HPA-D Diagnosis - Part 3

Let's move on to DHEA. For DHEA and DHEA sulfate, the options are similar to cortisol; we have serum, saliva, and urine. Note that the concentration of DHEA sulfate is 300 times higher than DHEA in serum. In fact, DHEA sulfate is the most abundant hormone in the blood. For many years, DHEA sulfate was thought of as the storage form of DHEA, which would be converted to androstenedione or other androgens. But we now know that DHEA sulfate is a neurosteroid with a direct effect in sulfated form. In serum, DHEA and DHEA sulfate are often combined into a measurement called DHEA (S), with the S in parentheses after the DHEA, I know this is somewhat confusing but I didn't set this up. Both DHEA and DHEA sulfate are used in studies, but there's been a trend more recently to use either only DHEA sulfate or DHEA total, which is DHEA (S) in parentheses. DHEA does have a slight diurnal rhythm, but it's difficult to measure in any body fluid, so it's not currently recommended.

Testing of DHEA in saliva is somewhat controversial, in part because we understand so little about DHEA. DHEA is a neural steroid and passes rapidly from the blood to saliva via passive diffusion. It's a charged molecule and can't easily enter saliva. That means that salivary DHEA (S) sulfate levels are only 2.5 times higher than salivary DHEA, the normal DHEA, versus 300 times higher DHEA sulfate than normal DHEA in the serum. So as you can see, the ratios are very different. Nevertheless, recent studies have shown that DHEA sulfate can be a useful marker of HPA axis function in saliva. If saliva is used, make sure the patient doesn't use gum to stimulate saliva production, as chewing can mix gingival fluids or serum with saliva. Also, levels of DHEA (S) drop quickly in the first hour after awakening, and this can make timing difficult, as you'll soon see when we discuss saliva cortisol timing in more detail.

In urine, DHEA sulfate can be measured, but not DHEA. Urine can also test for other androgens like androsterone and etiocholanolone. Some studies suggest that a combination of DHEA sulfate in these other two markers may be a more accurate indication of total DHEA production.

The cortisol-to-DHEA ratio is another important marker to consider. It can be seen as an indicator of catabolism versus anabolism. Anabolism means building things up, tissue repair, growth, and recovery. It's defined as the set of metabolic pathways that construct molecules from smaller units. Catabolism means breaking things down, tissue destruction and remodeling, and it's defined as breaking down molecules into smaller units that are oxidized to release energy. Sometimes, absolute cortisol levels may not tell the whole story, but the cortisol-to-DHEA ratio will.

One study compared healthy Alzheimer's caregivers to controls, and cortisol levels were similar despite the caregivers having much higher stress levels, anxiety, and depression, but the cortisol-to-DHEA ratio was significantly higher in the caregivers than controls, and that was mainly driven by lower DHEA levels. High cortisol-to-DHEA ratios have been observed in

treatment-resistant depression and relapse to smoking cessation. One study found that "individuals with IBS showed an elevated cortisol to DHEA ratio after awakening compared with individuals without IBS," and the elevated ratio peaked under the prolonged stress. The cortisol to DHEA ratio is higher in infertile women, again driven primarily by low DHEA. Conversely, listening to music, which has known stress reduction benefits, has been shown to reduce both cortisol and the cortisol-to-DHEA ratio. There's no consensus on an optimal ratio in the literature, but some have suggested four to one as a ratio if you're using DHEA sulfate, and ten to one for total DHEA. Clinically, I just use the ratio as a rough relative guide; if DHEA is low or low-normal and cortisol is high or high-normal, the ratio is out of whack.

Now let's talk about which method of assessment is best to use in clinical practice. We can rule out serum and 24-hour urine pretty easily as routine tests. Hair is not commonly offered and is more of a marker of longer-term HPA axis function, which could be useful, but not for real-time assessment. Serum can be useful for getting a single time point if both total and free are measured, but not for diurnal rhythm or total production, and it lacks the metabolites. Twenty-four-hour urine gives great info on cortisol and its metabolites and DHEA sulfate, but there's no diurnal rhythm or specific time point information, just average collection, so this leaves saliva and DUTCH as the two remaining possibilities.

Saliva Pros

- Non-invasive
- Can be done at home
- Not stress-inducing
- Reflects specific time points
- Measures free/unbound cortisol
- Has potential of capturing C.A.R.
- Measures cortisol metabolites
- Measures cortisone results

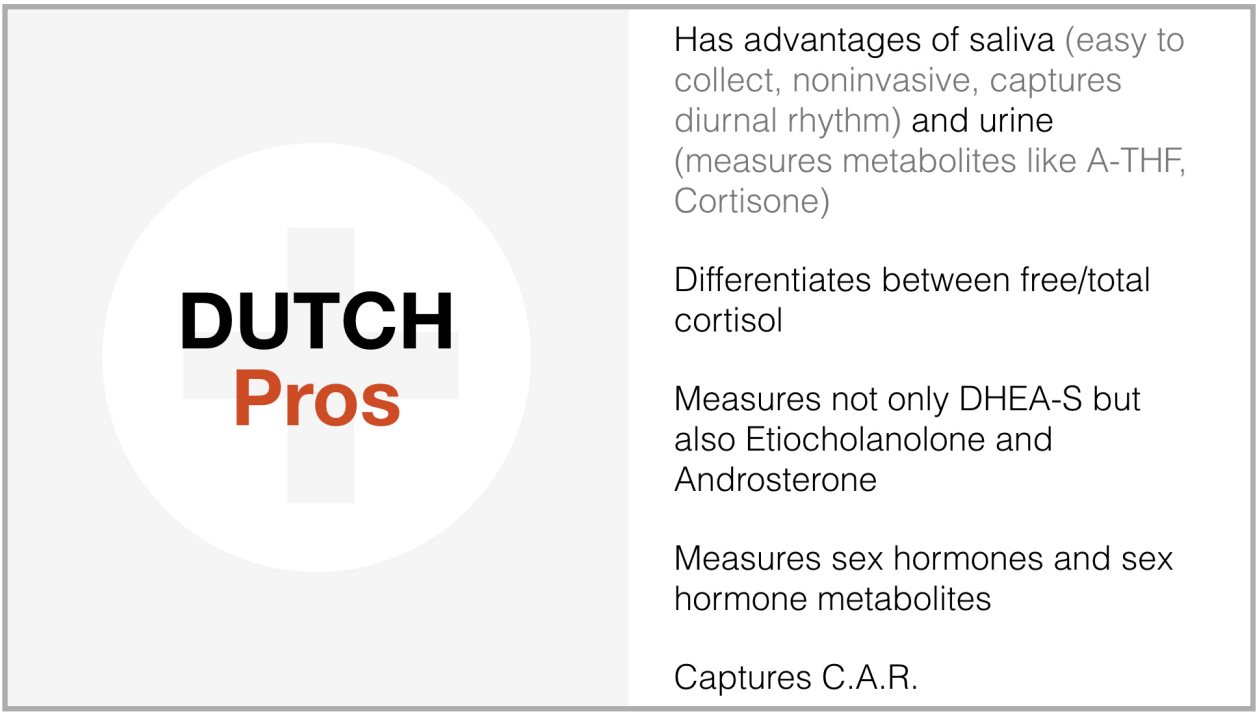
As mentioned, saliva has been the default for cortisol testing mostly because it has several advantages. It's non-invasive, can be done at home, it's not stress inducing, it reflects specific time points, it measures free cortisol (cortisol that is unbound to a carrier protein), it can capture the cortisol awakening response (if the timing of the test is done in such a way that it can do that), and can also measure free cortisone, which can be helpful when evaluating the full picture.

Saliva Cons

Non-standardized

Potential for Improper test collection methods

The con of saliva is that it's non-standardized. You see different units, different ranges, and different methodologies for testing amongst different labs. Test collection can be a little bit difficult. Patients, for example, with a four-point saliva cortisol collection are advised to collect any time within the 6 to 8 a.m. window instead of a specific time after awakening, and now that you understand the cortisol awakening response and how much cortisol is produced so quickly after opening our eyes, you can understand why this would be a problem. The results are often highly dependent upon when the first sample was taken. If a patient didn't sleep well and they were restless in the morning, sometimes they've already had the cortisol awakening response before they actually take the test, so the results can look falsely low. It is a little bit tricky to get the saliva testing right.



The pros of DUTCH include that it has the advantages of saliva, it's easy to collect, it's non-invasive, it captures the diurnal rhythm and the CAR, and the advantages of urine, it measures the metabolites of cortisol like the A-THF and cortisone. DUTCH also differentiates between free cortisol and total or metabolized cortisol, which can allow for a much more clear assessment of cortisol production, and also the identification of conditions like subclinical hypothyroidism that can be identified using the ratio of free cortisol and total cortisol. DUTCH measures not only DHEA-S but also etiocholanolone and androsterone, which gives a much more complete picture of total DHEA production. Sometimes you'll see high or normal DHEA-S and low total DHEA or vice versa, and that gives you important information that you can't get from other labs. And then as I mentioned, the DUTCH now is able to capture the cortisol awakening response, which is the most evidence-based way of assessing HPA axis function.

DUTCH
Cons

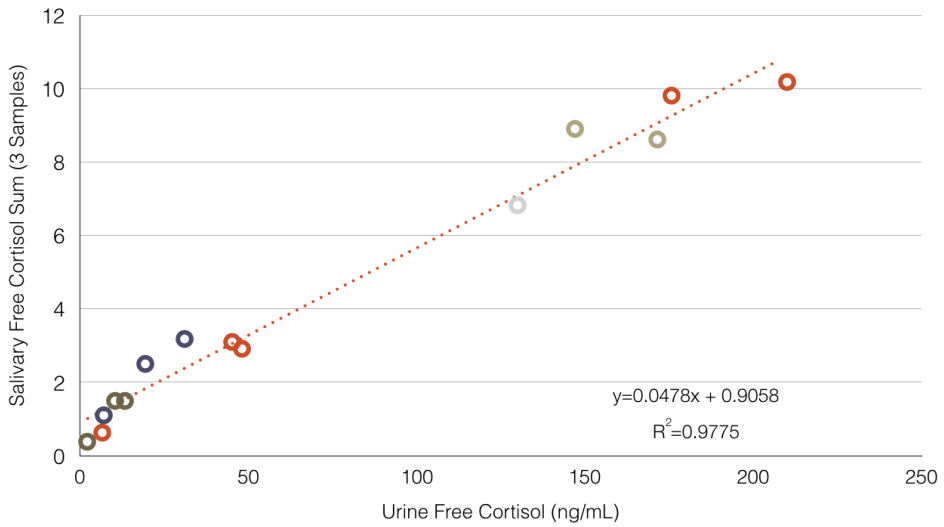
Not as much research correlating urine cortisol with disease (though there is quite a bit on obesity, depression, chronic fatigue, etc.)

Not much research on dried urine

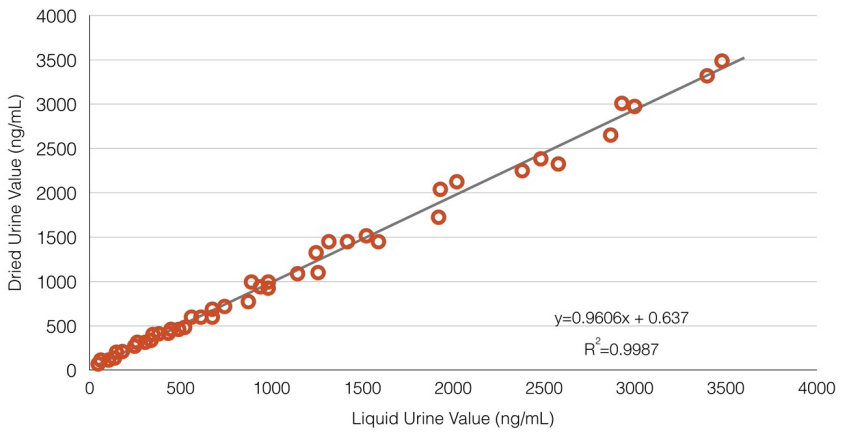
Each sample represents 2-hour previous average, rather than single time point

Cons of DUTCH include that there's not as much research correlating urine cortisol with disease, though there is quite a bit on obesity, depression, chronic fatigue, etc. And there's new research that has just come out recently in support of all of this. There's not as much research on dried urine and its correlation with 24-hour urine collection or more complete urine collection and saliva, although again, there is recent research supporting these correlations. And then each sample represents a two-hour previous average rather than a single time point, so it's perhaps a little bit less precise in that way.

Saliva and Urine Free Cortisol Comparison



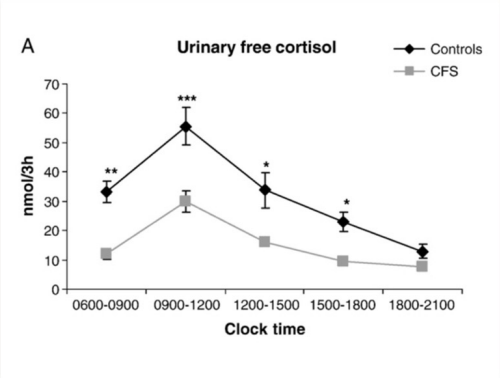
But how significant are those cons of the DUTCH test? Though there's less research correlating urine cortisol with disease outcomes overall, there's quite a bit on obesity, depression, and chronic fatigue, as I mentioned, many papers showing low free cortisol, lower normal free cortisol, and high cortisol metabolites in patients with metabolic dysfunction. Also, studies in the literature have shown that urine cortisol and saliva cortisol correlate quite well, and internal research at Precision Analytical with split urine saliva samples has shown a good correlation as the chart on this slide indicates. Patients collected three saliva samples over two hours and then one dried urine sample which reflects 90 to 120 minutes prior to the collection time, due to how cortisol is metabolized. The idea was to see if one dried urine correlates well with three saliva samples over that 90- to 120-minute time period, and as you can see, it correlated very well. Urine is recognized as a valid marker of cortisol in the research literature, but its shortcoming has always been its inability to capture diurnal rhythm with a 24-hour collection, and the dried urine testing has changed that.



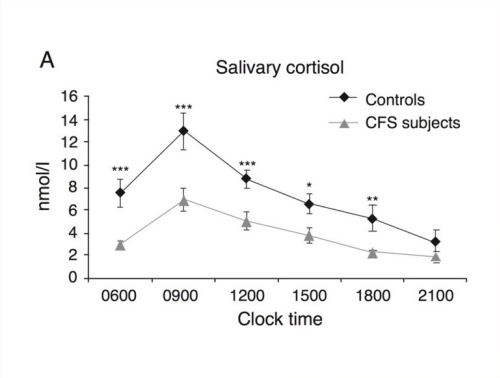
Unpublished data, Precision Analytical

There's no research in the literature in dried urine testing and cortisol because it's only been recently developed. However, there is unpublished data from Precision Analytical indicating a very strong correlation between dried urine and liquid urine cortisol as you can see on this chart. A study was recently published as of 2019 showing a very strong correlation between dried urine sex hormone values and liquid urine sex hormone values, and I believe serum as well. This study did not include cortisol values, but this is good news in terms of assessing dried urine's correlation with liquid urine.

Urine



Saliva



Jerjes et al. J Psychosom Res. 2006 Feb;60(2):145-53, Jerjes et al. J Affect Disord. 2005 Aug;87(2-3):299-304

What about the criticism that urine is cumulative rather than real-time, so therefore it doesn't accurately reflect the diurnal rhythm? If you look in the research literature, you'll see that the diurnal rhythm measured by urine is actually very close to the diurnal rhythm measured with saliva. There are two papers from Jerjes et al. looking at diurnal cortisol rhythm in CFS (chronic fatigue syndrome) patients versus controls. The chart on the left here is urine, and the right is saliva, and as you can see, they're essentially identical. Both showed a similar rhythm for both CFS patients and controls. What's more, you could argue that having a sample that represents the previous 90 minutes rather than just a single moment in time is an advantage rather than a disadvantage. For example, what if a patient gets a stressful phone call just minutes before a saliva sample on the test day? That will show up as a spike in saliva, but in urine it would be averaged with other values over the prior 90-minute period, so a single isolated stressful event like that is less likely to affect the test results.

As for the DUTCH not capturing the CAR, that's a valid criticism, but to be fair, no saliva labs are offering it currently either. CAR would be a really useful marker to have, and I've talked with Mark Newman from Precision Analytical, and he's even considering offering it. This would be the best of both worlds—DUTCH for the broader view of the HPA axis with the greater amount of info it offers, and then the CAR as a mini-stress test, which is a great marker of the ability of the HPA axis to respond to stress.