KRESSER INSTITUTE FOR FUNCTIONAL AND EVOLUTIONARY MEDICINE

HPA-D Diagnosis - Part 2

"Allostatic load battery"

Blood sugar
Blood pressure
Insulin
Waist circumference
Interleukin-6
Heart rate variability
Cortisol
Norepinephrine
DHEA

Okay, now let's move on to laboratory testing. Given the range of effects that HPA-D can produce, there are not surprisingly many biomarkers that can be used to detect dysfunction. These have been collectively referred to as an allostatic load battery by Bruce McKuen. It includes markers like blood pressure, fasting and post-meal glucose, insulin, waist circumference, interleukin-6, heart rate variability, C-reactive protein, heart rate variability, urinary norepinephrine, cortisol, and DHEA. But from a practical perspective, when we're doing a case review, we're often assessing many of these markers together. In this unit, we're going to focus on the markers that research suggests are most effective for evaluating HPA-D, and these are free cortisol, metabolized cortisol, the diurnal free cortisol and free cortisone rhythm, the cortisol awakening response, or CAR, DHEA and the DHEA-to-cortisol ratio.

Let's start with cortisol. There are four primary methods of assessing cortisol levels: serum, hair, saliva and urine. Serum total cortisol levels represent both bound cortisol, which is 95 percent of the cortisol in the body, and unbound or free cortisol, which is 3 to 5 percent of the cortisol in the body. Total serum cortisol is still often used in clinical trials, but it's problematic because most of that cortisol is bound to cortisol-binding albumin or globulin and is not bioavailable. We know that several conditions and medications affect cortisol-binding globulin levels, so using total serum cortisol is not a great idea. Free cortisol can be separately measured in the serum; it's usually calculated from measurements of total cortisol plus cortisol-binding globulin, so direct measurement is possible with some labs. It's not used in clinical trials much anymore; it's been

replaced by saliva cortisol as a way of measuring free cortisol levels. One of the main problems with serum cortisol, whether you're talking about free or total, is that it's not really practical as a means of capturing the diurnal rhythm of cortisol production. You're not going to send a patient to a lab four times in a day to have their blood drawn, that's not going to be successful, and it's impossible to capture the 30 to 45-minute post-waking sample and nighttime sample that way in the majority of cases.

Hair cortisol is a more recent tool; as with many drugs and toxins, cortisol accumulates in the hair via passive diffusion from the blood. Hair samples are non-invasive and can be stored at room temp for years. Because human hair is known to grow at an average of one centimeter per month, hair cortisol is being investigated as a historical measure of HPA-D. It's almost like a hemoglobin A1c for cortisol, where you're looking at average cortisol levels over a period of time, rather than a single time point. This is likely only possible for about six centimeters worth of hair, equivalent to six months of cortisol production, and of course, it's only practical when the person has at least six centimeters of hair. Some studies have shown that high hair cortisol is associated with a history of cardiovascular disease. One study showed people with normal body mass index had hair cortisol of 36 picograms per milligram, and those who were overweight with a high BMI had hair cortisol of 61 picograms per milligram, and this correlates with what we see in terms of urine cortisol metabolites and BMI. Hair cortisol is not commonly offered, and I'm uncertain as to its clinical value, because there's not a ton of research or clinical experience with it so far, but one thing it could be helpful with is providing the ability to easily track the progress of treatment over time.

The next method of assessment is saliva. It's now commonplace in scientific studies. For example, midnight salivary cortisol is now considered reliable as a tool for diagnosing Cushing's syndrome, and diurnal salivary testing is being investigated as a tool for predicting the progression of dementia and other cognitive disorders. Saliva's also the most commonly used anolyte in functional and integrative assessments of so-called adrenal fatigue. For example, the adrenal stress index, or ASI, has been the mainstay of functional and integrative medicine practitioners for years. Saliva cortisol is a surrogate marker for serum free cortisol. Saliva only contains free cortisol, so you don't have to worry about binding proteins in the saliva. It's a non-invasive, non-stress-inducing time-specific marker that allows you to capture the diurnal rhythm of cortisol production in a convenient setting, usually when the patient's at home, and you can, through those four different readings, estimate total cortisol production. Cortisol in saliva is generally lower than serum due to parotid activity of 11-Beta-HSD2, which converts cortisol to cortisone, and having said that, there's still a linear relationship between salivary and free serum cortisol, and they are quite well correlated in studies.

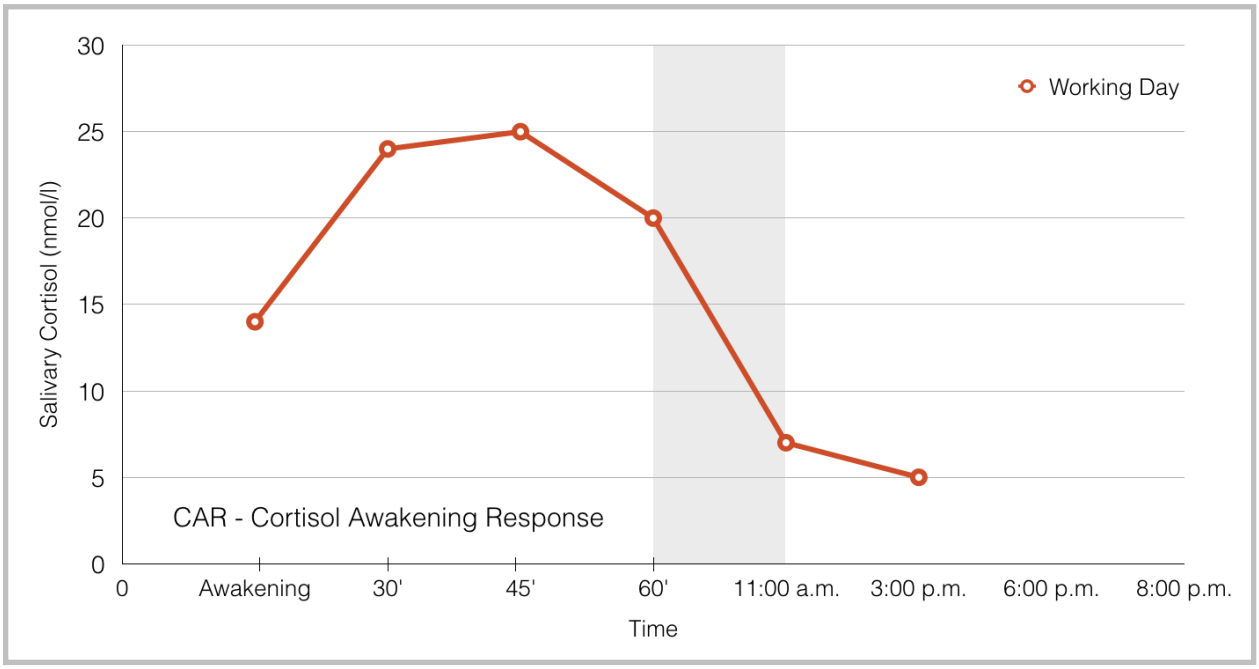
Relative Concentrations of Cortisol and DHEA(S)

	Total Serum (95% bound)	Serum Free (5% of total)	Saliva
Cortisol (High)	~500 nmol/L (180 µg/L)	25 nmol/L* (9 µg/L)	~20 nmol/L* (7 µg/L, 7 ng/ml, 0.7 µg/dL)
Cortisol (Low)	<100 nmol/L (37 µg/L)	<5 nmol/L* (1.8 µg/L)	<4 nmol/L* (1.44 µg/L, 1.44 ng/ml, 0.14 µg/dL)
DHEA	20 nmol/L (5.76 µg/L)	1 nmol/L (288 ng/L or pg/ml)	1 nmol/L* (288 ng/L or 288 pg/ml
DHEA-S	5 µmol/L (1.85 mg/L)	250 nmol/L (92 µg/L or ng/ml)	2.5 to 5 nmol/L* [0.9 µg/L (pg/ml) to 1.8 µg/dL (pg/ml)]

Adapted from Guilliams, T. The Role of Stress and the HPA Axis in Chronic Disease Management. Point Institute, 2015. p. 54

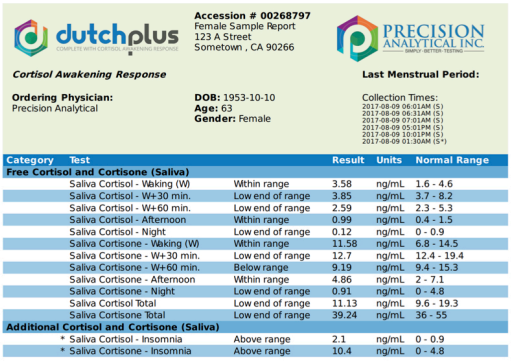
Here's a table showing the relative concentrations of cortisol and DHEA. This is adapted from Dr. Guilliams' book; as you can see, it's not simple to directly compare saliva and serum cortisol levels. They're different units, and they need to be converted and different labs have different ranges, and this is really one of the biggest challenges, is that different labs will use different units and different ranges, and so you have to convert to the same units in order to be able to compare them. So we can provide a handout of this slide to make it easy for you, something to refer to if you're getting lab results from different sources.

Finally, we have urine cortisol. Traditionally, this has been measured in a 24-hour collection, where a patient just carries a jug around with them, for 24 hours at home. Urine contains free cortisol, but it also contains many cortisol metabolites, like cortisone or 5-alpha-tetrahydrocortisol, 5-beta-tetrahydrocortisol, tetrahydrocortisone, etc. Assuming normal cortisol clearance rates, most cortisol synthesized by adrenal glands will be metabolized in the liver and cleared in the urine within 90 minutes. So, urine cortisol is a reflection of cortisol production over the 90 minutes prior to when the sample was collected, if you're doing a spot urine cortisol test. Precision Analytical developed a new method of urine cortisol measurement called DUTCH—Dried Urine Test Comprehensive Hormones—and this involves collection of a small amount of urine on filtered paper four times a day, and in this way, the DUTCH test is able to estimate diurnal production, like saliva, but also provide information on both total free cortisol and overall cortisol using metabolites as well as free cortisone and metabolized cortisone.



One of the most commonly used markers for HPA axis function in the scientific literature is called the cortisol awakening response, or CAR. Until recently this was not available to clinicians outside of a research setting. The CAR is the predictable increase of cortisol that occurs in the morning just after awakening. It's the result of the momentum of rising cortisol that begins several hours before awakening due to normal HPA axis activity. And then when you wake up and open your eyes in the morning and light hits the retina, you see a huge and rapid increase in cortisol, up to 50 percent of the total cortisol production for the day, in fact, in that 30- to 45-minute period just after opening your eyes. The CAR has more research behind it as a marker than the four-point overall diurnal cortisol rhythm test offered by most saliva testing labs. The CAR is a marker of how the HPA axis responds to stress. For example, a lower CAR is seen in people with PTSD, chronic fatigue, and burnout, and a higher CAR is observed in people with ongoing job stress and higher perceived stress.

C.A.R. testing options



dutchplus

Accession # 00268797
Female Sample Report
123 A Street
Sometown , CA 90266

PRECISION ANALYTICAL INC

Cortisol Awakening Response

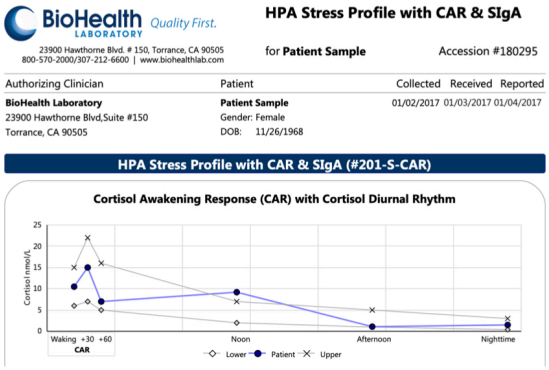
Ordering Physician:
Precision Analytical

DOB: 1953-10-10
Age: 63
Gender: Female

Last Menstrual Period:

Collection Times:
2017-08-09 06:01AM (S)
2017-08-09 06:31AM (S)
2017-08-09 07:01AM (S)
2017-08-09 05:01PM (S)
2017-08-09 10:01PM (S)
2017-08-09 01:30AM (S*)

Category	Test		Result	Units	Normal Range
Free Cortisol and Cortisone (Saliva)					
	Saliva Cortisol - Waking (W)	Within range	3.58	ng/mL	1.6 - 4.6
	Saliva Cortisol - W+30 min.	Low end of range	3.85	ng/mL	3.7 - 8.2
	Saliva Cortisol - W+60 min.	Low end of range	2.59	ng/mL	2.3 - 5.3
	Saliva Cortisol - Afternoon	Within range	0.99	ng/mL	0.4 - 1.5
	Saliva Cortisol - Night	Low end of range	0.12	ng/mL	0 - 0.9
	Saliva Cortisone - Waking (W)	Within range	11.58	ng/mL	6.8 - 14.5
	Saliva Cortisone - W+30 min.	Low end of range	12.7	ng/mL	12.4 - 19.4
	Saliva Cortisone - W+60 min.	Below range	9.19	ng/mL	9.4 - 15.3
	Saliva Cortisone - Afternoon	Within range	4.86	ng/mL	2 - 7.1
	Saliva Cortisone - Night	Low end of range	0.91	ng/mL	0 - 4.8
	Saliva Cortisol Total	Low end of range	11.13	ng/mL	9.6 - 19.3
	Saliva Cortisone Total	Low end of range	39.24	ng/mL	36 - 55
Additional Cortisol and Cortisone (Saliva)					
	* Saliva Cortisol - Insomnia	Above range	2.1	ng/mL	0 - 0.9
	* Saliva Cortisone - Insomnia	Above range	10.4	ng/mL	0 - 4.8



BioHealth LABORATORY *Quality First.*
23900 Hawthorne Blvd. # 150, Torrance, CA 90505
800-570-2000/307-212-6600 | www.biohealthlab.com

HPA Stress Profile with CAR & SIgA

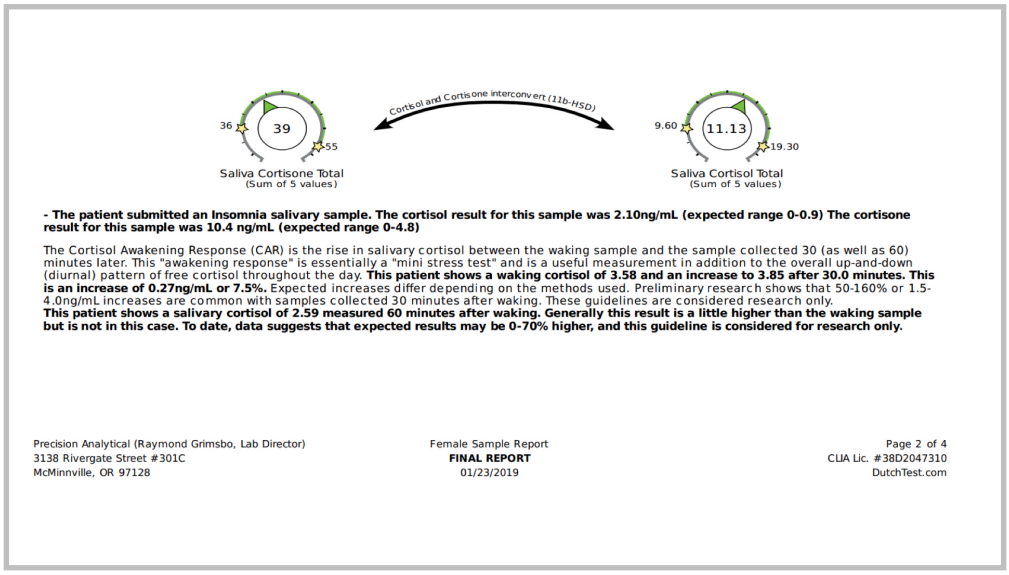
for **Patient Sample** Accession #180295

Authorizing Clinician	Patient	Collected	Received	Reported
BioHealth Laboratory	**Patient Sample**	01/02/2017	01/03/2017	01/04/2017
23900 Hawthorne Blvd,Suite #150	Gender: Female			
Torrance, CA 90505	DOB: 11/26/1968			

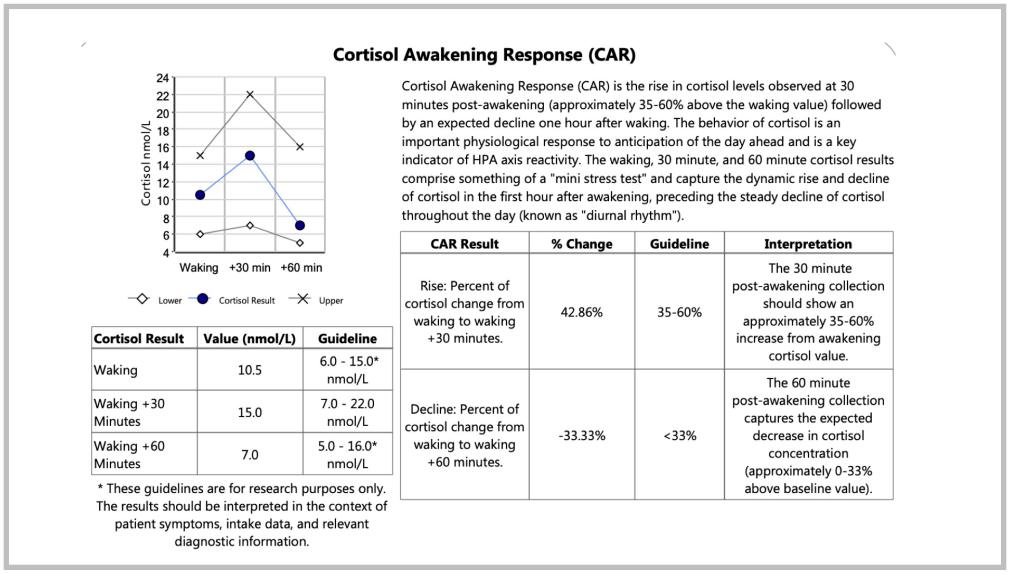
HPA Stress Profile with CAR & SIgA (#201-S-CAR)

Cortisol Awakening Response (CAR) with Cortisol Diurnal Rhythm

There are two options for testing the CAR from functional testing labs. The first is an add-on for the Precision Analytical DUTCH test, which we're going to be talking a lot about in this unit, and the second is a separate panel from BioHealth. The DUTCH test collects six saliva samples at waking, 30 minutes after waking, 60 minutes after waking, before the evening meal, between 10:00 p.m. and midnight, and an optional sixth collection overnight if the patient wakes up during the middle of the night. BioHealth also advises collections of six samples with mostly the same time intervals, with the exception of the afternoon and evening collection.

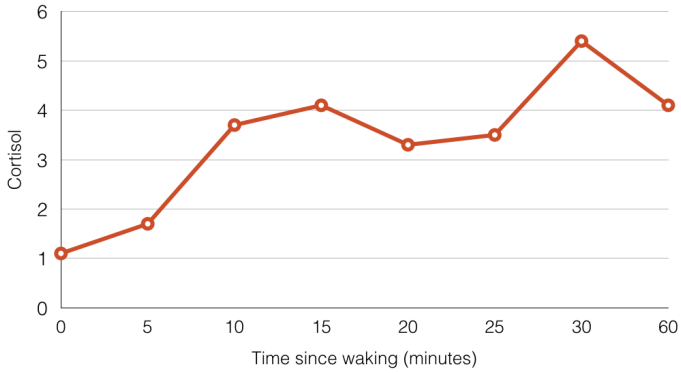


According to Precision Analytical, the expected increases differ depending on the methods used, but preliminary research shows that 50 to 100 percent increases are common with samples collected 30 minutes after waking. And to date, the data suggests that expected results may be 0 to 70 percent higher for 60 minutes after waking.



BioHealth uses a different range that shows a normal 30 minute post-awakening collection of approximately 35 to 60 percent increase and a 60 minute post-awakening collection with a decrease in cortisol concentration of approximately 0 to 33 percent above the baseline value.

C.A.R. Saliva Research



Cortisol	Minutes	% Increase	Molar units
1.1	0	0	3.01
1.7	5	55	4.66
3.7	10	236	10.14
4.1	15	273	11.23
3.3	20	200	9.04
3.5	25	218	9.59
5.4	30	391	14.79
4.1	60	273	11.23

There is a way that you can hack a CAR using a pre-existing saliva test from any of the major labs. You would just order a kit from one of the labs, have the patient rinse their mouth and immediately collect saliva for two to three minutes right after waking, and then you would have them collect again at 30 minutes, 45 minutes and 60 minutes, so that's using the four tubes that they send you. It's best for the patient to drink a little bit between each collection, but not eat, and when you get the results back you're going to have to ignore the report, because the report is predicated upon the idea that the patient is taking four samples throughout the day, but you can just look at the numerical values, and a healthy CAR should show a 350 to 600 percent increase in the first 30 minutes. So the table and chart on this slide depict a CAR that Mark Newman, who's the head of Precision Analytical, did on himself using these guidelines, and as you can see, he hit 391 percent at 30 minutes, which is right in the expected range. Some studies on CAR use molar units and others use nanograms per milliliter, but either way, the percentage increase would be the same, so you can calculate it regardless of what the units are.