Chapter 4. LABORATORY ASSESSMENT OF TESTICULAR FUNCTION

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Revised 1 1 2016

ABSTRACT

Since the symptoms of hypogonadism may be nonspecific, and the signs of testosterone deficiency can be subtle and slow to develop, the assessment of testicular function relies heavily on laboratory testing. The laboratory diagnosis of hypogonadism is based on a consistent unequivocally low serum total testosterone level, but normal ranges vary with different methods and among laboratories. Moreover, many men who present for possible adult onset testosterone deficiency have a low level of sex hormone-binding globulin (SHBG) associated with obesity, insulin resistance and type 2 diabetes. In these men, tests for the albumin bound and free testosterone fractions (non-SHBG or bioavailable testosterone) are essential for an accurate diagnosis. If testosterone deficiency is confirmed, differentiating between primary and secondary hypogonadism by measuring LH and FSH is important. With many disorders, however, both the testes and the hypothalamic-pituitary unit are affected. Other tests such as estradiol, inhibin-B, and mullerian inhibitory hormone, and provocative tests using hCG, GnRH or its analogs, or antiandrogens or antiestrogens are sometimes useful.

INTRODUCTION

The evaluation of men for suspected hypogonadism begins with a detailed medical history and a careful physical examination. Laboratory tests are an essential component of almost all evaluations, and proper interpretation of the results obtained requires an understanding of methodology as well as an awareness of the impact of endocrine rhythms, age, race, body composition, and nutrition on the various tests of endocrine testicular function. Endocrinologists rely on clinical laboratories to provide accurate and precise, and in some cases sensitive assays are essential for accurate diagnoses. We expect the reference ranges to be based on large normal control populations, and for some assays, normative data must be stratified by age. Unfortunately, these expectations are not always fulfilled. Endocrinologists should examine the protocols for each assay, and discuss them with the laboratory director. In recognition of these potential shortcomings, the U.S. Endocrine Society issued a position statement in 2007 [1] that calls for

standardizing the methods by which testosterone assays are validated, and provided recommendations to physicians who order and interpret the results of androgen assays. The U.S. Centers for Disease Control has established a voluntary Hormone Standardization Program to help clinical, research, and public health laboratories maintain and enhance the quality and comparability of their results.

TESTOSTERONE

Testosterone, the major androgen in men, is necessary for fetal male sexual differentiation, pubertal development, and the maintenance of adult secondary sex characteristics and spermatogenesis. Testosterone also regulates gene expression in most extra-genital tissues, including muscle and bone, and the immune system. The testes are the source of more than 95% of the circulating testosterone in men although the adrenal cortex produces large amounts of the testosterone precursor steroids, dehydroepiandrosterone (DHEA) and androstenedione.

Following birth there is a period of activation of the gonadotropin-releasing hormone (GnRH) pulse generator that stimulates testosterone secretion to peak levels of 200-300 ng/dl (7-10.5 nmol/L) at ages 1-2 months with elevation until age 4-6 months [2]. Thereafter, GnRH secretion declines, causing a fall in testosterone to very low levels until puberty begins. In early puberty, LH and testosterone secretion increase dramatically during sleep which gradually transitions to sustained secretion throughout the day and night [3]. The nocturnal rise in testosterone levels in early puberty can be used clinically to evaluate boys with delayed puberty because it may precede pubertal testis growth, and indicates that puberty has begun [4]. Figure 1 illustrates age-specific median values and ranges for testosterone in morning blood samples from 138 boys [5].

Hypothalamic neurons that express kisspeptin, neurokinin-B, and dynorphin, are thought to activate and up-regulate GnRH and initiate pubertal development [6]. Adult testosterone levels are usually achieved by age 16 years, and generally range from 300-1000 ng/dL (10-35 nmol/L).

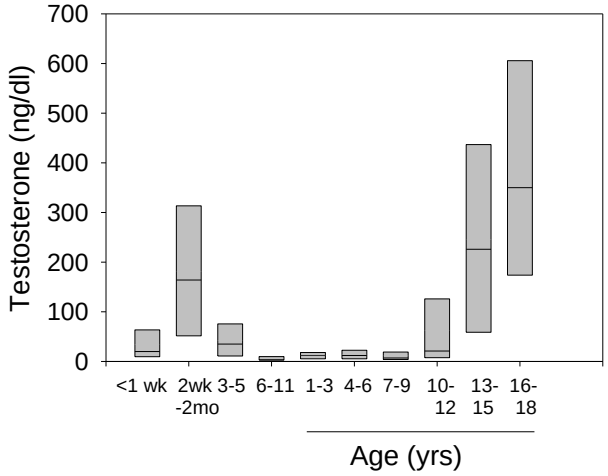

Figure 1. Serum testosterone levels (median and range) by LC-MS in leftover samples from blood checks before minor surgery or for the exclusion of endocrine diseases. Data from Kulle et al [5].

The secretion of testosterone is periodic with oscillations occurring over hours, days, and months. Frequent sampling of peripheral blood in adult men reveals small frequent moment-to-moment fluctuations in testosterone [7] whereas spermatic venous blood sampling reveals robust episodes of testosterone secretion occurring about once per hr [8]. Presumably because of this rapid pulse frequency and a plasma half-life of 60-90min, only small testosterone fluctuations are generally observed in peripheral blood. Therefore a single blood sample is usually an adequate assessment of testosterone production on a given day. There is a diurnal variation in testosterone in adult men, with highest levels in the early morning, followed by a progressive decline throughout the day, reaching lowest levels in the evening (Figure 2). Thus, the time of day of blood sampling is an important consideration, and a blood sample drawn in the morning between 0800 and 1000h is recommended because reference ranges are generally based on morning values. Nadir evening values are generally 15-20% lower than morning values in young men, although the differences can be as great as 50% [9]. The diurnal testosterone rhythm is blunted as men grow older [10] as well as in young men with primary testicular failure. On the other hand, the diurnal variation in testosterone is exaggerated in hypogonadal men with hyperprolactinemia [11] much like in normal adolescents. There may be a seasonable variation in testosterone as well [12].

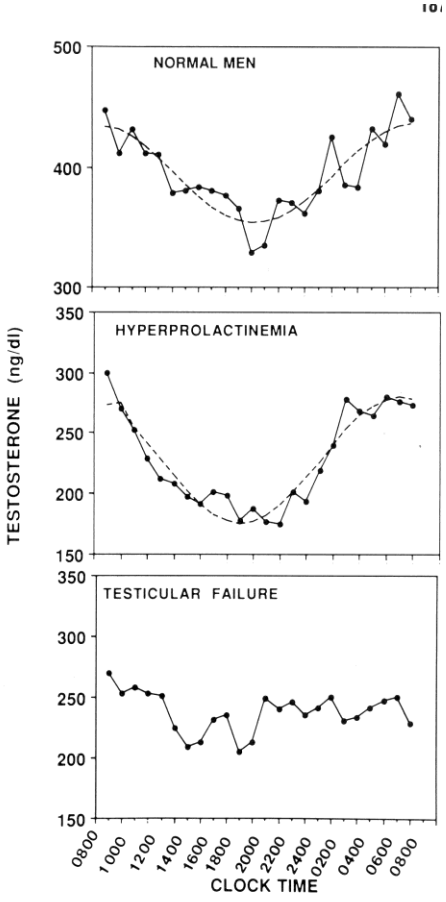

Figure 2. 24-hour testosterone concentration profiles in eugonadal men (n=5), untreated hypogonadal men with PRL-producing pituitary adenomas, and men with testicular failure (n=5). Adapted from Winters, SJ [11].

In general, the results of one abnormal test should be confirmed. In one study in which multiple blood samples were taken from middle-aged men over one year, there was a relatively good correlation (r=0.85) between the plasma testosterone level in the first sample and the mean value of seven subsequent samples; however, as many as 15% of men were misclassified, with either low or normal values that were not reproducible [13].

Testosterone deficiency can be an obvious clinical diagnosis, and

laboratory tests are often merely confirmatory; however, the diagnosis of hypogonadism is sometimes less straightforward. Many authors feel that the diagnosis of hypogonadism requires the presence of symptoms of hypogonadism as well as a low serum testosterone level. On the other hand, the symptoms of hypogonadism lack specificity, and are subjective. Thus the diagnosis by laboratory testing may be more objective, and less controversial. While men with low testosterone levels tend to have symptoms of hypogonadism [14], many studies have shown that symptoms are often unrelated to testosterone levels, and many men with low testosterone levels do not complain of symptoms [15].

The level of total testosterone in serum is the best single test to screen for hypogonadism because methodology has been optimized and normative data are widely available. While early assays extracted plasma steroids into organic solvents and separated them by column chromatography, those research methods generated radioactive waste and are too costly for clinical purposes. Currently most hospital laboratories use automated platforms with either a competitive or proportional two-site format. These platforms use small sample volumes to avoid matrix effects and a monoclonal antibody with a chemi-luminescent label for detection. The light flash emitted, measured by a photomultiplier tube, produces a signal proportional to the amount of hormone in the sample. The accuracy of the result is highly dependent on the affinity and specificity of the antibody used, and the literature unequivocally demonstrates that platform immunoassays provide variable results (Figure 3) especially for the low values in children and women. When the manufacturer provides a reference range which is based on a large number of samples, automated assays are probably sufficient for most clinical purposes in adult men although results can vary by as much as two-fold among different assay methods (Figure 3). There is a substantial positive bias at low values, however, so that automated assays are not recommended for clinical purposes in women and children. Lipemia may produce high values.

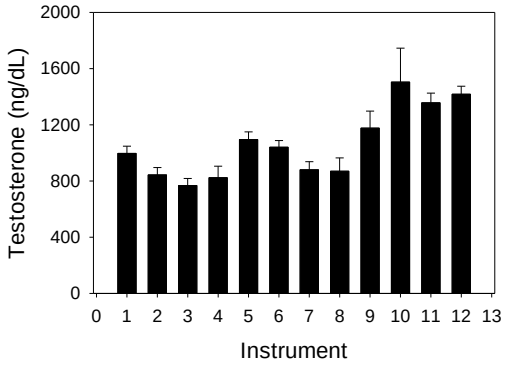

Figure 3. Total testosterone levels (±SD) in a proficiency sample (Y-02) distributed by the College of American Pathologists, and assayed using 12 different instruments in 1505 participating laboratories in 2015. The most frequent method (#8) was used by 358 laboratories; method #4 is mass spectrometry

Reference laboratories are increasingly employing automated liquid (LC) or gas chromatography (GC)-tandem mass spectrometry (MS)

methods [16, 17]. This approach combines the resolution of chromatography with the specificity of MS, and is now viewed as a gold standard. Larger aliquots of serum are extracted with organic solvents, steroid hormones are separated by chromatography, in some assays following derivatization, and values are determined by peak area integration of testosterone-containing fractions of the column eluate. Accuracy is gained by adding an internal standard as a stable isotope of the same compound being measured in order to correct for procedural losses. LC-MS accurately detects testosterone concentrations as low as 1.0 ng/dl with a within assay coefficient of variation of <10% [18, 19]. The equipment needed is costly, however, and each LC-MS assay requires substantial analytical development and optimization, and highly trained personnel. When compared to the coefficient of variation among various immunoassay methods (23%), the coefficient of variation was 10% among those laboratories (n=38) that used mass spectrometry methodology in the CAP survey (Figure 3).

Most cross-sectional and longitudinal studies indicate that testosterone levels peak in the third decade, and are lower as men grow older. On the other hand, one study of men who described their health status as very good or excellent found no impact of age on total testosterone levels in men between the ages of 40-80+ [20]. One reference range for total testosterone from normal volunteers assayed by high-turbulence flow liquid chromatography (HTLC)-MS [17] is shown in Table 1.

Table 1. Impact of age on total testosterone by HTLC–MS/MS in normal men. (ng/dl)

Age (yrs)	*20-29*	*30-39*	*40-49*	*50-59*	*60-69*	*70-79*	*>80*
Mean	*590*	*546*	*573*	*534*	*559*	*417*	*404*
SD	*232*	*206*	*209*	*194*	*226*	*177*	*234*
95th percentile	*1052*	*910*	*901*	*909*	*928*	*755*	*716*
5th percentile	*283*	*319*	*310*	*296*	*290*	*168*	*92*
N	*61*	*76*	*55*	*51*	*19*	*44*	*19*

Results from Quest Diagnostics, San Juan Capistrano, CA, courtesy of M.P. Caulfield. To convert total testosterone levels to nmol/L multiply by 0.0347

Table 2 shows a second reference range with values that are 20-40% higher for a combined population of men from the Framingham Heart Study analyzed by LC-MS, and the European Male Aging Study and Osteoporotic Fractures in Men Study analyzed by GS-MS [21].

Table 2. Impact of age on total testosterone levels measured by LC-MS or GC-MS) from Bhasin et al [21]†.

Age (yrs)	20-29	30-39	40-49	50-59	60-69	70-79	80+

Mean	713	656	617	611	569	567	570
95thpercentile	1104	1084	1001	1059	965	1097	1079
5th percentile	375	343	304	286	276	254	238
N	220	660	872	788	493	289	26

†To convert total testosterone levels to nmol/L multiply by 0.0347

Testosterone in Saliva

The level of testosterone in saliva is positively correlated with the free testosterone concentration in serum [22] , and salivary samples are easily collected, usually by a non-stimulated drool. Both extraction and non-extraction immunoassay methods are available although LC-MS is being increasingly employed [23]. Usual values in adult men are 150-500 pmol/L (40-145 pg/ml). Salivary testosterone assays are a useful research tool for field studies and other settings in which blood sampling is impossible or impractical [24, 25]. Method artifacts may occur, however, and careful assay validation and quality control are essential. Salivary testosterone assays are not recommended for clinical purposes [26].

SHBG

Sex hormone-binding globulin (SHBG) is a glycoprotein of molecular weight 90-100,000 KDa that is produced by the liver. SHBG binds testosterone and other steroids, prolongs their metabolic clearance, and regulates their access to target tissues [24]. SHBG had been measured indirectly by radio-ligand binding assays, but two site immune-radiometric and enzyme-linked assay kits are now widely available, and automated versions have been developed. There is little effect of meals or time-of-day on SHBG.

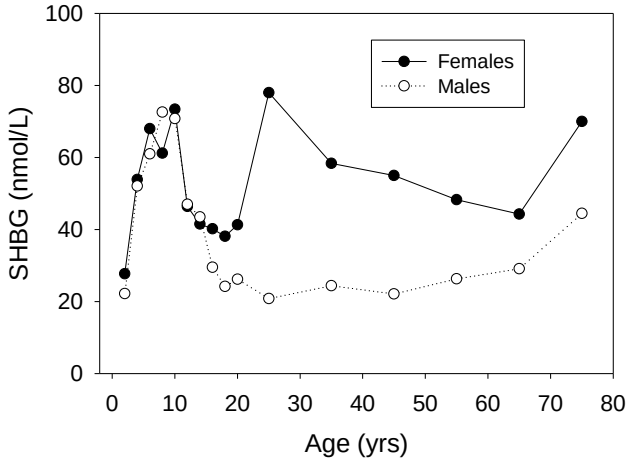

Figure 4. SHBG levels from birth to old age in males and females. Redrawn from Elmlinger et al [27].

The impact of age on SHBG in males and females is shown in Figure 4. SHBG is found in cord blood and in the plasma of newborns. This study found an increase following birth to peak values at ages 4-5 years followed by a decline that is greater in males than females [27]. SHBG levels in adult male plasma range from 20-100 nmol/L, and levels tend to rise slightly in old age.

In normal men, SHBG binds 40-60% of the circulating testosterone with high affinity, and the level of SHBG is a major determinant of the total testosterone level (Figure 5). Single nucleotide polymorphisms in the SHBG gene appear to influence the affinity of SHBG for testosterone and affect the level in plasma [28].

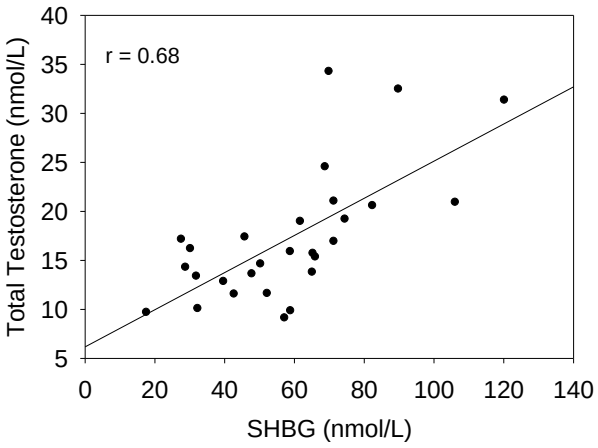

Figure 5. Levels of SHBG and total testosterone in 28 normal men whose BMI ranged from 23-40 kg/m^2. From Winters SJ et al [29].

A variety of clinical conditions is associated with reduced or increased SHBG levels, and thereby tends to lower or increase total testosterone concentrations (Table 3). High SHBG levels in men with HepC or hyperthyroidism may lead to very high total testosterone levels and can be a diagnostic dilemma. Serum SHBG levels are inversely related to BMI [30], and are low in men with T2DM or the Metabolic Syndrome (MetS) [31]. In fact, low SHBG predicts the development of the MetS as well as type 2 diabetes mellitus [32]. SHBG levels rise with weight loss [33] and when insulin resistance improves, e.g. with exercise. Some studies suggest that the mechanism for low SHBG with insulin resistance and obesity may be through hyperinsulinemia [34, 35], and other studies suggest a mechanism involving liver fat, cytokines, and the transcription factor HNF4α [36, 37].

Table 3. Factors that influence the level of SHBG in plasma

Increase	Decrease
hyperthyroidism	hypothyroidism
hepatitis C	Insulin resistance
GH deficiency	growth hormone excess
alcoholic cirrhosis	Glucocorticoids
acute intermittent porphyria	Androgens
first generation anticonvulsants	progestins
estrogens	nephrotic syndrome
thinness	genetic mutation
	obesity

FREE AND NON-SHBG (BIOAVAILABLE) TESTOSTERONE

The total testosterone level is sometimes inadequate to determine whether testosterone deficiency is present. This occurs with a borderline value, or when the clinical findings and the total testosterone concentration do not agree. This situation occurs most

often in obese men, those with type 2 diabetes, or the metabolic syndrome whose total testosterone levels are generally low. In older men, on the other hand, the total testosterone level may be within the normal range even though testosterone production is reduced. The total testosterone level may also be elevated, most often in men with hyperthyroidism,or hepatitis-C, or taking first generation anticonvulsants. These values occur primarily because the primary condition causes a marked decrease or increase in SHBG levels, and in these conditions diagnoses based on total testosterone levels may be misleading.

Under these circumstances, assessment of free or non-SHBG bound testosterone assists in diagnosing androgen deficiency. This approach stems from the hypothesis that the bioactivity of circulating testosterone is due to the small percentage (1-4%) of total testosterone that circulates unbound (free testosterone), or to the 40-50% of testosterone that circulates unbound as well as loosely bound to albumin (non-SHBG testosterone) and is often designated as "bioavailable-testosterone". The dissociation of albumin-bound testosterone is very rapid, and a short dissociation time is thought to allow the albumin-bound fraction to be available for uptake by cells [38].

The free testosterone concentration can be calculated from the product of the total testosterone level and the percentage that is free. The latter can be determined by equilibrium dialysis. This procedure examines the distribution of radiolabeled-testosterone between two compartments, one containing the tracer added to the serum sample, and the second containing buffer or an albumin solution. The compartments are separated by a semi-permeable membrane with a low-molecular weight cut-off. Dialysis is generally performed overnight at 37C by which time the concentrations of radiolabeled-testosterone in the inner and outer compartments reach equilibrium. With centrifugal ultrafiltration, dialysis is accelerated by centrifugation. The percentage of counts per minute (cpm) of radiolabeled-testosterone within the inner compartment is determined, and is multiplied by the total testosterone level. The % cpm in the inner compartment containing the plasma is proportional to the concentration of SHBG. Since the % free testosterone in men is usually 1-4%, the free testosterone level commonly ranges from 4-20 ng/dL (140-695 pmol/L). Equilibrium dialysis is associated with potential error due to temperature, sample dilution and tracer impurities, among other problems. The level of free testosterone has also be measured in the dialysate directly using a highly sensitive RIA [39] or by LC-MS [40].

A second and technically simpler approach to adjust the total testosterone level for a high or low SHBG concentration is to calculate the free testosterone level from the levels of total testosterone and SHBG, using binding constants for SHBG and albumin. The K_D for testosterone for SHBG is most often defined as 1×10^9 L/M and for albumin, 3×10^4 L/M, and the use of different constants will affect the result. Differences in the level of albumin in

the sample have little impact on the calculated free testosterone, and are usually ignored. Both the free and non-SHBG bound testosterone can be computed using an internet program (www.issam.ch/freetesto.htm[1]). There is an excellent correlation between the level of free testosterone obtained by equilibrium dialysis and the calculated free testosterone level [41], and between the calculated free testosterone and non-SHBG testosterone levels. Calculated free testosterone concentrations vary with the binding constants and algorithms employed, however, and SNPs of the SHBG gene may influence the affinity of SHBG for testosterone [28], and thereby the accuracy of the calculated free testosterone level. Because the result is calculated from testosterone and SHBG levels, measurement error for the two hormones impact directly on the measured free and non-SHBG testosterone.

Published reference ranges indicate that free testosterone levels decline as men grow older but results vary between reference laboratories.

Table 4. Reference for free testosterone (pg/ml) by equilibrium dialysis in normal men†.

Age (yrs)	20-29	30-39	40-49	50-59	60-69	70-79	>80
Mean	102	86	74	84	84	80	65
SD	29	33	22	30	22	27	26
95.00%	148	144	114	136	111	131	101
5.00%	57	45	37	35	55	47	34
N	49	55	48	36	14	36	17

†Results from Quest Diagnostics, San Juan Capistrano, CA, courtesy of M.P. Caulfield. ^{3}H-testosterone was added to a sample diluted 1:5 in assay buffer and incubated in a dialysis chamber for 20 h at 37 °C to allow the tracer to reach equilibrium with the endogenous testosterone and binding proteins. To convert total testosterone levels to nmol/L multiply by 0.0347.

Table 5. Influence of age on free testosterone levels (pg/ml) in community men†.

Age	**20-29**	**30-39**	**40-49**	**50-59**	**60-69**	**70-79**	**>80**
Mean	148	132	116	99	82	72	58
5th Percentile	79	70	61	50	45	33	29
95th Percentile	229	212	199	164	135	132	93
n	220	660	872	788	493	289	26

†Total testosterone was measured in community dwelling men in Framingham, MA using LC-MS. SHBG was measured using a two-site immunofluorometric assay from DELFIA-Wallac, Inc. Free

testosterone was calculated by the law-of-mass-action equation using of *K* SHBG-T of 0.998×10^9 L/mol and a *K* Alb-T of 3.57×10^4 L/mol. Data from Bhasin et al, [21].

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Non-SHBG-testosterone is called "bio-available" because adding SHBG to an androgen-containing sample reduces its androgen receptor binding activity [42] and there is experimental evidence that both the free and albumin bound testosterone fractions are biologically active [43]. The non-SHBG-testosterone (bioavailable) level can be determined by adding a tracer amount of ^{3}H-testosterone to the serum sample, and selectively precipitating the SHBG-bound ^{3}H-testosterone by adding 50% ammonium sulfate. Concanavalin-A Sepharose can alternatively be used. The ^{3}H-testosterone that remains in the supernatant is presumed to be either free or albumin-bound, and is counted. The percentage of counts added that is in the supernatant is multiplied by the total testosterone level in order to determine the non-SHBG testosterone. The assay is technically easy to perform, but is a two-step procedure requiring specialized reagents and ^{3}H-testosterone, and is not readily automated. Differences in reagent purity can affect the results, and the complete separation of SHBG from albumin is presumed, but not verified. A published range for bioavailable testosterone by age in which testosterone was measured in the supernatant following ammonium sulfate precipitation is shown in Table 6.

Table 6. Age-dependent reference range for non-SHBG (bioavailable) testosterone (nmol/L) †.

Age (yrs)	30-<35	35-<40	40-<45	45-<50	50-<55	55-<60	60+
Median	5.4	5.1	4.8	4.5	4.0	3.5	3.0
2.5 percentile	3.6	3.3	3.0	2.7	2.4	2.0	1.7
97.5 percentile	8.1	7.9	7.7	7.4	6.8	6.1	5.4
n	16	20	23	31	29	17	18

† Morning blood samples were drawn from Canadians of European or South Asian origin. Bio-testosterone was determined by competitive chemiluminometric immunoassay (Siemens Centaur) after ammonium sulfate precipitation (from Zimmerman et al [44].

The free testosterone index (FAI, free androgen index) represents the ratio: total testosterone/SHBG (both in units of nmol/L). This value is easy to calculate, and is believed to be valid in serum samples from women. It is less useful in men [45] because most of the SHBG in men is bound to testosterone. Like the calculated free testosterone, the FAI is dependent on accurate values for testosterone and SHBG

The direct free testosterone assay was developed as a single-step, non-extraction method in which a ^{125}I-labeled testosterone analog competes with unbound testosterone in plasma for binding to a testosterone-specific antiserum that has been immobilized on a polypropylene assay tube. The basis for the test is that the analog has a low affinity for SHBG and for albumin. Values for normal men with this method, as a percentage of the total testosterone (0.2-0.64%), are substantially lower than the 1.0-4.0% determined by other methods. While this difference alone does not cause a problem if adequate reference ranges are available, it immediately prompted speculation concerning the accuracy of the method. Subsequent studies revealed that analog free testosterone assay results are related to the level of SHBG, much like total testosterone[29], and that free testosterone is un-measurable by analog assays in a dialysate of normal adult male serum [46]**.** Thus, the free testosterone level determined with analog assays appears to provide essentially the same information as the total testosterone level, and is not recommended. The 2015 College of American Pathologists survey revealed that 10% of participating laboratories continue to use this method; howeve**r,** it is slowly disappearing from research articles related to testosterone.

Cell-based reporter bioassays have been developed to analyze androgen bioactivity in biological samples. A stable cell line is created by transfection with plasmids encoding the human androgen receptor and a reporter system containing an androgen-responsive gene such as the mouse mammary tumor virus (MMTV)-luciferase reporter. When cells are stimulated with androgens, luciferase activity is increased dose-dependently [47, 48]. These assays remain investigational.

DIHYDROTESTOSTERONE

Testosterone is converted to the more potent ligand dihydrotestosterone (DHT) by at least two steroid 5α-reductase (5AR) isoenzymes, 5AR types 1 and 2. 5AR-1 is found in liver, skin, brain, ovary, prostate, and testis whereas 5AR-2 is expressed in prostate, seminal vesicle, epididymis and skin. Approximately 20% of the circulating DHT in men is secreted by the testes, and the remainder is derived from the bioconversion of testosterone in tissues. Because of a high level of expression of 5AR-2 in prostate, testosterone is effectively converted to DHT in that tissue, in which the level of DHT exceeds the peripheral blood concentration by 5-10 fold. The concentration of DHT in adult male serum is only about 10% of the value for testosterone. Therefore an assay with negligible cross-reactivity with testosterone is needed for an accurate result. A mass spectrometry method is now used by most reference laboratories. Patients with 5α-reductase deficiency type 2 have ambiguous genitalia, and are detected as neonates, although a few patients have only microphallus or cryptorchidism. A rise in

testosterone but not DHT following hCG stimulation, producing a ratio of more than 10:1, is characteristic in most of these patients [49]. However, DHT production by 5AR1 can reduce the reliability of the ratio for diagnosis [50]. The diagnosis is most often made by urinary steroid profiling by GC-MS together with mutational analysis. DHT levels are often measured in epidemiological studies and in clinical research on prostate cancer and its treatment, but are probably not useful in most clinical situations.

ESTRADIOL AND ESTRONE

Serum levels of estradiol and estrone are often measured in men with gynecomastia [51], or with unexplained gonadotropin deficiency. These conditions are rarely but occasionally due to estrogen-producing tumors, or to acquired or genetic abnormalities in which estrogen production is increased. Moreover, accurate measurement of estradiol may be helpful when SERMs, aromatase inhibitors or hCG are used are used to increase testosterone levels. Understanding the physiological function of estrogens in men is also of substantial research interest [52, 53]. Estradiol is produced from testosterone, and estrone is produced from androstenedione, by aromatase P450, the product of the CYP19 gene. This enzyme is expressed in Leydig cells and in the adrenal cortex, as well as in adipose- and skin-stromal cells, aortic smooth muscle cells, kidney, skeletal muscle cells, and the brain. The promoter sequences of the P450 aromatase genes are tissue-specific, but the translated protein appears to be the same in all tissues. Increased aromatase expression in adipose and skin stroma with obesity is the most common cause for mild estrogen excess in men. Interestingly, most studies of the age-associated decline in testosterone do not find a parallel fall in plasma estradiol levels perhaps because of increasing aromatase activity and fat mass as men grow older [54].

Traditional immunoassays for estrogens employed large volumes of plasma (2-5 ml) that are extracted with organic solvents. Because those assays are time-consuming and expensive to perform, non-radioactive automated methods that were optimized for the higher values normally found in pre-menopausal women are often employed. However, the very small sample volumes used in automated assays may produce unexpectedly high values. There is poor agreement between laboratories as shown in the results for the low potency proficiency sample distributed in 2015 by the College of American Pathologists to 1450 participating laboratories (Figure 6). While several methods produced similar results, overall values ranged from 73.7 to 307 pg/ml (258-1127pmol/L), which represents a coefficient of variation of 38%, compared to the goal of ±12.5%.

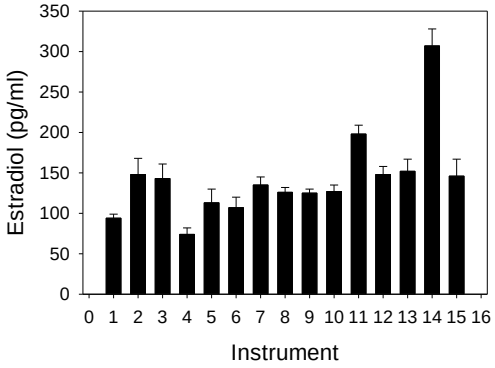


Figure 6. Estradiol levels (±SD) in a low range proficiency sample (Y-03) distributed by the College of American Pathologists assayed using 15 different instruments in 1459 participating laboratories in 2015. Mass spectrometry assays (n=10) are represented in column 4.

Mass spectrometry assays for estradiol and estrone, with a limit of detection of 1 pg/ml, have replaced conventional radio-immunoassays in most reference laboratories [55], and are recommended. Figure 6 shows that the result with these assays is much lower than with immunoassays which presumably also detect interfering substances. Moreover, the between laboratory coefficient variation for those laboratories using MS methods was also acceptably low at 10.4%.

Most of the circulating estradiol in men is loosely bound to albumin or is unbound [56], and only about 20% is bound to SHBG. Because estradiol binds to SHBG with lower affinity than does testosterone, the serum level of SHBG was not predicted to influence substantially the actions of estradiol. In many research studies, however, non-SHBG bound estradiol levels correlated more strongly with low bone mineral density and with indexes of high bone turnover in older men than did levels of total estradiol [57]. Non-SHBG (bioavailable) estradiol levels can be determined by ammonium sulfate precipitation using ^{3}H-estradiol, or by equilibrium dialysis. Non-SHBG- and free- estradiol can be calculated from the total level of estradiol, the level of SHBG, and binding constants of estradiol to SHBG and albumin of 0.3×10^{9} L/M, and 3×10^{4} L/M, but this calculation is complex because it ignores the testosterone binding to SHBG. Reference ranges are not available, and these measurements are primarily applicable to research studies.

GONADOTROPINS

FSH and LH, together with TSH and hCG, form a closely related family of heterodimeric glycoprotein hormones. Each consists of a common α-subunit that is non-covalently linked to a specific β-subunit. The α–β dimer is held together by a 'seatbelt' structure formed by the C-terminal amino acids of the β-subunit wrapped around the α-subunit. Both subunits have asparagine-linked carbohydrate chains (2 for human α-subunit, 2 for FSH-β, and 1 for LH-β). The oligosaccharides project from the peptide skeleton, and

by shielding of epitopes and altering the tertiary structure of the hormone, the sugars may impact receptor activation and bioactivity as well as antibody binding. Glycosylation also prolongs hormone clearance.

Most laboratories utilize fully automated, commercial assay systems for peptide hormones including LH and FSH. Detection is generally by chemi-luminescence, avoiding the use of radioactive tracers. Many assays achieve specificity by utilizing a biotinylated monoclonal antibody to the α-subunit as a capture antibody. A second monoclonal antibody to the β-subunit is labeled with an organic ester that produces chemiluminescence in the presence of hydrogen peroxide (indicator antibody). Various pituitary and recombinant preparations are used for the standard curve but most assays are calibrated in terms of IU/L of International Reference Preparations (IRP) of highly purified human LH and FSH. The various standards have differing sugar sequences and branch patterns producing some variation in results between laboratories and assays, however, differences are relatively small. When defined as a level of precision of replicate determinations of <10%, the sensitivity of these assays approximates 0.1 U/L.

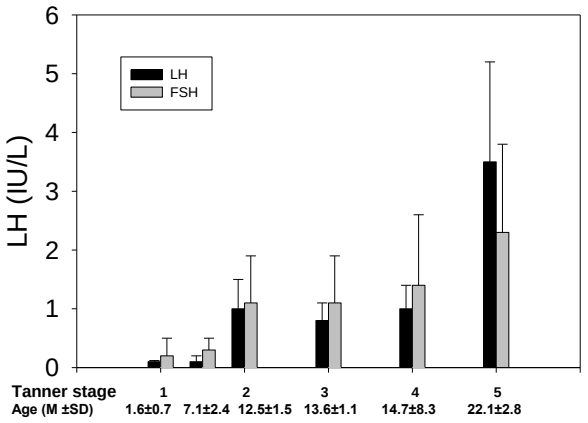


Figure 7. Serum LH and FSH levels in normal males measured by an ICMA assay. Redrawn from Resende et al. [58].

Figure 7 shows mean levels of LH and FSH across the stages of pubertal development in boys measured with an automated ICMA. Values with an immunofluometric assay were slightly higher [58]. FSH levels tend to exceed LH before puberty, and both gonadotropins rise progressively during puberty with substantial overlap among the various pubertal stages.

Prepubertal children have low amplitude but discrete pulsatile patterns of LH secretion that are amplified during sleep as puberty begins [59]**.** In adult men, LH is released in robust pulses every 1-2 h throughout the day and night with within subject variation in pulse height and between-pulse interval (Figure 8). In part because of this pulsatility**,** the normal range for LH is wide, with typical ranges for adult men in terms of 2nd IRP of 1.6–8.0 IU/L for LH and 1.3–8.4 IU/L for FSH. Because of this pulsatility, pooling of 3 samples taken

20–30 min apart may provide a more accurate estimate of a person's LH value than does a single sample.

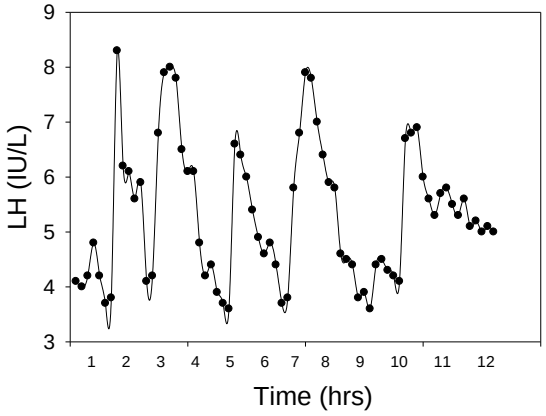


Figure 8. Pulsatile patterns of LH secretion. Blood samples were drawn every 10 min for 12h starting at 0800 h from a 32 year old normal man whose testosterone level was 474 ng/dL. Winters, SJ (unpublished).

Elevated levels of LH and FSH generally signify testicular damage. The testes are usually small and testosterone levels are low. Circulating estradiol levels are generally normal, however, in part because testicular aromatase is stimulated by LH. This observation contrasts with studies in men treated with estrogen antagonists or aromatase inhibitors, and observations in a few men with inactivating mutations of the estrogen receptor-α in whom LH is elevated and testosterone levels are normal or elevated which imply that estradiol is the major mediator of testicular negative feedback control of GnRH-LH secretion. Nevertheless, elevated LH levels in patients with androgen insensitivity syndromes and men treated with antiandrogens reveal additional inhibitory control by androgen receptor signaling. FSH levels are generally normal in patients with androgen insensitivity because of intact feedback regulation by estradiol and inhibin.

FSH levels may be increased selectively. FSH production is regulated not only by GnRH and gonadal steroids but also by a paracrine control mechanism that involves pituitary activin and follistatin, and gonadal inhibin (see below). When Sertoli cells fail to function normally, inhibin-B production declines, and pituitary activin stimulation of FSH-β gene expression is unopposed. Most men with high FSH levels have small testes and oligo- or azoospermia. FSH levels are inversely associated with indices of spermatogenesis in testicular biopsy specimens [60]; however, FSH levels may be within the reference range in infertile men with severe damage to the germinal epithelium who may have normal sized or small testes Therefore, testicular sperm extraction or open testicular biopsy may occasionally be needed to further distinguish men with obstructive from non-obstructive azoospermia [61].

An elevated FSH level may rarely indicate an FSH-producing pituitary gonadotroph adenoma [62]. These tumors are generally macroadenomas, and often co-secrete free α-subunit. LH is usually

suppressed even when immunochemistry reveals that the tumors are LH-β immunoreactive, and testosterone levels are generally low. However, LH and testosterone levels may be normal. A high LH level due to LH secretion by a pituitary tumor is very rare [63]. FSH hypersecretion can lead to testicular enlargement [64]. FSH levels tend to rise as normal men grow older, and may exceed the normal range [65.]

The diagnosis of hypogonadotropic hypogonadism is based upon finding a low serum testosterone level with inappropriately low/normal LH and FSH concentrations. It is important to verify that testosterone deficiency is present since LH levels may overlap with the reference range because of the pulsatile nature of LH secretion. The differential diagnosis is broad and includes congenital HH including Kallmann syndrome, and acquired disorders such as prolactinoma, other sellar and suprasellar tumors or cysts, infiltrative diseases and vascular causes, iron overload, head trauma and others. HH in childhood limits pubertal development while in adults it results in the regression of secondary sexual characteristics, albeit slowly. Three men have been reported with isolated deficiency of FSH due to mutation in the *FSH-β* gene sequence. Affected men have been azoospermic, with borderline or low testosterone and increased LH levels [66]. A SNP in the 5′ untranslated region of *FSH-β* that reduces binding by the transcription factor LHX3 has been associated with reduced serum FSH levels in men from the Baltics, Italy, and Germany [67].A few hypogonadal men with inactivating mutations of the LH-β gene have also been described. LH may be present or absent in serum depending on the nature of the mutation [68]. As placental hCG, rather than LH, stimulates male sexual differentiation, affected males have a normal phenotype at birth while deficiency of bioactive LH prevents normal puberty.

Two-site assays can occasionally be "too specific". Polymorphisms that impact on immunoassay detection by monoclonal antibodies may lead to misdiagnoses. For example, there is a relatively common polymorphism in the LH-β gene that is characterized by two point mutations in codons 8 and 15 resulting in two amino acid substitutions and an extra glycosylation site [69]. Even though men and women with the LH variant appear to be normal and fertile, the serum LH level is low or undetectable using certain monoclonal antibodies, whereas the result with other assays is normal. When a low or undetectable result, or a disparity between LH and FSH levels cannot be readily explained, a second assay method should be used.

Various glycoforms of LH and FSH with structurally heterogeneous glycans are found in the pituitary and in the circulation, and glycosylation has been shown to influence hormone clearance and biological activity. Both sex steroids and activin have been reported to affect glycosylation. Highly sialylated glycoforms, with an acidic pH, tend to have a longer circulating half-life, whereas the more alkaline forms tend to exhibit greater bioactivity in vitro. The finding

that LH and FSH glycosylation is under physiological control increases further the interest in the gonadotropin glycoforms. The ideal immunoassay would detect accurately only the total bioactive LH or FSH in the sample, but clearly this goal is difficult to accomplish. As an alternative approach, in vitro bioassays for LH and FSH can be used to assess the function of the gonadotropins. An in vitro bioassay based on the production of testosterone by cultured mouse or rat Leydig cells [70] can assess LH function, and production of estradiol by rat granulosa cells or immature Sertoli cells has been used to assay for FSH bioactivity [71]. Bioassays based on cAMP production by cell lines stably expressing gonadotropin receptors, with quantification using cAMP-responsive promoters linked to a luciferase reporter, have also been developed [72]. While useful for the study of the biological properties of recombinant or purified proteins, the clinical use of these assays is limited by the nonspecific effects of serum. In fact, many findings reported in patients using in vitro LH bioassays were found subsequently to be methodological artifacts.

Glycoprotein α-Subunit

Glycoprotein α-subunit is secreted in bursts that coincide with LH secretory episodes, implying pre-eminent regulation by GnRH [73]. Accordingly, serum α-subunit levels increase at puberty [74] and are elevated in men with testicular failure and in postmenopausal women. α-Subunit is also expressed in thyrotrophs, and levels are increased in patients with primary hypothyroidism [75].

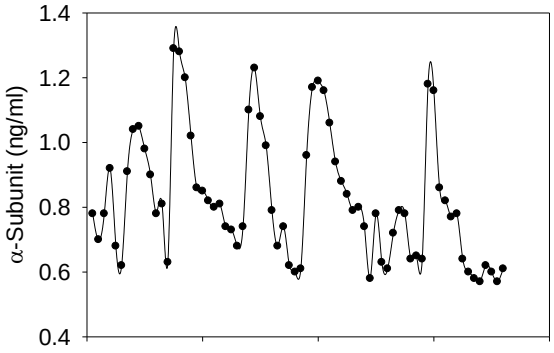


Figure 9. Pulsatile patterns of α-subunit secretion. Blood samples were drawn every 10 min for 12h starting at 0800 h from a 32 year old normal man whose testosterone level was 474 ng/dL. Winters, SJ (unpublished)

α-Subunit levels are low, but measurable, in normal children and in patients with congenital hypogonadotropic hypogonadism. Because peak α-subunit levels after GnRH stimulation tend to be lower in IHH patients than in prepubertal boys, this test has been used to distinguish between these two patient groups, although some overlap occurs [76]. α-Subunit is produced by as many as 20% of pituitary adenomas [77] often together with FSH. Because it is cleared by renal excretion, α-subunit levels are high in patients with renal failure.

INSULIN-LIKE FACTOR 3

Insulin-like factor-3 (INSL3) is a peptide hormone member of the relaxin-insulin hormone family that is secreted by Leydig cells

following LH stimulation. It affects testicular descent through effects on the gubernaculum [78]. At the time of puberty, INSL3 levels rise in parallel with testosterone INSL3 concentrations in the blood of normal adult men are approximately 1 ng/mL. Patients with Leydig cell dysfunction have lower levels, and the very low concentrations in men with hypogonadotropic hypogonadism increase following hCG administration [79].

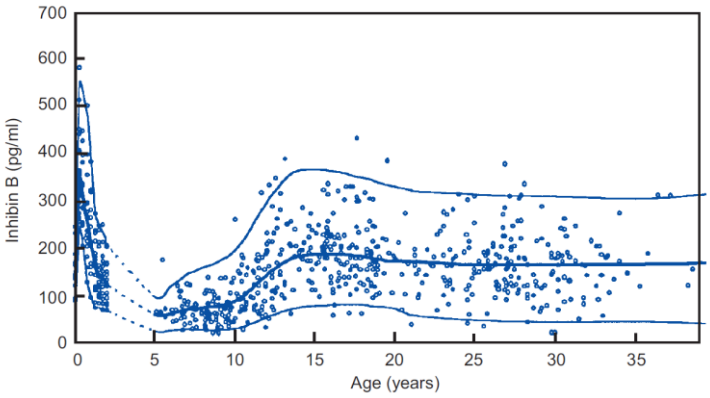
INHIBIN

Inhibin, a glycoprotein hormone produced by the testes as well as the ovaries, is responsible for the selective negative feedback control of FSH secretion, and functions as an intra-gonadal regulator [80]. Inhibin decreases FSH-β mRNA levels by blocking activin-stimulated transcription. Inhibin is a 32-kDa heterodimer composed of an α-subunit, and one of two β subunits, $\beta_A$or β_B. Inhibin-B (α-β_B) is the form produced by testicular Sertoli cells, whereas inhibin-A (α-β_A), produced by the corpus luteum and placenta, is undetectable in men. Higher molecular weight forms of the uncombined inhibin α-subunit that lack bioactivity are released into the circulation in excess of dimeric inhibin-B. The expression of the α- and β-subunits in Sertoli cells is regulated by cAMP and by a range of transcription factors, including CREB, SP-1, Smads, and GATA.

Current two site inhibin-B ELISA assays use a capture antibody that was raised to a peptide from the βB-subunit of inhibin, and a biotinylated detection antibody that was raised to a peptide from the inhibin α-subunit, [81] Capture can be facilitated by a methionine (in the β-subunit) oxidation step with hydrogen peroxide. One assay requires a specificity enhancing step in which samples are heated with sodium dodecyl sulphate solution. In the following step, wells are incubated with a streptavidin-labeled horseradish peroxidase and then with the substrate tetramethylbenzidine which produces a colorometric signal that is proportional to the amount of inhibin-B in the sample. The ELISA was updated in 2010 (Gen II version).

Inhibin-B is produced by the fetal testis and is measurable in serum at term. Levels increase substantially in newborns coincident with the rise in gonadotropins and testosterone, and remain elevated for 2-4 months and then decline [82]. In contrast to barely detectable levels of gonadotropins and testosterone, circulating inhibin-B is readily measurable in sera from prepubertal boys suggesting that production is partly gonadotropin-independent. Serum inhibin-B levels increase to adult values at the time of puberty, and decline as men grow older [65]. Serum inhibin levels are partly determined by gonadotropin stimulation [83], but also correlate positively with Sertoli cell number [84]. The lower limit for normal men has varied from 48 to 105 pg/ml in different studies.

Figure 10. Inhibin B levels in males in relation to age. Reprinted from Andersson A-M [85] with permission of the publisher (Elsevier).



Although there is no remarkable pulsatile fluctuation in circulating inhibin-B levels, there is a diurnal variation in adult men with the highest values in the morning and nadir values approximately 35% lower in the evening. This diurnal pattern of secretion parallels that of testosterone [86].

In keeping with its function to suppress FSH production, circulating inhibin-B levels are inversely correlated with FSH levels in adult men and are more strongly correlated inversely when values from men with primary testicular failure are included in the analysis [83]. There is also a demonstrable, albeit weak, positive correlation with sperm count [87], and with the germ cell score in testicular biopsy specimens among infertile men [88]. Inhibin-B levels are low in men with testicular failure, and are very low in men with Klinefelter syndrome in whom seminiferous tubules are hyalinized and Sertoli cells are absent [89]. Low but measurable inhibin-B levels in men following chemotherapy [90] or testicular irradiation [91] who develop germinal cell aplasia although Sertoli cells are present, suggest that germ cell factors regulate SC inhibin production. Serum inhibin levels changed little in normal men who participated in a male contraceptive clinical trial of testosterone together with a progestin, and developed azoospermia or severe oligospermia [92].

Inhibin has been extensively studied as a biomarker of spermatogenesis. Low inhibin-B levels predict persistent azoospermia in men with testicular cancer who undergo orchidectomy, chemotherapy and irradiation [93]. Inhibin-B levels have been studied in the male partners of infertile couples undergoing testicular sperm extraction (TESE) for intra-cytoplasmic sperm injection (ICSI). While mean inhibin B levels tend to be lower (and FSH higher) in men with no sperm found at TESE, there is no level of inhibin-B that reproducibly predicts either the presence or absence of spermatozoa in TESE samples, or successful in vitro fertilization [94]. Although mean inhibin-B levels are lower in men with impaired spermatogenesis, there is substantial overlap between normospermic and oligospermic men [83]. Thus measurement of inhibin-B is not recommended in the decision making for fertility

potential among azoospermic men undergoing TESE and ICSI. Further, very large doses of FSH are needed to increase circulating inhibin-B levels in men, so that an FSH stimulation test for inhibin-B is also not helpful in a clinical evaluation of hypospermatogenesis.

Inhibin-B levels are reduced in gonadotropin-deficient men [95]. In those with the complete form of congenital hypogonadotropic hypogonadism, values are lower than in men with partial gonadotropin deficiency who have some spontaneous pubertal development. This quantitative difference is partly due to a presumed larger mass of Sertoli cells (as reflected by larger testicular size) in the latter group of men. Low inhibin-B levels have been reported to differentiate boys with constitutional delay of puberty (CDP) from those with congenital hypogonadoptropic hypogonadism (CHH) with 80-100% sensitivity and specificity [96]. While plasma inhibin B concentrations in CHH and CDP may overlap, in one study of boys age 14-18 years with delayed puberty, among those who were genital stage 1 (testis volume ≤ 3ml), inhibin-B levels <35 pg/ml predicted persistent hypogonadism (testosterone levels < 3 nmol/L), and presumably the diagnosis of CHH, after 2 years of follow-up [97]. Inhibin-B is undetectable in most boys with congenital anorchia as in castrates, and is therefore a useful test to help distinguish these patients from boys with intra-abdominal testes [98].

High inhibin-B levels have been reported in a few boys with FSH-producing pituitary tumors [99], in patients with Sertoli cell tumors [100] and in boys with McCune Albright syndrome with macro-orchidism and autonomous function of Sertoli cells [101]. Adrenal tumors often express the inhibin α-subunit, and there is one report of high serum inhibin level in an adult man with an adrenal neoplasm [102].

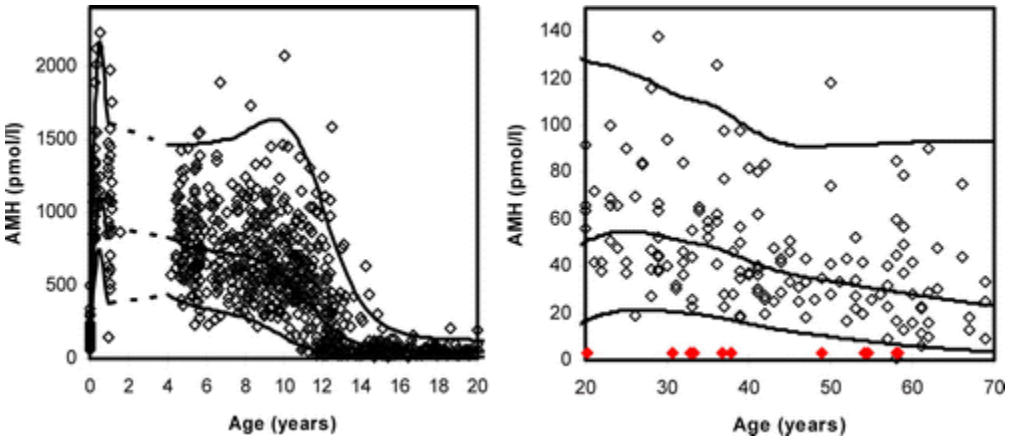
Current inhibin-B ELISAs detect not only 31K inhibin, but also larger (55-100 K) molecular weight forms [103, 104]. The bioactivity and significance of the larger forms are not known. In monkeys, differences in 90-100 K inhibin-B levels among prepubertal, juvenile and adult animals paralleled those of 31 K inhibin-B. The detection of multiple forms of inhibin-B could partly explain overlap in values among normal and oligospermic men. Accordingly, more specific assays for 31K inhibin-B are being developed.

ANTI- MULLERIAN HORMONE

Anti-mullerian hormone (AMH, also known as mullerian inhibitory hormone) is a 140 KDa homodimeric member of the TGF-β family of growth and differentiation factors. It is produced by fetal Sertoli cells and causes regression of mullerian structures during male development [105]. In males deficient in AMH, the persistence of Müllerian derivatives is accompanied by the normal development of male external genitalia which is regulated by androgens. AMH is readily detectable in the serum of prepubertal boys in concentrations of 10-70 ng/ml, but declines to levels of 2-5 ng/ml with entry into

adolescence due to Sertoli cell maturation following suppression by testosterone [106], although AMH remains detectable at low values throughout adulthood [104]. The physiological function of AMH in males beyond fetal life is uncertain. AMH levels are elevated in untreated men with congenital hypogonadotropic hypogonadism and decline following treatment with hCG [107]. AMH is absent from the plasma of most prepubertal boys with congenital anorchia, but is generally detectable in boys with bilateral cryptorchidism [108]. Therefore measuring AMH is useful in evaluating boys with non-palpable gonads. AMH levels are low in men with seminiferous tubular failure but overlap with normal in men with oligospermia, and do not predict testicular sperm retrieval for ICSI [67].

Figure 11. Serum concentrations of AMH in healthy males from birth (cord blood) to age 69 years. From Aksglaede L et al [109]. Red dots are values from men with bilateral anorchia. Republished with permission of The Endocrine Society.



FUNCTIONAL TESTS

hCG Stimulation

Human chorionic gonadotropin (hCG) can be used as a test agent to examine Leydig cell steroidogenesis in prepubertal boys who secrete little or no endogenous gonadotropins. A variety of protocols have been used. In one study, serum testosterone levels rose to greater than 300 ng/dl (10.5 nmol/L) in healthy prepubertal boys administered hCG 1500 IU intramuscularly every other day for seven doses. The effect of hCG to increase circulating levels of testosterone and precursor steroids, and DHT can be used in conjunction with mutation analysis to help evaluate patients with a 46,XY karyotype with ambiguous genitalia due to androgen insensitivity, defects in testosterone biosynthesis, or 5α-reductase deficiency. Androgen insensitivity syndrome is the most frequent 46,XY disorder of sexual differentiation. Serum testosterone

concentrations increase following hCG stimulation, although the increment is variable [110]. When the basal and hCG-stimulated profile indicates accumulation of steroid precursors upstream of an enzymatic defect, sequencing can be performed to identify the disorder. The biochemical diagnosis of 5α-reductase type 2 is based on a raised serum testosterone to dihydrotestosterone ratio. However, DHT production by 5α-RD1 can reduce the reliability of the ratio for diagnosis [50]

Testosterone levels are generally unaffected by hCG in boys with congenital bilateral anorchia but increase in boys with bilateral intra-abdominal testes [111]. Therefore, this test, together with measures of inhibin-B and AMH , help establish which boys have intra-abdominal testes and should undergo laparoscopy, and orchidopexy or orchiectomy [98].

Adult men with primary testicular failure have elevated endogenous serum LH concentrations, and hCG will predictably increase serum testosterone levels less in these men than in normal men. In gonadotropin-deficient men, the testosterone response to short-term administration of hCG is also blunted because Leydig cell steroidogenic enzymes are down-regulated. Overall, hCG testing provides little clinically useful information in either group of adult men.

Blockade of Steroid Hormone Biosynthesis and Action

Lowering circulating sex steroid levels with pharmacological inhibitors, or blocking steroid hormone action with receptor antagonists, can be used to assess the integrity of the hypothalamic-pituitary-testicular unit as a research tool, and can be used clinically in adult men with borderline low testosterone levels and possible hypogonadotropic hypogonadism.

Ketoconazole, a competitive inhibitor of cytochrome P450 cholesterol side chain cleavage (P450scc) and C17/20 lyase in the biosynthetic pathway to testosterone, causes a dose-dependent reduction in circulating testosterone and estradiol levels. A dose of 900-1200 mg for one week increased LH and FSH levels 2-fold and 1.6-fold, whereas a loading dose of 1.0 g followed by 400 mg every 6 hrs for five days increased serum LH levels 3-fold [112]. Lowering testosterone production with ketoconazole has been used to examine pulsatile gonadotropin secretion under conditions of reduced androgen negative feedback inhibition [113]. Because ketoconazole also lowers cortisol production, glucocorticoids are co-administered with ketoconazole to prevent the symptoms of cortisol deficiency.

Estrogens produced by the testes, peripheral tissues and the CNS play an important role in the physiological feedback regulation of gonadotropin secretion in men. Selective estrogen response modulators (SERMs) bind to estrogen receptors (ER-α and/or ER-β and exert estrogen-like effects, or inhibit estrogen effects, in various tissues. SERMs also have non-classical extra-nuclear (membrane) signaling mechanisms in certain cells. By blocking estradiol negative

feedback, most SERMS, such as clomiphene [114] and tamoxifen, increase plasma LH and FSH levels. Similarly, aromatase inhibitors [115] such as anastrazole, reduce circulating estradiol levels, and increase LH in men. Dosages of 100 to 200 mg/day of clomiphene for seven days produce a mean two-fold increase in serum LH levels in normal men, with peak increments of five-six fold at three weeks [114]. Two-fold increments in LH were produced by anastrazole 10 mg daily within 3-4 days. Both blocking estrogen negative feedback and decreasing estradiol production increased LH pulse frequency indicating an effect of estradiol on the GnRH pulse generator. A normal response implies functional integrity of GnRH-LH-testosterone pathways; however, responses among normal subjects are variable, and diagnostic tests using SERMS are not thought to be useful clinically. Men with the adult-onset hypogonadism can be treated with SERMs to increase LH and testosterone production [116].

The nonsteroidal antiandrogen flutamide, at a dose of 250 mg every 6 h for 14 days, increases serum LH levels 1.5-fold [113] by blocking the androgen negative feedback effect on GnRH secretion. Because LH levels rise, estradiol production increases, and gynecomastia may develop.

GnRH Test

GnRH is used as a research tool to examine the responsiveness of gonadotrophs to their physiological stimulus. In normal adult men, the intravenous administration of 100 µg of GnRH increases serum LH levels three- to- six-fold while serum FSH levels rise by about 50%. Generally, the total and incremental release of LH and FSH following GnRH administration is directly proportional to the basal hormone level, although exceptions do occur.

The GnRH test was introduced as a method to diagnose hypogonadism, and to distinguish hypogonadism due to hypothalamic from pituitary disorders. Because GnRH up-regulates its receptor on gonadotrophs, as well as the expression level of each of the gonadotropin subunit genes and thereby LH and FSH production, the gonadotropin response to stimulation with GnRH is diminished in patients with GnRH deficiency. Thus, the LH response to GnRH stimulation is attenuated in these patients even though the pituitary is essentially normal. The LH and FSH response to GnRH is also subnormal when gonadotrophs are damaged or destroyed by pituitary tumors or by other pathologies. Therefore, when evaluating gonadotropin deficient adult men, the GnRH test generally provides little information beyond that of the basal testosterone, LH and FSH levels, and is not recommended for clinical purposes.

GnRH analog (naferelin, leuprolide, buserelin, triptorelin) testing may help distinguish prepubertal boys with constitutional delay of puberty from those with congenital hypogonadotropic hypogonadism [117-119]. These agents bind the GnRH receptor with higher affinity and have a longer circulating half-life than native GnRH. The LH and testosterone responses at 4h and 24h in boys with CDP generally

exceed those of HH. In a recent study, prepubertal boys age 13.7-17.5 yrs with testicular volume ≤ 4ml whose LH level at 4h following 0.1 mg sc triptorelin exceeded 5.3 U/L all progressed to testicular enlargement, testis size ≥ 8 ml, over 18 months. Those boys with lower LH basal ≤ 2U/L and stimulated ≤5.3 mIU/ml values did not progress to this level. The CHH group also had low levels of inhibin-B (<111 pg/ml) at baseline [97]. Thus the test seems to be of use in evaluating prepubertal boys with delayed puberty. In patients with CHH who are homozygotes or compound heterogyzotes for mutations of the GnRH receptor gene, GnRH binding to its receptor may be either absent or reduced in affinity, or receptor signal transduction may be impaired, and the LH response to GnRH stimulation in these patients may be absent or reduced. The LH response in men with partial hypogonadotropic hypogonadism overlaps with the normal response.

Notes 1. http://www.issam.ch/freetesto.htm

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