Testosterone and the Free Androgen Index

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Testosterone measurements are very helpful in the evaluation of hypogonadal states in males, and hirsutism and virilism in females.

In males, increased testosterone levels can be found in complete androgen resistance (testicular feminization); and decreased levels in hypogonadism, orchidectomy, estrogen therapy, Klinefelter's syndrome, hypopituitarism and hepatic cirrhosis.

When testosterone levels are slightly elevated in females, hirsutism can develop. Hirsutism in women is associated with the administration of androgens and the overproduction of testosterone. There appears to be a correlation between serum testosterone levels and the degree of hirsutism, although approximately 25 percent of women with varying degrees of hirsutism have serum levels falling within the female reference range. Abnormal production of the androgen in women, to levels similar to those seen in men, can cause virilization.

Other conditions exhibiting increased testosterone levels include menstrual irregularities (oligomenorrhea, amenorrhea), Stein-Leventhal syndrome (polycystic ovary syndrome), acne, ovarian tumors, adrenal tumors, and adrenal hyperplasia. A slight overproduction of androgens can also be observed in patients with Cushing's disease, in postmenopausal patients, in pregnant subjects, and in patients treated with androgens.

Testosterone Physiology

Testosterone (17β-hydroxyandrost-4-ene-3-one, $C_{19}H_{28}O_2$), the dominant sex hormone in the male, is a C-19 steroid with a molecular mass of 288.41 daltons. This androgen has an unsaturated bond between C4 and C5, a keto group on C3, and a hydroxyl group at C17. (See Figure 1.)

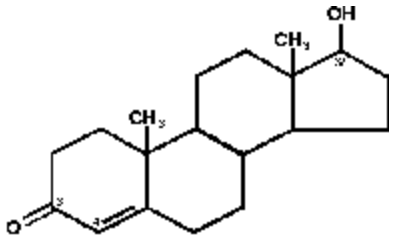

Figure 1. Structure of testosterone molecule.

In males, testosterone is secreted by the testes and the adrenal cortex. Synthesis occurs mainly in the interstitial Leydig cells of the testes in response to interstitial cell stimulating hormone (ICSH, also known as luteinizing hormone, LH) of the anterior pituitary gland.

Testosterone is responsible for the regulation of Wolffian duct differentiation, gonadotropin secretion and spermatogenesis. (In males, the Wolffian duct develops into the epididymal duct, vas deferens, and seminal vesicle; in females, a part remains as the vertical duct of the epoophoron, the rest regressing to rudimentary Gartner's duct). It is also responsible for the development of secondary sex characteristics such as accessory sex organs; the prostate; seminal vesicles; and the growth of facial, pubic and axillary hair after conversion to dihydrotestosterone. (See Figure 2.)

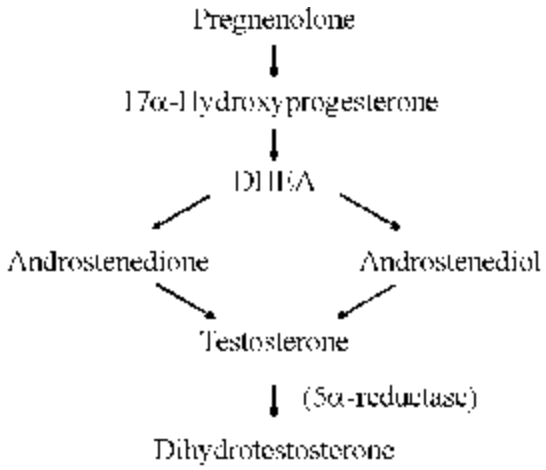

Figure 2. Biosynthetic pathway of testosterone and dihydrotestosterone.

Women normally have at least 3- to 4-fold lower testosterone levels than those encountered in healthy males. Testosterone in the female arises from three sources. The adrenal glands and the ovaries secrete small amounts, but most of the testosterone produced daily (about 50 to 60 percent) is derived from the peripheral conversion of the prehormones androstenedione and DHEA. (See tables 1 and 2.)

Haning, et al. have studied the role of testosterone as a follicular regulator. Testosterone of ovarian origin is secreted by the theca-interstitial compartment and stimulates FSH secretion (resulting in follicular growth), insulin-like growth factor I (IGF-I) production (resulting in granulosa cell

aromatase, an enzyme activity that catalyzes the conversion of testosterone to estradiol), and inhibin secretion. A simultaneous interaction of the hypothalamic-pituitary axis with the thecal and granulosa cell compartments results in the selection of a dominant follicle.

Table 1. Secretion rate of major androgens in normally menstruating women (mg/24 h).[1]

Androgen	Adrenal	Ovary
DHEA-SO_4	18	0
DHEA	< 1	< 1
Androstenedione	1.5	1.5
Testosterone	0.02	0.05

Table 2. Origin of testosterone in normally menstruating women (mg/24 h).[1]

Source	Testosterone Production
Ovarian secretion	0.05
Adrenal secretion	0.02
Peripheral conversion:	
androstenedione to testosterone	0.13
DHEA to testosterone	0.05
Total	0.25

Testosterone and its Binding Proteins

Once secreted, testosterone is almost entirely bound to transport proteins. Thus, in both males and females, testosterone is present in the peripheral circulation in three forms:

- free, unbound (corresponding to the free molecular form);
- weakly bound to albumin and to cortisol-binding globulin; and
- tightly bound to sex hormone-binding globulin (SHBG).

The free testosterone is biologically active, and the testosterone weakly bound to albumin can be rendered immediately active through its rapid dissociation from albumin. Therefore, the pool of free and weakly-bound testosterone is collectively called the "bioavailable" or "non-SHBG-bound" testosterone (NSB-T). Estimates of the amount of testosterone in each form are indicated in Table 3.

Table 3. Different forms of testosterone as a percentage of total testosterone.

	Transport Protein	Percent of Total
Free testosterone[a]	None	0.01–3.0 (females)
		0.16–0.68 (males)
Weakly bound[10]	Albumin	25–65 (females)
		45–85 (males)
Tightly bound[10]	SHBG	35–75 (females)
		14–50 (males)

[a] *Based on a DPC study on 81 females—ovulating, on oral contraceptives, and postmenopausal; and on 87 males, aged 20 to over 50 years. A somewhat different range of values for free testosterone as a percent of total testosterone is obtained with various methodologies used to estimate the proportions of the three testosterone pools (free, weakly bound, tightly bound).*[10]

SHBG, the principal transport protein for testosterone, also binds to estradiol and dihydrotestosterone. Accordingly, this protein is variously known as sex hormone-binding globulin (SHBG), testosterone-estrogen-binding globulin (TeBG), sex steroid-binding globulin (SSBG), or simply sex steroid-binding protein (SBP).

SHBG is the most important transport protein for three reasons:

- It carries (at least in females) a higher percentage of testosterone than either albumin or cortisol-binding globulin.
- It binds testosterone with a much higher affinity (1.5×10^{-9} mol/L) than do the two other transport proteins. (Albumin binds testosterone with an affinity of about 3.6×10^{-4} mol/L.)

- SHBG, unlike albumin, is sensitive to changes in the circulating estrogen/androgen ratio. Thus, SHBG plays a greater role in determining the level of free testosterone.

Total testosterone levels may change in concert with the SHBG levels to maintain a constant concentration of free testosterone. It is also expected that when the concentration of free testosterone increases, the total testosterone level will increase and the SHBG level will decrease. The SHBG level is significant because a decreased SHBG level in the presence of a normal or slightly elevated total testosterone level results in more bioactive testosterone, with higher peripheral androgen activity.

Free Androgen Index

True androgen status can be assessed either by measuring free testosterone or by calculating the ratio of the total testosterone (TT) concentration to the concentration (or binding capacity) of SHBG. This ratio, which is a useful indicator of an abnormal androgen status, is called the free androgen index (FAI) or, sometimes, the testosterone free index (TFI). It is typically calculated on a molar/molar basis and rescaled by a factor of ten, one hundred or one thousand, as shown below.

$$FAI = (TT \text{ in nmol/L} \div SHBG \text{ in nmol/L}) \times 10, \text{ or } \times 100, \text{ or } \times 1{,}000$$

The FAI is often increased in severe acne, male androgenic alopecia (balding), hirsutism, and other conditions in which a normal total testosterone level is found with a low SHBG level. In nonobese, nonhirsute oligomenorrheic women, an elevated FAI during the early follicular phase is reported to be a sensitive and specific indicator for polycystic ovarian diseases.

Many studies have found that the FAI corresponded well with clinical findings but correlated less well with other biochemical measurements. Moreover, NSB-T measurements did not provide additional diagnostic information in the hirsute patients studied.[2]

Preliminary Reference Ranges

The preliminary IMMULITE reference ranges presented below were determined from data obtained in recent multisite studies. Laboratories should consider these ranges ***as guidelines only****. It is important for each laboratory to establish the appropriateness of adopting the reference ranges suggested by these studies.*

IMMULITE Total Testosterone

- *Calibration Range:* 0.2 to 16 ng/mL
  Equivalently: 0.35 to 55.5 nmol/L
- *Detection Limit:* 0.1 ng/mL
  Equivalently: 0.35 nmol/L
  Values below this level are tabulated below as "ND".
- *Conversion:* ng/mL $\times$ 3.467 $\rightarrow$ nmol/L

A reference range study for IMMULITE Total Testosterone was conducted at two sites.

Testosterone, in ng/mL

Group	*n*	Median	Range
Females			
Ovulating	117	0.4	ND–1.0[L]
Oral contraceptives	39	0.4	ND–1.1[a]
Postmenopausal	103	0.3	ND–0.8[L]
Males	99	4.1	2.0–8.1[c]

a: absolute range, c: central 95% range, L: lower 95% range

Testosterone, in nmol/L

Group	*n*	Median	Range
Females			
Ovulating	117	1.39	ND–3.47[L]
Oral contraceptives	39	1.39	ND–3.81[a]
Postmenopausal	103	1.04	ND–2.77[L]
Males	99	14.2	6.93–28.1[c]

a: absolute range, c: central 95% range, L: lower 95% range

One of these sites also measured total testosterone levels in pregnant and postmenopausal women, with the following results.

Testosterone, in ng/mL

Group	*n*	Median	Absolute Range
Pregnant Females			
First trimester	20	0.7	0.3–2.3
Second trimester	20	0.9	0.3–2.0
Third trimester	19	1.1	0.3–1.9
Postmenopausal Females			
Untreated	29	0.2	ND–1.0
Treated	29	0.3	ND–1.0
Surgical	30	0.3	ND–0.6

Testosterone, in nmol/L

Group	*n*	Median	Absolute Range
Pregnant Females			
First trimester	20	2.43	1.04–7.97
Second trimester	20	3.12	1.04–6.93
Third trimester	19	3.81	1.04–6.59
Postmenopausal Females			
Untreated	29	0.693	ND–3.47
Treated	29	1.04	ND–3.47
Surgical	30	1.04	ND–2.08

IMMULITE SHBG

- *Calibration Range:* up to 180 nmol/L
- *Detection Limit:* 0.2 nmol/L
- *Calculation:* Free Androgen Index (FAI) = (total testosterone, in nmol/L) × 100 divided by (SHBG, in nmol/L)

In an independent study, total testosterone, sex hormone-binding globulin (SHBG) and the free androgen index (FAI) were determined in normal cycling women, untreated postmenopausal women, women on oral contraceptives, women with mild to moderately severe hirsutism, and normal males.

Note that the criteria adopted for selecting reference groups can have an effect on the resulting reference range limits. In this study, the following criteria were used to define "normal cycling" women: (1) a history of regular menstrual cycles of 25–34 days; (2) no obvious obesity, i.e. within about 20 percent of ideal body weight; and (3) no hyperandrogenic symptoms, such as acne, oily skin, or signs of hirsutism.

SHBG, in nmol/L

Group	*n*	Median	Range
Females			
Normal cycling	46	54.5	18.4–144[c]
Oral contraceptives	18	119	56.3–159[a]
Postmenopausal (untreated)	29	63.2	20.2–142[a]
Hirsute	24	40.6	19.9–84.8[a]
Males	50	32.3	7.2–100[c]

a: absolute range, c: central 95% range

Free Androgen Index

Group	*n*	Median	Range
Females			
Normal cycling	47	2.1	ND–8.5[c]
Oral contraceptives	18	1.2	ND–3.4[a]
Postmenopausal (untreated)	29	1.5	ND–6.6[a]
Hirsute	24	5.6	1.7–20.6[a]
Males	50	35.0	14.8–94.8[c]

a: absolute range, c: central 95% range

Available DPC Assays

DPC's IMMULITE system is the only automated system offering both total testosterone and SHBG assays.

IMMULITE® Total Testosterone is a solid-phase, ligand-labeled, competitive chemiluminescent immunoassay designed for the quantitative measurement of total testosterone in serum. The assay has a detection limit of 0.1 ng/mL (2 SD below the counts at zero dose) and a calibration range of 0.2 to 16 ng/mL. The assay shows close agreement with DPC's Coat-A-Count® assay for total testosterone. In a method comparison study on 79 samples with total testosterone values ranging from 0.1 to 8 ng/mL, linear regression analysis yielded the following statistics: (IML) = 0.92 (CAC) + 0.45 ng/mL; r = 0.983. IMMULITE Total Tesosterone is available in 100- and 500-test sizes.

IMMULITE® SHBG, used in combination with IMMULITE Total Testosterone, allows the free androgen index to be calculated from results obtained with the ease and efficiency of an automated system. IMMULITE SHBG is a solid-phase, chemiluminescent enzyme immunometric assay designed for the quantitative measurement of sex hormone-binding globulin (SHBG) in serum. It is available in 100- and 500-test sizes.

Coat-A-Count® Free Testosterone is a solid-phase ^{125}I radioimmunoassay designed for the direct quantitative measurement of free testosterone in serum. It requires neither a preincubation step nor preliminary isolation of the free hormone. The assay is supplied with calibrators having free testosterone values ranging from approximately 0.55 to 50 pg/mL (1.9 to 173 pmol/L), and requires one 4-hour incubation. In the assay procedure, ^{125}I-labeled testosterone analog competes with free testosterone in the patient sample under conditions that leave the original equilibrium between free and protein-bound testosterone in the patient sample essentially unchanged. Coat-A-Count Free Testosterone is available in 100- and 200-tube sizes.

Coat-A-Count® Total Testosterone is a solid-phase ^{125}I radioimmunoassay designed for the quantitative measurement of total testosterone in unextracted serum or heparinized plasma. Coupled with an acid hydrolysis procedure, it is also suitable for assaying urine samples. The assay has a single 3-hour incubation and is equipped with calibrators having total testosterone values ranging from 20 to 1,600 ng/dL (0.7 to 55 nmol/L). Coat-A-Count Total Testosterone is available in 100-, 200- and 500-tube sizes.

IRMA-Count® SHBG (available outside the US) is a solid-phase immunoradiometric assay designed for the quantitative measurement of sex hormone-binding globulin (SHBG) in serum. The assay is based on ligand-coated tubes and monoclonal antibodies, one ^{125}I-labeled, the other ligand-labeled. The assay has a total incubation time of just 90 minutes and is supplied with calibrators having SHBG values ranging from approximately 1 to 180 nmol/L. IRMA-Count SHBG is available in a 100-tube size.

In addition, DPC's complete panel of assays for the evaluation of hirsutism includes IMMULITE assays for DHEA-SO_4, FSH, LH, and prolactin; Coat-A-Count IRMAs for FSH, LH, and prolactin; and Coat-A-Count assays DHEA-SO_4, prolactin, and 17α-hydroxyprogesterone.

Summary

Testosterone measurements are very helpful in the evaluation of hypogonadal states in males, and hirsutism and virilism in females.

In males, testosterone is secreted by the testes and the adrenal cortex. The dominant male sex hormone, it regulates Wolffian duct differentiation, gonadotropin secretion and spermatogenesis, and is responsible for the development of secondary sex characteristics.

In women, testosterone is present at levels three or four times lower than those in healthy males. Small amounts of testosterone are secreted by the adrenal glands and ovaries, but most of the hormone is derived from the peripheral metabolism of androstenedione and DHEA. Testosterone in females acts as a follicular regulator.

Most of the circulating testosterone is bound to transport proteins, with only a small percentage biologically active as the free, or unbound, fraction. Sex hormone-binding globulin is the major transport protein for testosterone. True androgen status can be assessed with the help of either free testosterone measurement or the ratio of the total testosterone (TT) concentration to the concentration (or binding capacity) of SHBG. This ratio, typically calculated on a molar/molar basis, is called the free androgen index (FAI).

The FAI is often increased in severe acne, male androgenic alopecia (balding), hirsutism, and other conditions in which a normal total testosterone level is found with a low SHBG level. In nonobese, nonhirsute oligomenorrheic women, an elevated FAI during the early follicular phase is reported to be a sensitive and specific indicator for polycystic ovarian diseases.

DPC offers IMMULITE assays for total testosterone and SHBG, and isotopic assays for free testosterone, total testosterone, and SHBG.* IMMULITE is currently the only automated system to offer assays for both total testosterone and SHBG. When these assays are used in combination, the free androgen index can be calculated from results obtained with the ease and efficiency of an automated system.

* IRMA-Count SHBG is available outside the US.

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