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EDUCATIONAL COMMENTARY – TESTOSTERONE AND SEX-HORMONE BINDING GLOBULIN

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LEARNING OBJECTIVES

On completion of this exercise, the participant should be able to

- explain reasons for testosterone and sex hormone-binding globulin testing.
- describe the forms of testosterone.
- identify methods available to measure testosterone, free testosterone, bioavailable testosterone, and sex hormone-binding globulin.
- interpret test results for testosterone, free testosterone, bioavailable testosterone, and sex hormone-binding globulin.

Introduction

The testes in males and the ovaries in females are the primary sites of production of the steroid hormone testosterone, although small amounts are produced in the adrenal cortex. This production is regulated through the hypothalamic-pituitary axis by luteinizing hormone (LH). Testosterone, the major androgenic hormone, is responsible for the development of the male external genitalia and secondary sexual characteristics, such as growth of body hair and increased muscle and bone mass. One of the main roles of testosterone in females is as an estrogen precursor, but excess production of testosterone in women can have masculinizing effects. Production of testosterone in both men and women declines with age, and after menopause, ovarian production decreases significantly. In circulating blood, approximately 60% of testosterone is bound to sex hormone-binding globulin (SHBG), approximately 38% is loosely bound to albumin, and only up to 4% is unbound or free testosterone (FT). Because binding to albumin is weak, this fraction is available for uptake into tissue, and the combination of free and albumin-bound testosterone is considered bioavailable testosterone (BAT). Testosterone bound to SHBG is not considered BAT.

Reasons for Testosterone Testing

Testing is performed in men and women to detect abnormal testosterone levels. In boys, testosterone testing may be ordered to investigate delayed or early puberty. In men, the primary reason for testosterone testing is investigation of androgen deficiency, which produces symptoms that may include erectile dysfunction, decreased sex drive, gynecomastia, small or shrinking testes, and loss of body hair. Testosterone testing may be used in women to investigate irregular or absent menstrual periods, development of masculine features (e.g., excessive facial and body hair or low voice), suspected

polycystic ovarian syndrome (PCOS), or adrenal gland dysfunction. Infertility testing usually includes measurement of testosterone levels in both the man and the woman. Testosterone levels may also be assayed to monitor testosterone replacement and antiandrogen therapy.

Tests Available

Total Testosterone

For situations in which testosterone testing is recommended, measurement of total testosterone is the most frequently performed test. Results are routinely reported as testosterone concentrations in ng/dL of serum or plasma. The most frequently used method is immunoassay, and many combinations of reagents/kits and analytic platforms/instruments are available. The use of tandem mass spectrometry after liquid chromatography (LCMSMS) is increasing.

The reference method developed by the Centers for Disease Control and Prevention (CDC) Hormone Standardization Program uses an isotope dilution LCMSMS method calibrated with a pure testosterone reference material. This program collaborates with immunoassay manufacturers to calibrate their assays, certifies laboratories for testosterone testing, and develops commutable sera. Standardization of testosterone testing has many benefits, including enabling the use of common reference ranges.

Free Testosterone

When total testosterone levels are equivocal or do not correlate with symptoms or anticipated levels, measurement of FT may be helpful. There are several different methods available for measuring FT, but only two of these, equilibrium dialysis and ultra-centrifuge-accelerated methods, are considered to accurately measure the FT concentration. Because both of these methods are expensive, labor- and time-intensive, and not easily automated, alternatives have arisen. Several analog-based automated immunoassays have been developed for FT testing. In these assays, a labeled testosterone analog competes with FT to bind to an antibody. Several studies have demonstrated that values obtained using these immunoassays correlate with the total testosterone concentration and not with the FT as measured by the reference method, equilibrium dialysis. In its clinical practice guidelines for testosterone therapy in adult men, the Endocrine Society recommends determining FT levels by equilibrium dialysis or by calculations using total testosterone, SHBG, and albumin, rather than by analog-based FT assays.[1]

Bioavailable Testosterone

Bioavailable testosterone may be defined as the testosterone not bound to SHBG, or conversely as the total of the FT and albumin-bound testosterone. The most common method for measuring BAT uses

ammonium sulfate to precipitate the testosterone bound to SHBG. Measurement of BAT is then performed using LCMSMS. Alternatively, BAT may be calculated using total testosterone, SHBG, and albumin concentrations.

Sex Hormone-Binding Globulin

A glycoprotein, sex hormone-binding globulin is synthesized in the liver and binds several sex hormones, including testosterone and estrone/estriol, with high affinity. It binds testosterone with a higher affinity than estrogens and thus has a significant effect on the balance between bioavailable androgens and estrogens. Immunoassay methods for SHBG are available on automated instruments. Sex hormone-binding globulin levels inversely affect BAT levels. When SHBG levels are increased, there is less BAT than indicated by the total testosterone concentration. Similarly, when SHBG levels are decreased, more of the testosterone is available to tissues. Measurement of SHBG levels allows calculation of FT and BAT in laboratories without access to assays for these two tests.

Calculated FT and BAT

Both FT and BAT may be calculated using total testosterone, SHBG, and albumin concentrations. Several calculations have been developed, and each may give a slightly different result. Some formulas use an albumin concentration of 4.3 g/dL (to convert to g/L, multiply by 10) if the albumin concentration is not measured. The formulas incorporate known binding constants for the proteins, and free online calculators are available. As with all calculated test results, the reliability of the calculated FT and BAT results depends on the accuracy of values used in the calculation.

Interpretation

Total Testosterone

Although reference ranges may be method and population dependent, a generally accepted lower limit of the total testosterone reference range in young men is approximately 300 ng/dL (to convert to nmol/L, multiply by 0.0347). There is a greater likelihood of symptoms of hypogonadism when the testosterone level is below this concentration than when it is above it. Conditions that may cause a low testosterone level include hypothalamic or pituitary disease, genetic diseases (Klinefelter, Kallman, and Prader-Willi syndromes), testicular failure, metabolic disorders (e.g., hemochromatosis, liver failure), and damage to the testes from alcoholism, physical injury, or viral diseases such as mumps. Primary hypogonadism (testicular failure) is associated with increased LH and follicle-stimulating hormone levels, whereas LH and follicle-stimulating hormone levels are typically low in secondary/tertiary (pituitary/hypothalamic) hypogonadism.

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The upper limit for total testosterone reference ranges in healthy men is approximately 900-1000 ng/dL. Significantly increased testosterone levels in men may be caused by testicular or adrenal tumors, hyperthyroidism, early puberty, congenital adrenal hyperplasia, or use of anabolic steroids.

Testosterone levels may be used to monitor testosterone replacement therapy with the goal of achieving normal testosterone and LH levels. In the Endocrine Society guidelines, a target testosterone concentration of 400 to 700 ng/dL midway between injections is suggested.[1]

In women, testosterone levels are normally low. Ovarian or adrenal tumors often produce testosterone levels greater than 200 ng/dL. Other causes of increased testosterone levels include PCOS, congenital adrenal hyperplasia, and precocious puberty. In PCOS, testosterone levels may be normal or mildly elevated but usually not greater than 200 ng/dL. In congenital adrenal hyperplasia, levels of androgens such as 17-hydroxyprogesterone are elevated in addition to testosterone.

Decreased testosterone levels may be observed in women with primary or secondary ovarian failure and in those who have had an oophorectomy.

Sex Hormone-Binding Globulin

Increased SHBG levels result in less BAT than is indicated by the total testosterone level. The resulting conditions may be associated with signs and symptoms of hypogonadism in men. Conditions in which increased SHBG occur include aging, liver disease, hyperthyroidism, human immunodeficiency virus infection, anorexia, and estrogen use (hormone replacement therapy and oral contraceptives).

Decreased levels of SHBG can result in androgenization in women. Conditions associated with decreased SHBG levels include obesity, diabetes, nephrotic syndrome, thyroid disease, PCOS, hirsutism, androgen (steroid) use, acne, and Cushing's disease.

Sex hormone-binding globulin levels may be measured to monitor antiandrogen therapy when clinical assays are not available to measure the sex steroids and gonadotropins used in this therapy. In this instance, increases in SHBG levels indicate successful antiandrogen or estrogen therapy.

Free and Bioavailable Testosterone

Usually, FT and BAT levels parallel total testosterone levels. However, any condition or medication that affects the SHBG concentration changes total testosterone concentration without necessarily changing FT or BAT levels. In some cases of mild hypogonadism in pubertal boys and adult men, determination of either FT or BAT may be more helpful than measurement of total testosterone levels. In PCOS there is

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often significant insulin resistance, which is associated with low SHBG levels. In such cases, either FT or BAT testing should be used to supplement total testosterone testing. In older men with elevated SHBG levels and albumin levels affected by coexisting illnesses, BAT is the preferred test.

Factors Affecting Test Results

Testosterone secretion occurs in circadian rhythms, with reported peak levels varying from 6 AM to 10 AM. In young men, the difference between AM and PM levels may be as much as 50%. Reference ranges are usually based on early morning collections. For this reason, the time of specimen collection for testosterone testing can affect results and interpretation. Unless specifically indicated otherwise, specimens should always be collected in the morning. Also, because testosterone levels can fluctuate between days, assessment should be based on more than one measurement.

As previously mentioned, testosterone and SHBG levels may be affected by several conditions and diseases. During pregnancy, placental tissue produces SHBG, raising levels. A genetic variant of SHBG causes slower degradation and results in higher than expected SHBG levels. Many medications can alter testosterone and SHBG levels. Such medications are too numerous to list, but their effect should be considered whenever testosterone or SHBG results do not correlate with symptoms or expected results. Some medications known to cause increased testosterone levels include anticonvulsants, barbiturates, estrogens (in women), and clomiphene. Medications that cause decreased testosterone levels include digoxin, anabolic steroids and estrogens (in men), and opioids. Men with advanced prostate cancer are often treated with androgens that lower testosterone levels. Medications causing increased SHBG levels include anticonvulsants and estrogens. Medications causing decreased SHBG levels include glucocorticoids, progestins, and androgenic steroids.

Summary

Testosterone, the major androgenic hormone, is responsible for the development of the male external genitalia and secondary sexual characteristics. In women, it is an estrogen precursor.

Testosterone testing may be ordered to investigate delayed or very early development of puberty in boys; in adult men, the primary reason for testosterone testing is investigation of androgen deficiency. In women, testosterone testing may be used when a woman exhibits signs of masculinization, has menstrual irregularities, or when PCOS is suspected. Measurement of total testosterone is the most frequently performed test, but in situations where the results do not correlate with the clinical manifestation, additional tests include FT, BAT, and SHBG assays.

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References

1. Bhasin S, Cunningham GR, Hayes FJ, et al. Testosterone therapy in adult men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2010;95(6):2536-2559. http://www.endo-society.org/guidelines/final/upload/final-androgens-in-men-standalone.pdf. Accessed January 5, 2013.