Diagnosis of male hypogonadism: what is low testosterone

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Summary

In contrast to primary hypogonadism, whereby the rise of gonadotropin levels helps establishing a diagnosis, the diagnosis of secondary hypogonadism relies for the most part, on the identification of a low testosterone level in the right clinical context. Establishing that a patient indeed has a low testosterone level requires taking into account several physiological as well analytical aspects. The vast majority of testosterone in men circulates in plasma bound to albumin (50%), sex-steroid binding globulin (SHBG) (44%) and other proteins (4%), while only 2% is found free. The biologically active fraction consists of the free and the albumin-bound fractions. Testosterone levels are highest in the early morning and lowest during the summer. Both total and free testosterone decline with age, as SHBG levels increase. Conditions such as obesity and diabetes also result in diminished testosterone concentrations, while they are accompanied by low SHBG levels. Currently, measurement of total testosterone in hospital laboratories is usually performed on fully automated immunoassay analyzers. Although tandem mass spectrometry methods after gas or liquid chromatography are the most accurate means for testosterone measurement, they are still not widely available in most clinical laboratories. In some cases the concentration of total testosterone may not always represent a true reflection of the patient androgen status and therefore an estimate of the non-SHBG or bioavailable fraction may be a more appropriate measure. Methods of assessment of the non-SHBG-bound fraction of testosterone include estimation of the free concentration by methods including the calculation of the free androgen index (FAI), equilibrium dialysis, centrifugal ultrafiltration, direct analog RIA or calculation of the free fraction.

KEY WORDS: testosterone; free testosterone; SHBG; hypogonadism.

Introduction

Testosterone is the main androgen synthesized and secreted by the normal adult male testicle (1,2). The regulation of the gonadal axis begins at hypothalamic nuclei where highly specialized neurons “awake” during puberty and start synthesizing and releasing GnRH (Gonadotrophin releasing hormone) in a pulsatile manner, although higher brain nuclei and sites including the neocortex, also play an important role (1,2) (Fig. 1). GnRH acts through specific Gs-protein coupled membrane receptors on the gonadotroph cells of the anterior pituitary, resulting in the transcription of the genes that encode the specific beta subunits of LH and FSH as well as the common alpha subunit, and subsequently in the secretion of these gonadotropins into the systemic circulation (1,2) (Fig. 1). LH exerts its biological effects, stimulating the testosterone-producing Leydig cells, whereas FSH targets the Sertoli cells and is more involved in spermatogenesis as well as in the synthesis and secretion of inhibin (1,2) (Fig. 1). The testosterone secreted by the testicle, in turn, inhibits the release of LH and FSH via a negative feedback loop at both the hypothalamic and pituitary levels,

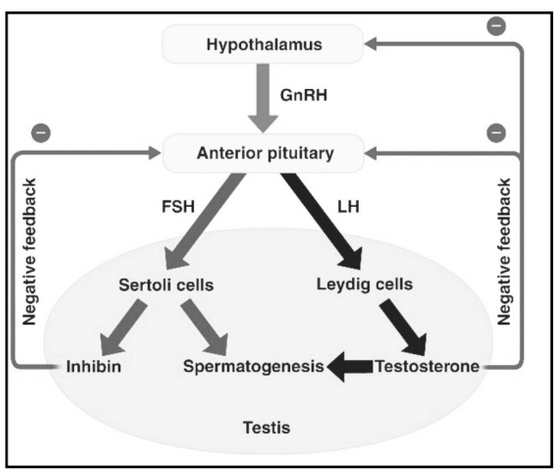

Figure 1 - Regulation of the male hypothalamic-pituitary-testicular axis.

Table 1 - Etiology of primary male hypogonadism.

Primary Male hypogonadism	
Congenital	**Acquired**
• Klinefelter Syndrome	• Mump·s and other infections
• FSH/ILH receptor mutations	• XRT and alkylating agents
• Varicocele	• Ketoconazole
• Cryptorchidism	• Glucocorticoids
• Disorders of androgen synthesis	• Environmental toxins
• Myotonic dystrophy	• Trauma
• Acquired	• Testicular torsion
	• Autoimmune
	• Hepatic & renal failure
	• HIV

Table 2 - Etiology of secondary male hypogonadism.

Secondary Male Hypogonadism	
Congenital	**Acquired**
• Kallman syndrome	• Suppression of gonadotropins
• DAXI and GPR54 mutations	- Hyperprolactinemia
• Leptin and leptin rec. mut.	- Gonadal steroids glucorticoid adm.
• Prader-Willy syndrome	- Opiates & GnRH analogs
• Gonadotropin SU mutations	- Critical illness
• Pituitary cytodifferentiation abnormalities	- Chronic systemic disease & DM
	• Gonadotrope damage
	- Pituitary adenomas
	- Craneopharyngiomas
	- Pituitary mets
	- Pituitary apoplexy
	- Trauma
	- Surgery and XRT to the sellar region

whereas inhibin acts only at the pituitary level (1,2) (Fig. 1). Irrespective of the etiology, in primary hypogonadism the loss of testosterone negative feedback results in a significant increment in gonadotropin secretion (1,2) (Tab. 1). In contrast, gonadal failure or insufficiency resulting from pituitary or hypothalamic causes is associated with low or inappropriately normal serum concentrations of LH and FSH (1,2) (Tab. 2). Thus whereas the diagnosis of primary hypogonadism is facilitated by the finding of high gonadotrophins in blood, the biochemical diagnosis of secondary hypogonadism relies only on the measurement of serum testosterone in the appropriate clinical context. Establishing that a patient has a low testosterone level is not always an easy task since several physiological and analytical aspects need to be taken into consideration. The purpose of this review is to summarize such aspects.

Basic physiology and biochemistry of circulating testosterone in men

The vast majority of testosterone in the adult male circulates in plasma bound to albumin (50%) and sex hormone binding globulin (SHBG, 44%), while only 2% is found free (3,4). The biologically active fraction consists of the free and the albumin-bound fractions (3,4). The precise role of these specific binding proteins remains incompletely understood. However, SHBG in particular appears to actively participate in the transport of testosterone into target tissues like the prostate and the testes themselves (5). Like with other steroid hormones such as estradiol and cortisol, the rise in binding proteins is associated with a concomitant increment in the measured total testosterone concentrations (3,4).

Testosterone secretion follows a circadian rhythm with the highest level found in the morning and the lowest in the evening (6). Such diurnal variation is clearly seen in males up to the seventh decade and appears to be distorted thereafter (6). The existence of a seasonal variation is controversial but it has been suggested that testosterone concentrations are higher during the winter and lower during the summer (6). Testosterone levels decrease progressively from early adulthood to older age (7-9). This is particularly true for free testosterone as SHBG concentrations rise with advancing age (7-9). The decline of testosterone with age is profoundly influenced by adiposity and the concomitant increased prevalence of conditions like diabetes that are also associated with low androgen concentrations (7-9). In contrast to menopause in women, andropause in men is more of a continuous age-dependent process with high inter-individual variability and overlapping serum testosterone concentrations between age groups (7-9). Serum concentrations of total testosterone are lower in obese men and this is for the most part due to a concomitant reduction in SHBG levels (7). Acute illness, HIV infection and malnutrition are associated with low total and free testosterone concentrations due to an adaptive state of secondary hypogonadism (7).

Analytical aspects of testosterone measurement

Total testosterone:

Testosterone measurement methods suffer from a lack of age- and gender-adjusted normal ranges and a universally recognized calibrating standard (10). Most clinical laboratories measure total testosterone (protein-bound plus free testosterone) by fully automated immunoassay analyzers (7,10,11). Prior to immunoassay, binding proteins need to be displaced from testosterone, be it by extraction of the steroid into an organic solvent or by the addition of a chemical agent with a higher affinity for the binding protein (7,10,11). The concentration of total testosterone in normal healthy men is at least four times higher than in normal adult women, and in most laboratories the quoted normal reference values range from 11 to 35 nmol/L (7,10,11). Compared to the original “manual” RIA, the currently in-use immunoassay platforms are capable of measuring many samples at the same time and allow for a quick turn-around of results (7,10,11). Inter-assay coefficients of variation among total testosterone im-

munoassays range from 2 to 8% (7,10,11). The main cross-reactant in testosterone assays is 5-alpha-dihydrotestosterone (DHT), which can still be detected after solvent extraction. However, the normal concentrations of serum DHT are considerably lower than those of testosterone and are rarely elevated enough to significantly interfere with testosterone measurement (7). Abnormally high testosterone concentrations (>40 nmol/L) are usually only found in patients receiving replacement therapy, since testosterone-secreting tumors are very rare. Spuriously low testosterone concentrations due to analytical causes are also very infrequent but can be found when the binding proteins in the sample are not properly extracted or even more rarely, by autoantibodies that recognize the labeled antigen (7,10,11).

Direct immunoassays without prior extraction for binding proteins tend to overestimate testosterone levels and have limited accuracy at concentrations below 11 nmol/L (7,10,11). Although these direct assays are currently fully automated and provide a rapid turnaround time, they are susceptible to matrix effects and the reference intervals are not standardized and totally method-dependent (7,10,11). RIA after chromatography extraction has been extensively used and there are well-documented reference intervals in different populations, however, it is technically cumbersome and very few clinical laboratories are qualified to do it (7,10,11). Mass spectrometry (MS) after extraction with either liquid or gas chromatography is highly accurate when properly validated and allows measurement of multiple steroids in the same aliquot, based on their chemical structures; tandem MS can increase the specificity of the method (12,13). The drawbacks of MS are their relatively high cost, lack of standardization and limited throughput (10).

Free testosterone:

As mentioned before, less than 2% of circulating testosterone is found free in plasma; the term bioavailable testosterone refers to the free and albumin-bound fractions, and appears to be biologically active. The reference methods for measuring free testosterone are equilibrium dialysis and centrifugal ultrafiltration (14,15). Both of these methodologies are technically cumbersome and thus, not widely available in clinical laboratories. Free testosterone is measured at many hospital laboratories by means of a tracer analog technique or direct RIA, which are rather inaccurate and non-validated (10). Bioavailable testosterone is measured in reference laboratories by the ammonium sulfate precipitation technique, which is also technically difficult (16).

Methods for estimating free testosterone:

In view of the difficulties in measuring free testosterone by robust and reliable methods such as equilibrium dialysis, several equations have been developed to estimate the percent of free testosterone in serum. Besides the total testosterone concentration, these formulae require the accurate measurement of SHBG concentrations, which can be done by immunoassays. The simplest of these equations calculates the free androgen index (FAI) as follows: FAI=[Total testosterone] x 100/[SHBG].

The FAI is useful in the diagnosis of hyperandrogenism in women but is not very reliable in discriminating hypogonadal from normal men (7). Another, more accurate way of calculating free testosterone based on the law of mass action also uses the measured total testosterone, SHBG and albumin but includes in the equation the dissociation constants (Kd) for testosterone with SHBG and with albumin (14,17). This method correlates very well with free testosterone measured by equilibrium dialysis (14, 17). These and other algorithms for the calculation of free and bioavailable testosterone are available at the website of the International Society for the Study of the Ageing Male (www.issam.ch).

Prevalence of androgen deficiency in men

Late-onset hypogonadism is a clinical and biochemical state with advancing age, characterized by particular symptoms and a low testosterone level (10). The exact prevalence and incidence of this syndrome in men is not known, as most of the published epidemiological studies used poorly validated immunoassay measurements of total testosterone (10). Such studies have used different definitions of hypogonadism based on the presence of symptoms, but the majority simply reports the prevalence of low testosterone levels (8,10). Thus, the precise testosterone level below which, symptoms of androgen deficiency emerge and adverse health outcomes ensue remains unclear.

Relatively recent publications of the European Male Aging Study (EMAS) provide a reasonable approximation to solving this issue (18-20). The EMAS has analyzed data from almost 4000 middle age and elderly men, recruited at 8 European centers (18-20). A cross-sectional analysis of over 3000 men spanning 4 decades of age from 40 to 80 years old found a decline in total and free testosterone concentrations of 8.6% and 33.1%, respectively, along with a 47% rise in SHBG levels (18). In this study, multiple factors altering the gonadotropic axis, such as obesity, smoking and the presence of co-morbidities like diabetes, are superimposed on the progressive testicular impairment associated with age (18). More recent reports from the same group, linked specific total testosterone-measured by GC-MS/MS-and calculated free testosterone levels with the appearance of symptoms of androgen deficiency (19,20). A decrease frequency of sexual thoughts, erectile dysfunction, a decreased frequency of morning erections and a reduced vigor were associated with total testosterone thresholds of 8, 8.5, 11 and 13 nmol/L, respectively; free testosterone levels of 160 pmol/L, were associated with a decreased frequency of sexual thoughts, whereas erectile dysfunction and decreased frequency of morning erections were linked to a free testosterone level of 280 pmol/L (19-20). Interestingly, psychological symptoms were not associated with a particular testosterone threshold. In this cohort,

4.1% and 17% of the population had total testosterone levels below 8 and 11 nmol/L, respectively (19,20). Overall, the prevalence of late-onset hypogonadism was estimated to be 2.1%, ranging from 0.1% in men aged 40-49 years, to 5.1% in men aged 70-79 years (19,20). Data from the Massachusetts Male Aging Study indicate that the prevalence of symptomatic androgen deficiency is between 6 and 12% (21,22). A similar figure has been reported in a population-based study derived from the Boston Area Community Health Survey and including Black, Hispanic and White men (23).

Another aspect that needs to be considered is the type of population studied, since the prevalence of androgen deficiency seems to be higher among men attending a primary care facility than among community-dwelling individuals (24,25). The Hypogonadism in Males Study estimated the prevalence of clinical and biochemical androgen deficiency in over 2000 men aged more than 45 years, visiting primary care practices in the United States (24). In this study, the prevalence of hypogonadism was close to 40%; conditions such as diabetes, hypertension, obesity, dyslipidemia and obstructive pulmonary disease, significantly increased the likelihood of androgen deficiency with odds ratios that ranged from 1.5 to 2.4 (24). Androgen deficiency is considerably more common in patients with diabetes. Biswass et al., have recently evaluated 115 subjects with type 2 and 93 with type 1 diabetes and compared them to an age-matched control group (26). These authors found that 45% and 61% of their patients with type 2 diabetes had very low levels of total (<11.9 nmol/L) and free testosterone (<260 pmol/L), respectively (26). The prevalence of a low androgen level was considerably lower among patients with type 1 diabetes, yet still significantly higher that in the control population (26). The frequency of low total and free testosterone in this study was strongly affected by age and central adiposity yet, androgen levels correlated rather weakly symptoms of sexual dysfunction and diabetes-related quality of life (26).

Concluding remarks

- Establishing the diagnosis of androgen deficiency in adult males can be a difficult task due to both physiological and analytical complexities of testosterone measurement.
- The initial hormonal evaluation of a man with probable hypogonadism should include measurement of early morning total testosterone, as well as LH, FSH and prolactin in order to differentiate primary from secondary hypogonadism. SHBG levels should also be measured in order to be able to estimate free testosterone levels.
- Ideally, total testosterone should be measured by tandem MS after extraction by either gas or liquid chromatography. Alternative methods include automated immunoassays that use an extraction step.
- A total testosterone value below 8 nmol/L is indicative of androgen deficiency and correlates well with the presence of symptoms. In subjects with values between 8 and 11 mmol/L, the free testosterone level is helpful in establishing a diagnosis.
- The prevalence of androgen deficiency among aging males varies according to the type of population studied. Among community dwelling populations it is reported to be between 6 and 12%, whereas among patients attending primary care facilities it can be as high as 30-40%.
- Normal aging is associated with a decline in testosterone levels, particularly in the free fraction, but symptoms of androgen deficiency such as sexual dysfunction do not always correlate with hormonal data.
- Conditions such as diabetes, obesity and insulin resistance are also associated with a reduced concentration of testosterone.
- The evaluation of the androgen status should be always carried out considering the clinical context.

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