



Measuring Testosterone Biomarkers in Serum and Saliva: *Are They Accurate?*

Clinicians are confronted daily with patients presenting with symptoms suggesting low levels of sex hormones such as testosterone. However, the best laboratory test to use to confirm this diagnosis is still controversial. This is partly due to two factors:

1. Clinical research so far has shown that symptoms may not correlate well to actual hormone levels. This is an area we need to study in greater depth.
2. Measuring hormones is complicated by the fact that hormone release is cyclical and influenced by many factors, including stress and the time of day.

Consequently, it is important that practitioners base diagnosis and therapeutic decisions on clinical assessment of the patient, with the aid of laboratory testing—rather than relying on laboratory tests alone. In today's litigious society, causing harm with treatment is difficult to defend. In general, there is support for treatment, particularly if biomarkers are indeed low and the patient is symptomatic. However, there is debate as to whether the test is performed in the blood or saliva.

Serum testing may be a useful tool to assist in diagnosis of low hormone levels. However, determining the “true” level of hormones may still be difficult, in part because hormones are biologically active, not only in the serum but also elsewhere, such as in the cell. There are no tests available at this time to ascertain true

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intracellular activity of hormones. My colleagues and I are actively involved in research on androgen deficiency in men, so I will use examples from this area to illustrate some of the difficulties.

Types of Testosterone

Determining the levels of different types of testosterone helps to illuminate the patient's clinical picture. To summarize, these levels are:

- Total testosterone—all measurable testosterone, including bound and unbound portions
- Free testosterone—testosterone that is not bound to proteins
- Bioavailable testosterone—the “active” form of testosterone, or what may be available for use by cells; includes both free testosterone plus that bound to sex hormone-binding globulin (SHBG) and usually correlates well with androgenicity.

Bioavailability of Free Levels of Testosterone

It is generally believed that free levels of testosterone represent the true androgenic status of an individual. However, there is an emerging concept that the bioavailable amount is

actually more accurate. Laboratories do not usually determine true levels of free testosterone, rather estimating the free amount based on what is bound to certain proteins.¹ Considering that free testosterone amounts to only 2% of the total amount, an error made in the measurement could lead to falsely high or low results.

As mentioned earlier, bioavailable testosterone refers to the “active” testosterone in the body and includes both free testosterone and that bound to SHBG. Testosterone is only loosely bound to SHBG and easily detaches from it, making it “free.” Thus, changes in protein concentrations can affect levels of hormones, leading to a misleading test result.

One assumption of the accuracy of biomarker measurement is that most sex hormones such as testosterone are circulated in the serum. Although this is partly true, it does not represent the whole picture and may not represent the *true* hormonal status of the patient. For example, it is impossible to measure what is stored in red blood cells; we don’t have assays that allow us to see what is going on at that level. A centrifuge typically spins down blood and the serum is sent for analysis. Red blood cells are generally discarded.

Another issue that is rarely considered is the action of hormones on their target receptors. Currently, there are

no commercially available laboratory tests to allow the practitioner to determine whether the intended hormone is truly working and locking onto receptors, and the failure to do so is sometimes the problem in a patient with symptoms. For example, some patients may feel better despite achieving a slight rise in their serum testosterone levels. Other patients may double their serum level of testosterone and feel no better. Neither serum nor saliva testing is able to determine whether the hormones and receptors are interlocking. Another limitation of saliva testing in following patients on testosterone treatment is that levels are often inappropriately high. Thus, results on a laboratory test may not reflect the true picture and it is sometimes more important to rely on how the patient feels.

Defining What Is Normal

Still other difficulties complicate the interpretation of test measurements. For example, most laboratories define the normal range for total testosterone as being from 260 to 1,000 ng/dL (9.02 to 34.7 nmol/L) and for free testosterone as being from 50 to 210 ng/L (174 to 729 pmol/L).² However, since decreased sensitivity to androgens occurs in target organs, absolute serum levels of testosterone can be misleading. In addition, the range is often *not age adjusted*, complicating interpretation.³ There is a natural decline of levels with normal aging, and most laboratories define normality as two standard deviations from the mean of the sample population with no regard to the age of the cohort.

Another challenge is that hormone levels can vary at different *times* during the day. Levels, even for one individual, do not remain constant. For instance, testosterone is secreted sporadically in men, with levels highest after awakening. In one study of 20 healthy men,⁴ when testosterone was sampled every 6 hours, levels ranged from 105 to 1,316 ng/dL (3.64 to 45.67 nmol/L). This demonstrates the difficulty of basing treatment solely on measurements, in that a normal person may be inappropriately committed to therapy, depending on what time the blood test was taken. Although this daily fluctuation is attenuated in older men, it can certainly complicate interpretation of results in younger patients, as demonstrated by a study at Boston.⁴

Correlating symptoms to levels of testosterone is still highly variable. As such, my colleagues and I have put forward a hypothesis of “relative hypogonadism” in aging men, involving individualization of normal levels for each patient.⁵ The concept is that levels are not always predictive of symptoms; consequently, it may be necessary to consider therapy in marginal cases of hypogonadism (that is, in patients with levels in the 300–400 range who are symptomatic).

Confounders to Low Testosterone

Other factors can also confound the clinical picture. For instance, issues that complicate diagnosis when considering the andropause syndrome can include clinical depression,

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obesity and certain medications. These medications include cimetidine, digoxin, spironolactone and steroids.^{1,6} The most sinister cause of low testosterone could be a pituitary tumor, and this has to be excluded before instituting testosterone therapy. Fortunately, these are rare.

In addition, psychological issues often appear at the same time as andropause syndrome.⁵ Long-term opioid use can also cause suppression of testosterone levels.⁷ Other confounding factors include stress and chronic illness (eg, arthritis, fibromyalgia), which can result in depressed testosterone levels. Diabetes, insulin resistance and obesity can also complicate the clinical picture and have been linked to hypogonadism.⁸

Methods Used to Measure Hormone Levels

There are several methods for measuring hormone levels such as testosterone. They can be summarized as follows:

- **Equilibrium dialysis** is based on separation of molecules to be measured from solutions that contain both cells and cellular products. It is one of the more accurate methods for determining *free* levels of testosterone. However, this is a very time-consuming process that is available mostly in research laboratories.
- **Ultracentrifugation** uses sedimentation velocity and equilibrium to characterize components. This methodology must be carefully monitored because time and temperature can distort results.
- **Radioimmunoassay** is one of the most commonly used tests to measure hormones, including testosterone. Sometimes it can present health hazards, and it is costly to comply with handling and disposal regulations for radioisotopes.

Total testosterone is generally determined by use of automated machines; results can be distorted by reactions of dihydrotestosterone; however, its impact is usually minimal.

Measurement of free testosterone usually gives a better measure of androgenicity than other tests;⁹ however, be aware that different laboratories may use different methods. In general, results of equilibrium dialysis and ultracentrifugation are usually comparable. Measuring the apparent free testosterone using equilibrium dialysis at 37°C is the best method for measuring free testosterone *in vivo*, although it is time consuming. A calculated free androgen index can also be used to measure active testosterone (usually calculated as the ratio of total testosterone divided by SHBG and multiplied by 100). Kits using a labeled testosterone analogue with a low-binding affinity for both SHBG and albumin can assist in directly estimating free testosterone in serum; they are easier to use than equilibrium dialysis or ultracentrifugation, require substantially fewer blood samples and allow direct estimation of free testosterone concentration without the requirement of measuring total testosterone. One study¹⁰ found that the analogue method correlates better with levels of total testosterone, rather than that which is bioavailable, leading researchers to conclude that free testosterone results with this method might be misleading in men with low SHBG concentration.

Bioavailable testosterone can be measured by determining the fraction of serum testosterone not precipitated by 50% ammonium sulfate concentration by direct radioimmunoassay in a supernatant after solvent extraction. This is a more expensive test, but one that is useful and accurate in older patients, as SHBG binding increases with age.

The Role of Saliva Testing

The role of saliva testing is a contemporary issue. Although it has been heavily marketed as a new test that is convenient and novel, it has yet to be accepted in mainstream medicine. And, although it is less expensive than blood tests, Medicare and insurance companies have generally not covered it. It is certainly more convenient than serum testing for the patient—especially since it spares the patient a needle prick. In the case of testosterone, if testing is performed correctly, results have been shown to correlate well with free testosterone levels in naive untreated patients.¹¹ However, in practice results may be somewhat confusing, particularly when treatment has been initiated. Sometimes there is a difference between serum- and saliva-testing values even before beginning treatment because of the way the test is performed, as patients may perform the chew test inadequately or may eat just before the test. Also,



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patients treated with testosterone often have very high levels, which may not represent the true androgen state. So far, recognized professional bodies such as the Endocrine Society, American Society of Clinical Endocrinologists and International Society for the Study of Aging Males have made consensus recommendations for treatment with testosterone based on blood testosterone levels rather than saliva levels. For medical and/or legal reasons, it is prudent at this point to base treatment on blood levels, as the consensus statements have been based on blood-testing levels. However, as there can be benefits with saliva testing, more research needs to be done on its utility before a final conclusion can be reached.

Other Tests to Assess Androgen Status

Other tests can also be used to assist in determining androgen status.

- Dynamic tests are sometimes used to assess the hypothalamic pituitary axis. For men, this may include the *human chorionic gonadotrophin (HCG) stimulation test* and the *clomiphene citrate test*. The former involves an intramuscular injection of, usually, 4000 IU of HCG for 4 days; this binds to luteinizing receptors in the Leydig cells and stimulates

the production and secretion of testosterone. A positive response usually causes testosterone levels to double and symptoms to improve, while no response indicates testicular failure. The latter test involves a test dose of 50 to 100 mg clomiphene, a nonsteroidal oral compound with estrogenic effects, which binds to estrogenic receptors in the body, causing the hypothalamus to secrete luteinizing hormone (LH). Healthy men usually have a 50% to 200% increase in levels of LH and testosterone. No response indicates testicular failure. These are, by and large, short-term tests—and long-term use is not established as yet.

- Measurement of LH and follicle-stimulating hormone (FSH) can be helpful in eliminating pathologic states that could be causing low hormones. It helps determine whether the low levels of testosterone are due to a primary or secondary process. When ordering a test to measure LH, it is sometimes necessary to distinguish between bioavailable and immunoreactive LH. Some laboratories do not evaluate the former. It is also inaccurate to rely on single values because of the pulsatile nature of LH. The secretion of FSH is less pulsatile. Although it is thought to be mainly regulating spermatogenesis, FSH may be an indirect measure of androgen regulation in aging men because it is secreted in conjunction with LH.

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The Importance of Symptoms in Diagnosis

As mentioned above, while laboratory assessments provide input to assist the clinician in the diagnosis of low hormone levels, they should be only one of the factors under consideration.³ A thorough history and physical examination is key, and it is imperative to evaluate patient symptoms before deciding to replace hormones. To use the analogy of building a house, when people are involved in such a project they want to see a picture of the whole house, not just a window. It is not possible to prescribe treatment accurately based only on a laboratory value. Often men and women suffering from low hormones may need an antidepressant, either in addition to or instead of hormones. They may need counseling. Sometimes a therapeutic trial with hormones may be necessary. The patient has to be evaluated as a whole and not in pieces. It is the symptoms that should lead to the treatment.

I often tell patients not to rely on numbers alone, as they tend to get fixated by them. Patients sometimes believe that they should feel three times better if the levels have tripled, but are disappointed when they are not feeling that way. You cannot look just at numbers alone. I ask patients to tell me how they feel because that will tell me whether treatment is successful. Clinical response is always a better indicator of dose requirements than serum testosterone levels.^{5,12}

Conclusion

Relying too heavily on laboratory assessment of hormone levels can result in misdiagnosis, lack of satisfaction on the

patient's part, and confusion; however, patients must be hypogonadal as defined by blood testing and symptoms before treatment. In the case of testosterone, physicians need to remember that (1) levels are not static, and (2) they change over time. The most important factor in optimal management is listening to the patient. Treatment for low testosterone should be based on symptoms superimposed on relative age-adjusted testosterone levels.⁴

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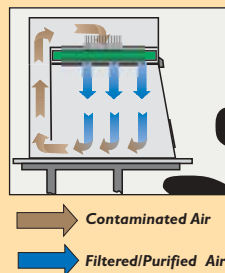
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