alpha lipoic acid
- CoQ10
- Curcumin
- Berries
- EGCG (Green Tea Extract)
- Glutathione
- Grapeseed
- Greens
- Minerals – Mag, Zinc
- Myoinositol
- NAC
- Pomegranate
- Quercetin
- trans-Resveratrol
- Rosmarinic Acid (Rosemary)
- Selenium
- SOD
- Taurine
- Vitamins – A, C, E

DUTCH Indicators of Insulin Resistance

Blood Sugar Supports

- R-alpha lipoic acid
- Berries
- Bitter Melon
- Carnosine
- Cinnamon
- Curcumin
- Eleuthrococcus
- EGCG (Green Tea Extract)
- Fenugreek
- Ginseng (Asian)
- Gymnema
- Minerals – Cr, Mag, Sel, Zinc/Copper
- Myoinositol
- Pomegranate Juice
- Quercetin
- Reishi
- Rosmarinic Acid (Rosemary)
- Vanadium
- Vitamin D

Low Metabolized Cortisol



Low Metabolized Cortisol

Can Indicate:

1. Low Cortisol *Production*
   (Free cortisol correlates low)

and/or

2. Low Cortisol *Clearance*
   (Free cortisol contrasts high)

Top Considerations:

Hypothyroid (even subclinical)

Anemia (iron deficiency)

Mitochondrial dysfunction

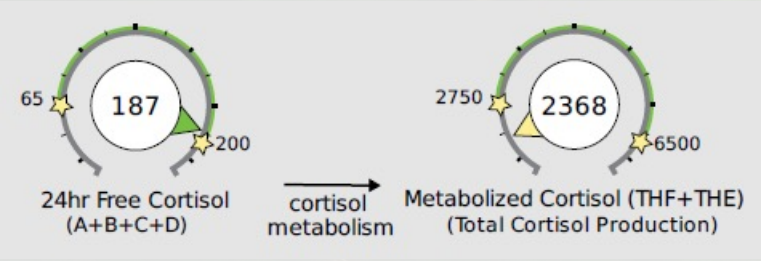
Liver/Gallbladder Stasis

Low caloric intake

HPA Axis Dysfunction

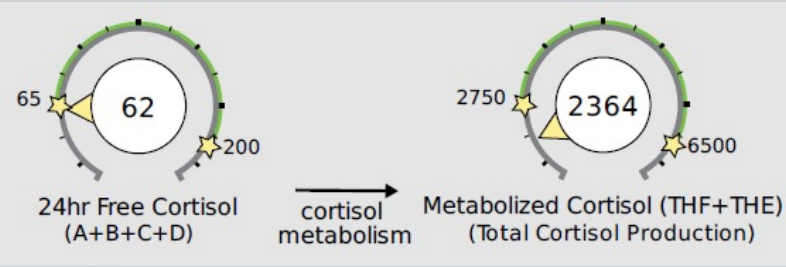
Medications (opiates, steroids, lamotrigine…)

Treatment Goals Hypometabolic Pattern – ↑ BMR



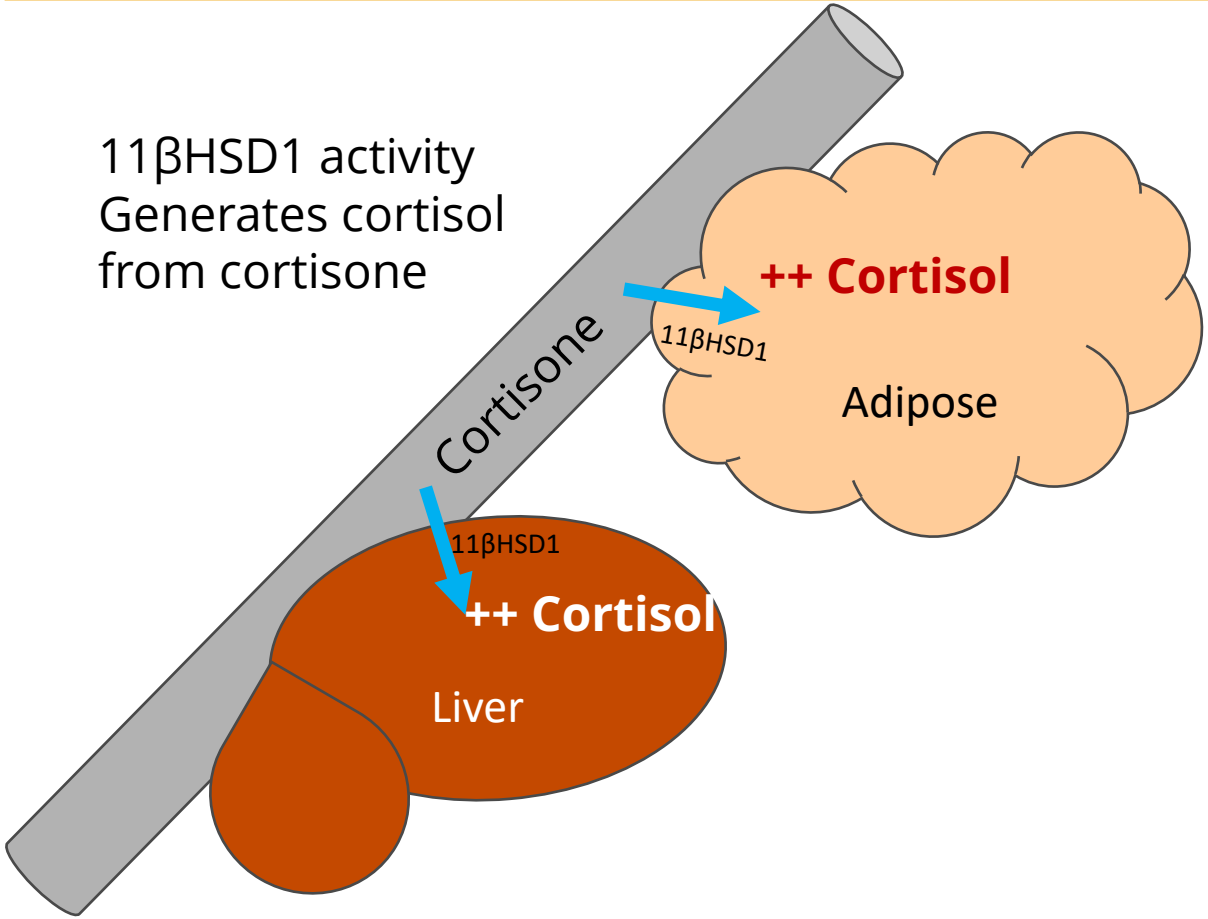
- Disturbed Sleep in this group may be due to:
  - Deficiencies: Iron, B12, Folate, Vitamins A and D
- Increase thyroid hormone activity
  - Thyroid hormone replacement
    - Iodine therapy if low dietary intake
  - T4→T3 conversion supports
    - Vitamins A, C, D, E, Selenium, Zinc, B vits
- Increase mitochondrial function
  - CoQ10, ALA, Carnitine, Antioxidants, NADH, Taurine
  - If there's toxicity, detox and rebuild
  - Exercise (from yoga → HIIT, trial and individualize, reverse dieting)
- Trophic supports – Feed, feed, feed
  - Targeted nutrition, increase caloric intake, adrenal glandulars, B complex, C, EFAs

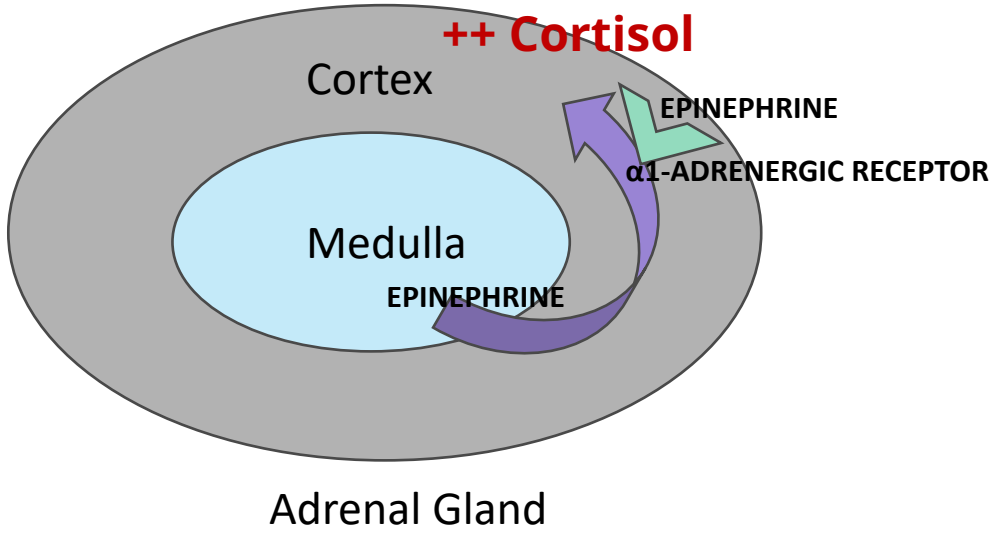
Treatment Goals – ↑ Production



- Treat chronic, underlying conditions
- Consider influence of concurrent medications known to lower HPA axis activity:
  - Examples: Corticosteroids (even hydrocortisone injections within the last 4-6 weeks prior to testing), Cannabis use, Lamictal/Lamotrigine, Opiates, SSRIs
- Disturbed Sleep in this group may be due to:
  - Deficiencies: Iron, B12, Folate, Vitamins A and D, lutein, zeaxanthin
- Mitochondrial Supports
  - CoQ10, ALA, Carnitine, Antioxidants, NADH, Taurine
  - Sex hormone and/or androgen replacement
- Trophic Supports
  - B6, B9, B12, C, EFAs, glandulars
- Increase cortisol effects
  - Licorice Extract, Grapefruit Juice, DIM
- Energetic Field Supports
  - Gemmos – Ribes/Sequoia/Quercus, Spagyrics, Homeopathics
- Energizing Adaptogens
  - Cordyceps, Ginsengs, Epimedium, Rhodiola, Maca, Shatavari
- Reconnect Brain-Body
  - Vibration, Biofeedback/HRV training, Meditation, Breathing, Gargling, Sleep Hygiene practices

Hypothalamus Isn't the ONLY Driver of Cortisol Production

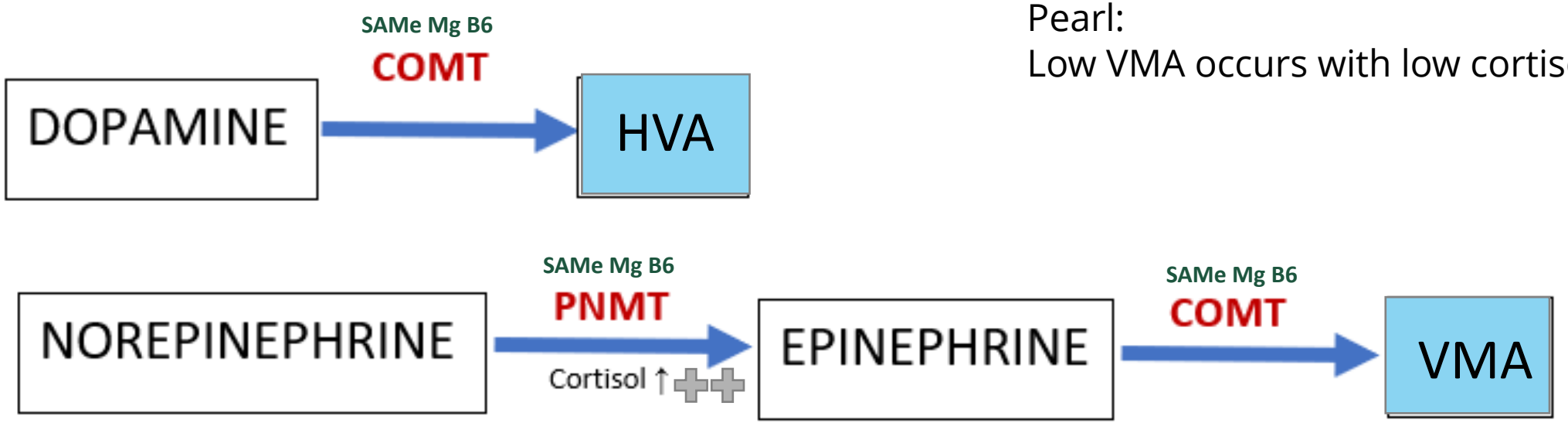




Adrenal Gland

Ehrhart-Bornstein M, et al. Neurotransmitters and neuropeptides in the differential regulation of steroidogenesis in adrenocortical-chromaffin co-cultures. Endocr Res. 2000 Nov;26(4):833-42

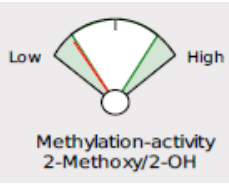
SNS Neurotransmitter Metabolism



Pearl:
Low VMA occurs with low cortisol

If HVA and VMA are LOW, before assuming low sympathetic tone, always go back and assess COMT activity on PAGE 3

If COMT activity on PAGE 3 is also low, consider supporting COMT activity to improve slow clearance *first*.



Inhibitory and Parasympathetic Nervous System Supports

- Sleep Hygiene Practices
- Regular Aerobic Exercise
- Stress Modulating Practices
  - Journaling, Meditation, Breathing, Mindfulness, HRV-Biofeedback, Emotional Freedom Technique, Music Therapy, Dance, Being Outside, Gargling, Havening…
- Probiotics
  - Bifidobacterium longum 1714, Lactobacillus plantarum PS128
- Melatonin and Serotoninergic Supports
  - Melatonin, 5-HTP, MTHF, B12, Rhodiola, Saffron, Tryptophan
  - Estradiol replacement inc serotonin production in brain (TrpH-2) and SERT exp, dec MAO-A/B; Testosterone replacement inc SERT expression and binding
- GABAergic Supports
  - Oral GABA, B6, Glycine, Taurine, Magnesium
  - Allopregnanolone-forming: Progesterone, Pregnenolone
- Herbal Adaptogens (modulate cortisol rhythm) and:
  - Promote GABA system: Ashwagandha, Relora/Magnolia, Bacopa, L-theanine, Lemon Balm, Holy Basil, Jujube, Skullcap
  - Nourish/Repair Hippocampus: Ginkgo, Bacopa, Reishi, Lion's Mane, Curcumin, Eleuthrococcus, Rhodiola, Schisandra
- Calming Herbs (including Adaptogens Relaxing to GABA system)
  - Catnip, Chamomile, Curcumin, Hops, Kava, Lavender, Passion Flower, Valerian
- Nutritional Adaptogens
  - Choline (PC, CDP-choline, lecithin, αGPC), DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid), Pantothenic Acid, Vitamin C, Zinc, Magnesium….

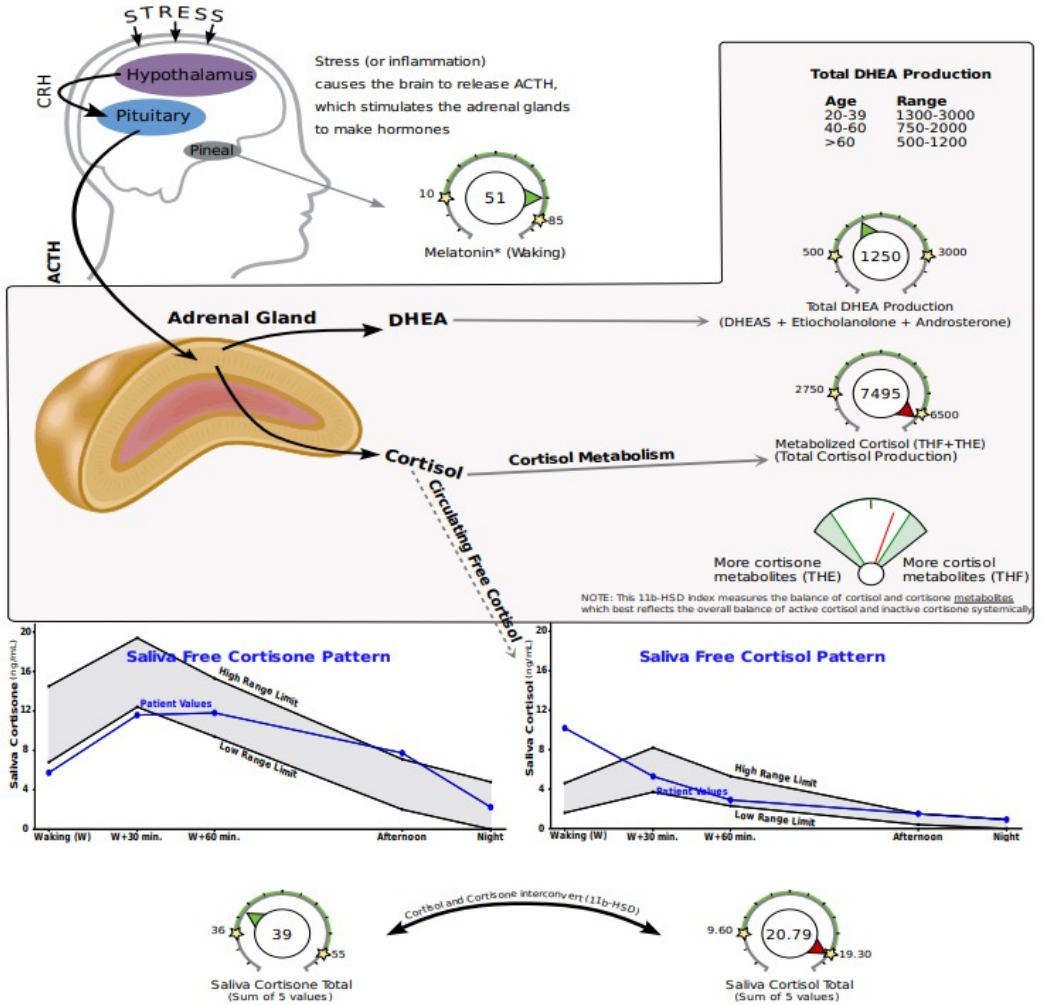
Acute Adrenal Stress

All the Highs

67 yo Female with high blood pressure + frequent and early waking

Page 5
DUTCH Plus

CORTISOL
PAGE



- The patient submitted an Insomnia salivary sample. The cortisol result for this sample was 2.49ng/mL (expected range 0-0.9) The cortisone result for this sample was 7.8 ng/mL (expected range 0-4.8)

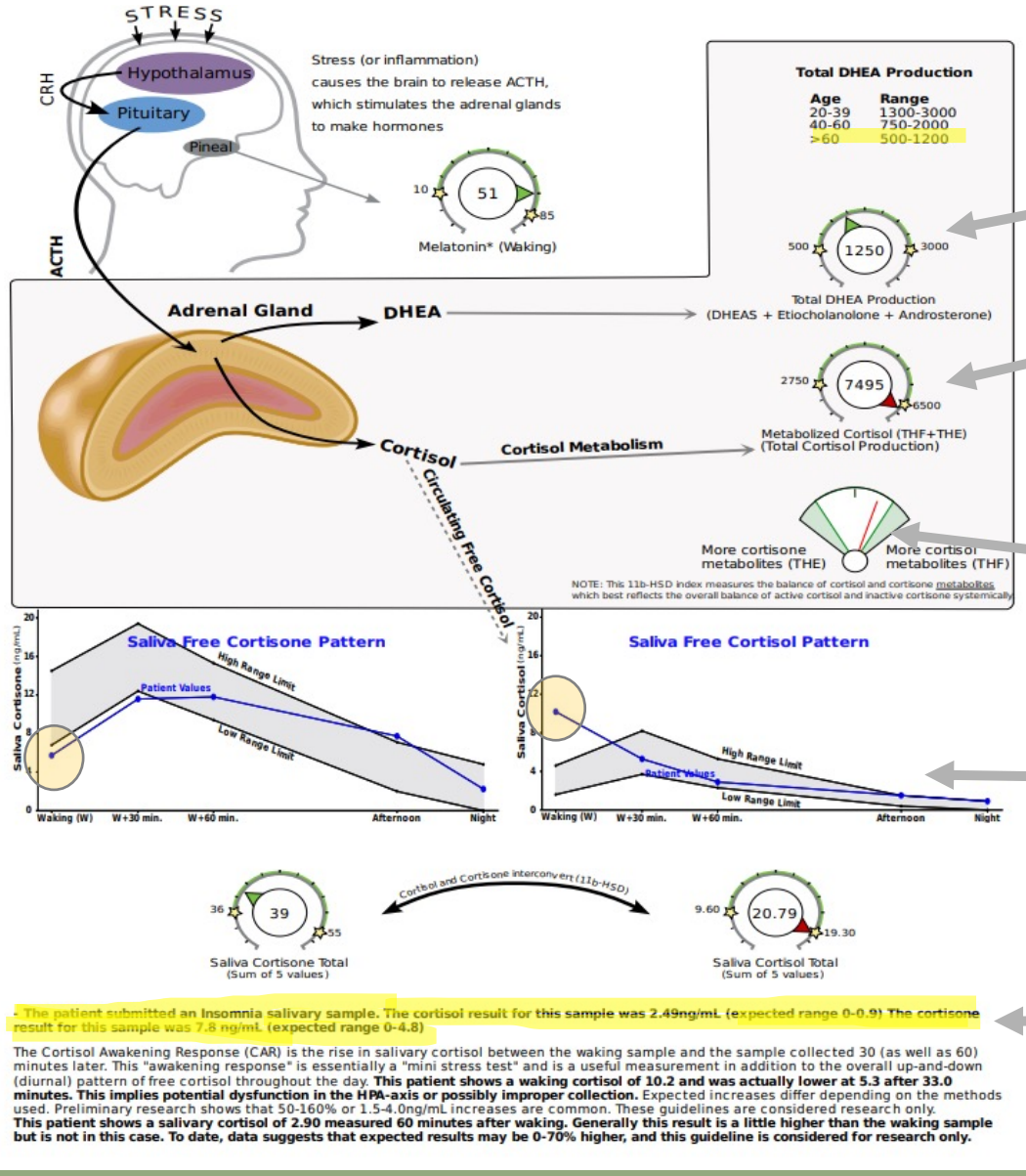
The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. **This patient shows a waking cortisol of 10.2 and was actually lower at 5.3 after 33.0 minutes. This implies potential dysfunction in the HPA-axis or possibly improper collection.** Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common. These guidelines are considered research only.
This patient shows a salivary cortisol of 2.90 measured 60 minutes after waking. Generally this result is a little higher than the waking sample but is not in this case. To date, data suggests that expected results may be 0-70% higher, and this guideline is considered for research only.

67 yo Female with high blood pressure + frequent and early waking

Page 5
DUTCH Plus

CORTISOL
PAGE

Acute
Adrenal
STRESS



Total DHEA is high for age

Cortisol metabolites elevated

Shift to THF = ACUTE STRESSORS

Free cortisols HIGH
Shift toward cortisol in the AM – aldosterone?

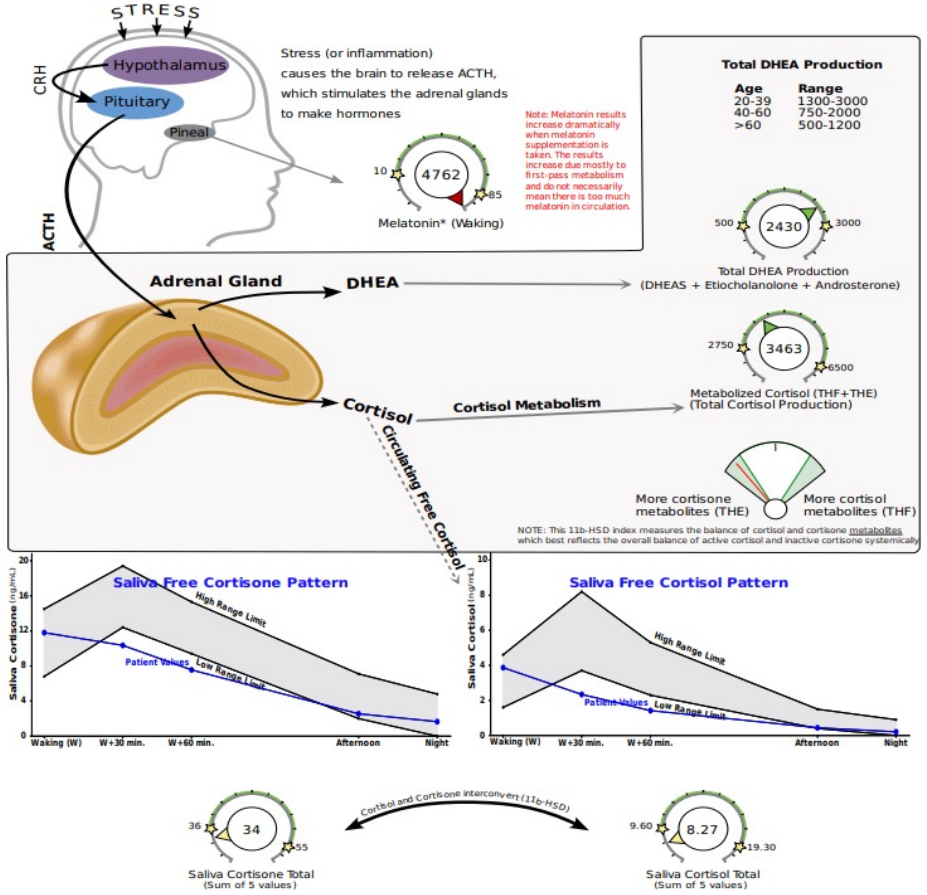
Cortisol and cortisone also high during the night

Practice Examples

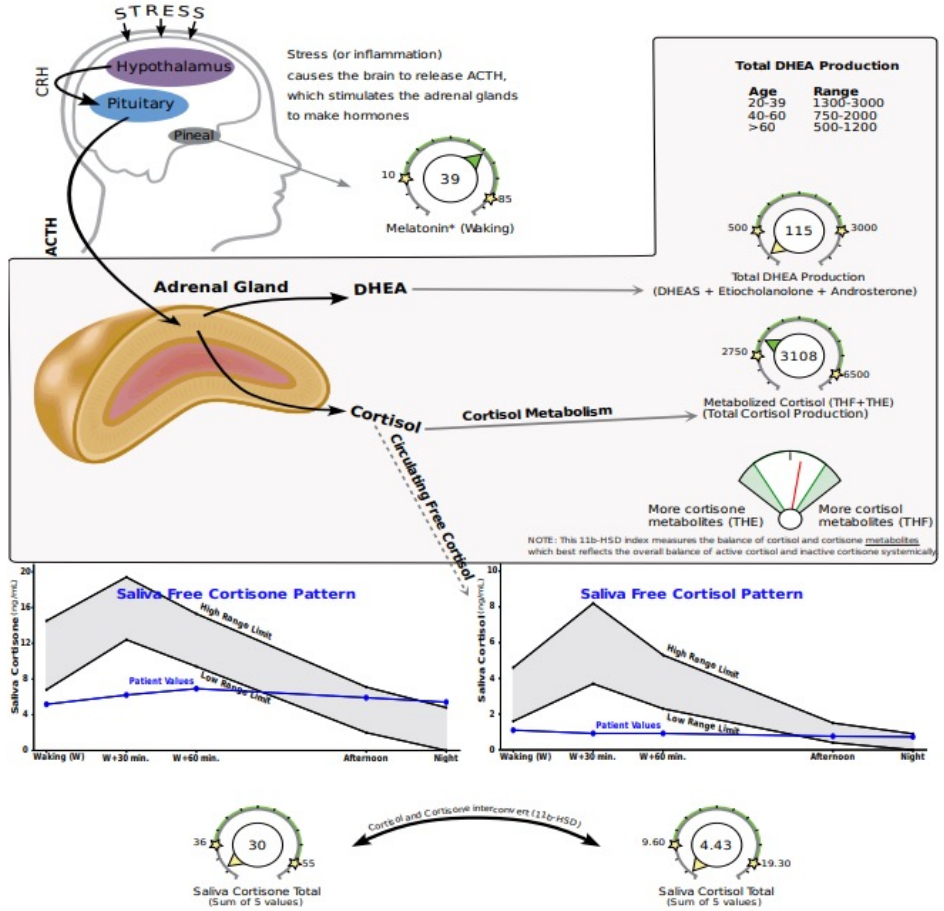
67yoF

Daytime Sleepiness

Working up for OSA



The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. **This patient shows a waking cortisol of 3.87 and was actually lower at 2.34 after 34.0 minutes. This implies potential dysfunction in the HPA-axis or possibly improper collection.** Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common. These guidelines are considered research only. **This patient shows a salivary cortisol of 1.42 measured 60 minutes after waking. Generally this result is a little higher than the waking sample but is not in this case. To date, data suggests that expected results may be 0-70% higher, and this guideline is considered for research only.**



The Cortisol Awakening Response (CAR) is the rise in salivary cortisol between the waking sample and the sample collected 30 (as well as 60) minutes later. This "awakening response" is essentially a "mini stress test" and is a useful measurement in addition to the overall up-and-down (diurnal) pattern of free cortisol throughout the day. **This patient shows a waking cortisol of 1.10 and was actually lower at 0.92 after 35.0 minutes. This implies potential dysfunction in the HPA-axis or possibly improper collection.** Expected increases differ depending on the methods used. Preliminary research shows that 50-160% or 1.5-4.0ng/mL increases are common. These guidelines are considered research only. **This patient shows a salivary cortisol of 0.92 measured 60 minutes after waking. Generally this result is a little higher than the waking sample but is not in this case. To date, data suggests that expected results may be 0-70% higher, and this guideline is considered for research only.**

46yoF

Menstrual migraines

Evening fatigue

Trouble falling asleep

Low libido

No known history of recent steroid use

Consider Melatonin Metabolite

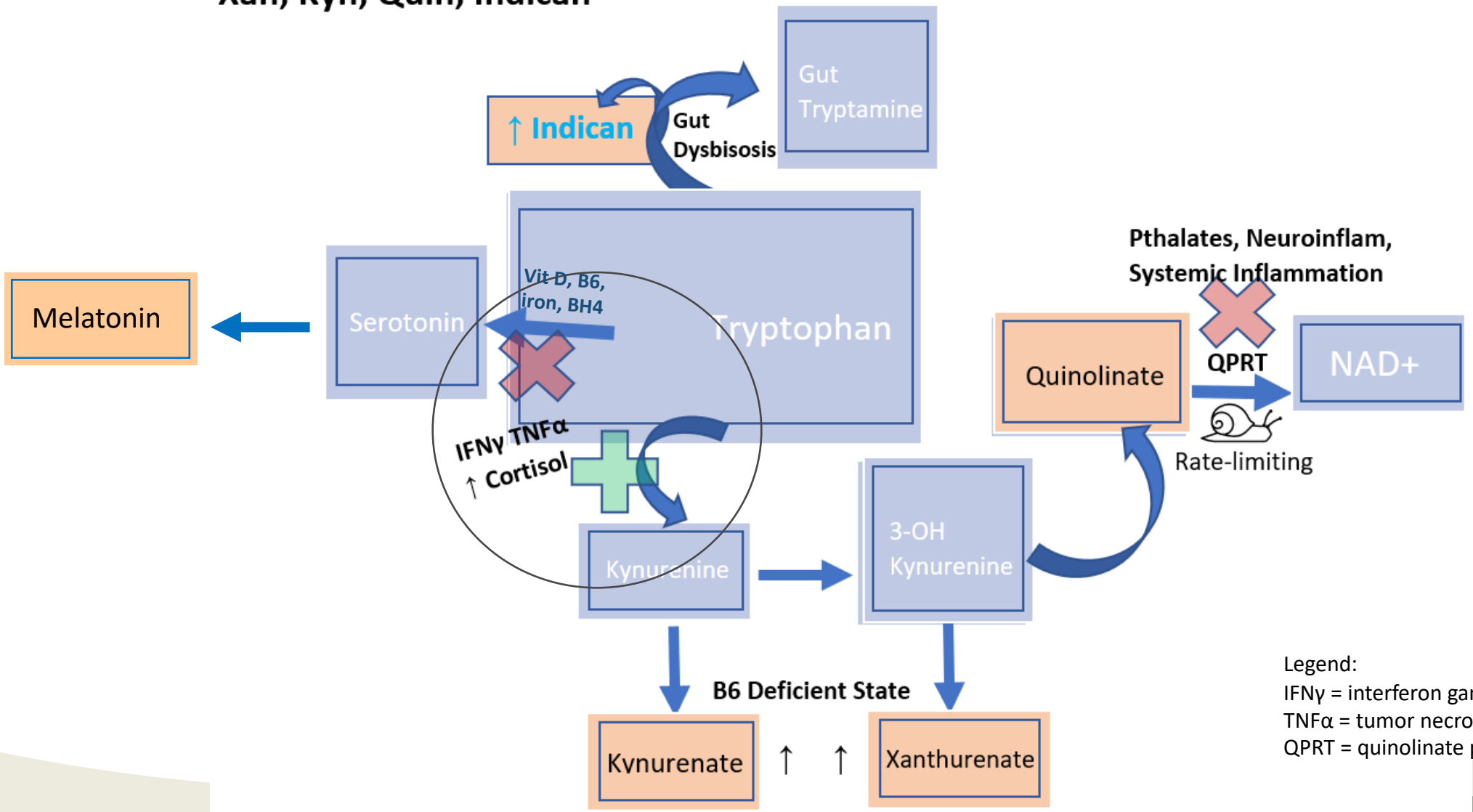
Melatonin Metabolite in Urine (MT6s)

- Reported from the 1st morning void sample
  - Provides overnight production, circulation, and clearance of melatonin
  - Good assessment of **night-time** melatonin activity
- Melatonin initiates Night-time gene expression relays
- Melatonin is high while cortisol is low and vice versa
  - Cortisol interrupts serotonin acetylation step, it needs to be low
- Disturbances in this balance may lead to sleep problems, insulin resistance, and high blood pressure

A Note on Melatonin Dosing for Sleep vs. “Other things”

- Direct replacement
  - Low range 0.3 – 3mg studied for sleep
  - Mid range 3 – 10 mg studied for metabolic syndrome and inflammation
  - High range 20 mg + studied for cancer
  - Caution re: possible MTNR1b gene variant carriers, monitor HgbA1c if prescribing melatonin
- Support serotonin (its precursor)
  - Tryptophan, Vitamin D, 5-HTP, B6; Herbs: Curcumin, Passiflora, Rhodiola, Saffron
- Support methylation step in melatonin synthesis
  - Methylated Bs, SAMe, Mag, B6, Choline, Methionine, etc.
- Keeping cortisol in check at night
  - Adaptogens, Phosphatidylserine, Choline, EFAs, L-Theanine…
- Sleep hygiene practices and diurnal support
  - Dim light starting 2 hours before bed, limit blue light
  - No lights in the room or masked sleeping
  - 10,000 lux Light therapy within 15 minutes of waking

Xan, Kyn, Quin, Indican



Legend:
IFNγ = interferon gamma
TNFα = tumor necrosis factor alpha
QPRT = quinolinate phosphoribosyltransferase

Hormone Players

Inflammation/Insulin/Metabolic

DUTCH Findings:

- CAR blunted (if later in disease process) Low
- CYP1B1 - 4OHE1 and 4OHE2 High/Predominant
- CYP3A4 activity
  - High if ***gut*** source inflammation/infections
  - Low if ***systemic*** source inflammation/infections
- 5α-Reductase - 5α-Androstanediol
- Free cortisol waking level High
- Kynurenate High
- Metabolized Cortisol (esp α-THF) High
- Sulfated DHEA low, DHEA metabolites high(er)
- Quinolinate High
- Xanthurenate High

Sympathetic Nervous System Activity

DUTCH Findings:

- CAR elevated and/or prolonged
- COMT activity low
- Free cortisol waking (early, later all free corts low)
- Metabolized Cortisol
- Quinolinate
- Vanillylmandelic Acid (VMA) high
  - Or may not show high if COMT activity low and/or cortisol is low due to **low NE clearance**

↑Histamine/Immune

DUTCH Findings:

- COMT activity low
- CYP3A4 activity high (high 16OHE1 in E2 dominant or histamine>prolactin-driven presentations)
- E2 dominance and/or Low Progesterone
- Free cortisol abnormals (high early, low late)
- Homovanillic Acid (HVA) low or low-normal
- Metabolized Cortisol
- Sulfated DHEA low
- Vanillylmandelic Acid (VMA) low or low-normal

Inadequate Melatonin

DUTCH Findings:

- COMT activity low
- Night cortisol high
  - Evening, Bedtime, and/or MOTN
- Melatonin metabolite (MT6s) <25
- Vanillylmandelic Acid (VMA) high

CAR = Cortisol Awakening Response
COMT = Catechol-o-methyltransferase enzyme
EPI = Epinephrine
MOTN = Middle of the Night
NE = Norepinephrine
THF = Tetrahydrocortisol

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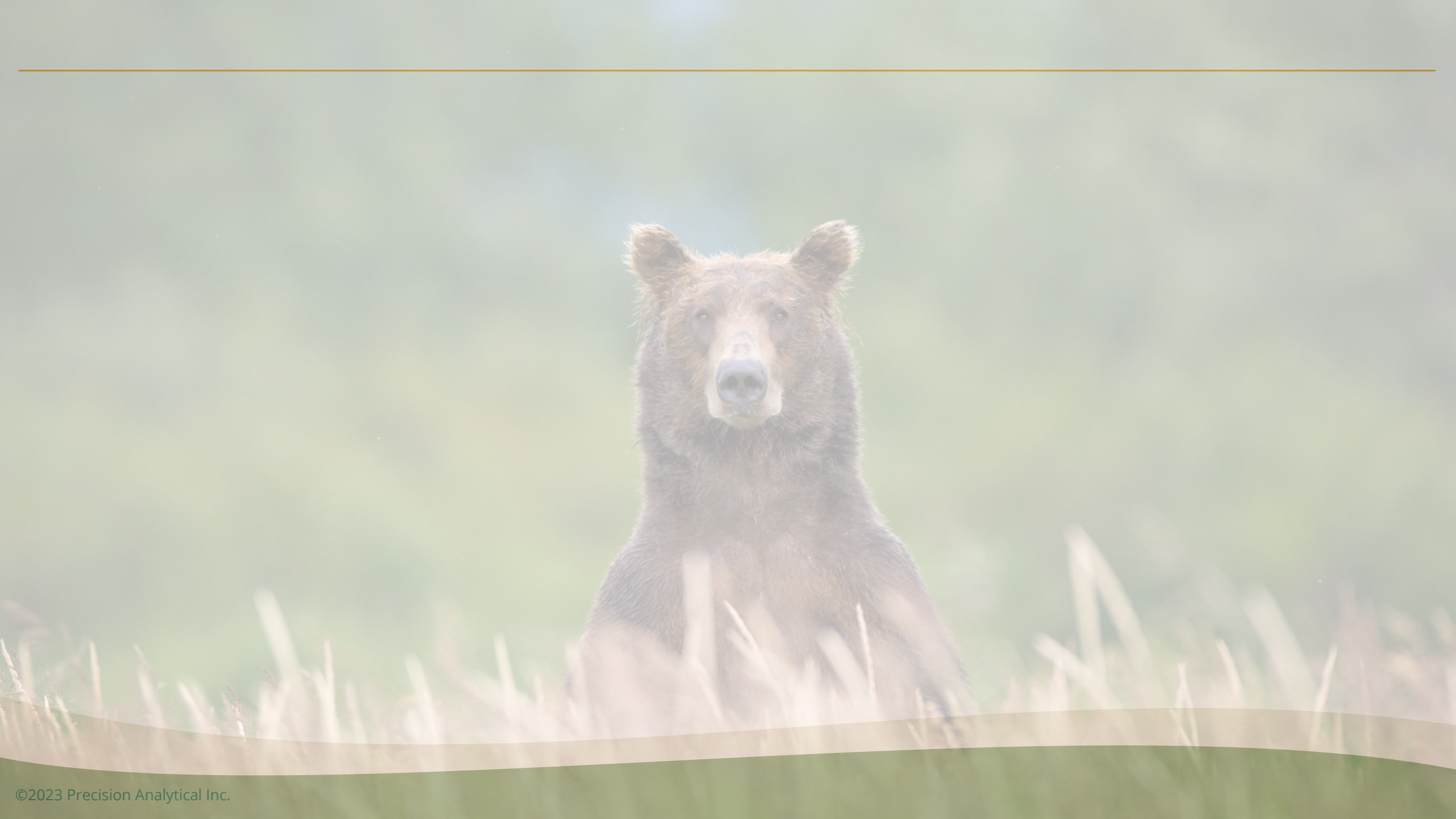

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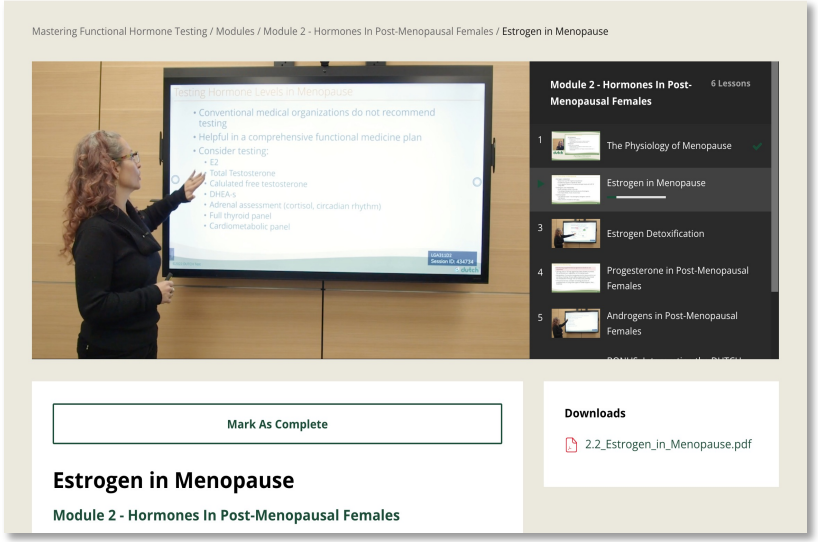

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