Reproductive

Testosterone Total/Free

Analyte Information

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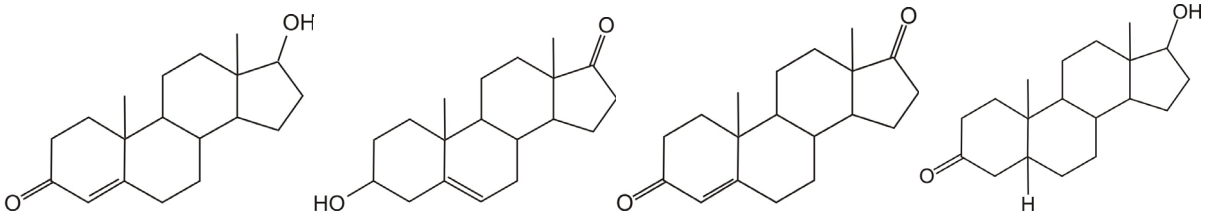
Introduction

Testosterone is the most important steroid hormone of the androgen group. Other important steroid hormones are dihydrotestosterone (DHT), androstenedione (ASD) and dehydroepiandrosterone (DHEA). Androgens are a group of C_{19} steroides that stimulate or control the development and maintenance of male characteristics. This includes the activity of the male sex organs and the development of secondary sex characteristics. Androgens are also precursors of all estrogens, the female sex hormones[1].
The chemical name of testosterone is 17β-hydroxy-4-androstene-3-one. Its summary formula is $C_{19}H_{28}O_2$ and its molecular weight (Mr) is 288.42 Da. The structural formulas of testosterone and related androgens are shown below:

Fig.1: Structural formulas of the most important androgens



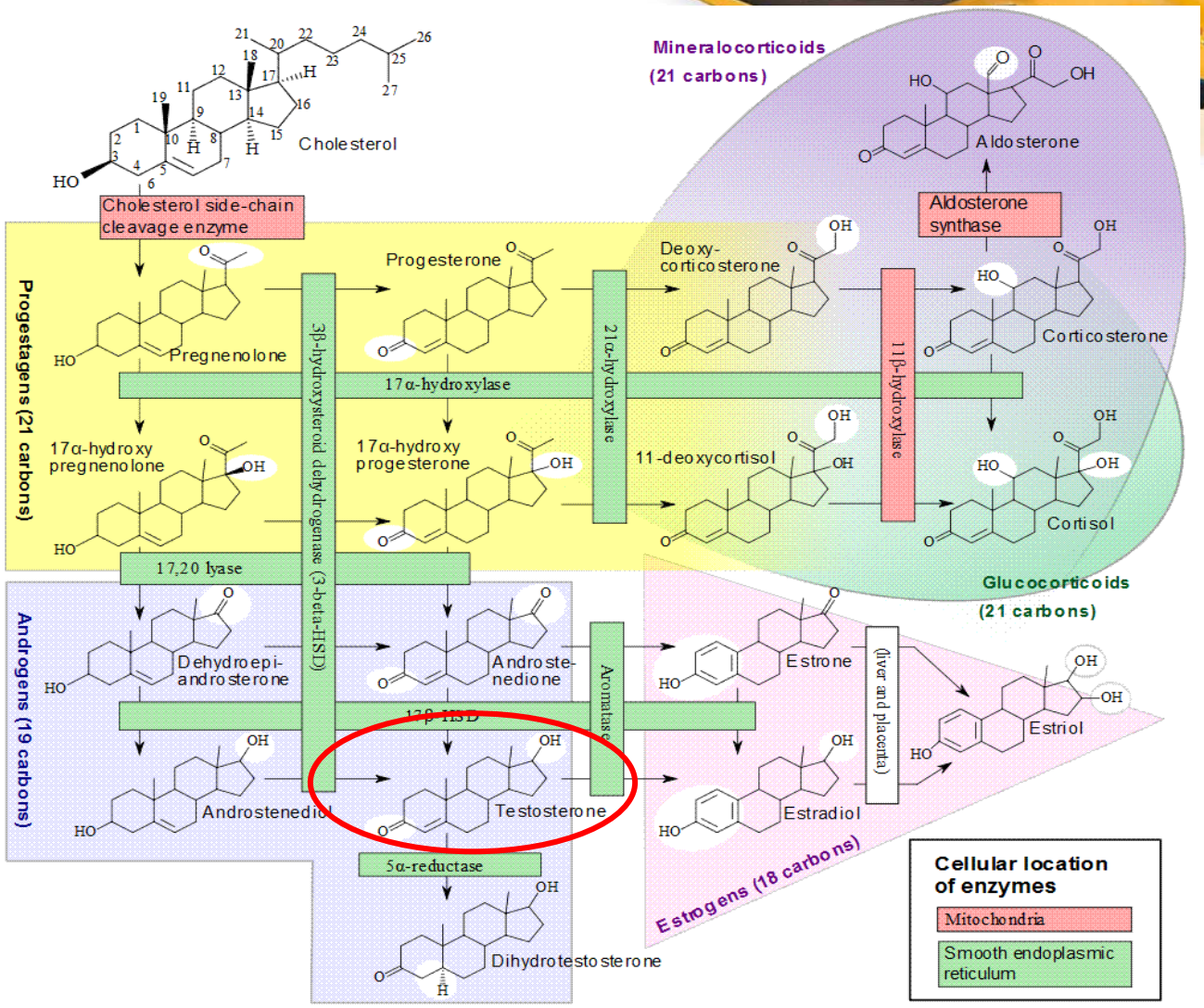
Testosterone DHEA Androstenedione Dihydrotestosterone

Over 80 other names for testosterone exist, including: 17-hydroxy-D4-androsten-3-one, 17β-testosterone and so on.

Biosynthesis

The synthesis of androgens begins with the mobilization of cholesterol from deposits of cholesterol esters stored in lipid droplets in cytoplasm. Cholesterol is converted to pregnenolone by the enzyme P450scc (side chain cleavage). This conversion appears to be a rate-limiting step in testosterone production. There are two subsequent mechanisms of testosterone biosynthesis: from pregnenolone via dehydroepiandrosteron (DHEA), or from progesterone and androstenedione (Fig.2).
In men, testosterone is secreted primarily by Leydig cells of the testes and to a minor extent by the adrenal cortex.
In premenopausal women, testosterone is produced mainly in the ovaries, with minor contributions by the adrenal glands and peripheral tissues. After menopause, ovarian testosterone production is significantly diminished.
Testosterone production in the testes and ovaries is regulated via pituitary-gonadal feedback mechanism involving luteinizing hormone (LH) and, to a lesser degree, inhibins and activins.

Fig.2: Steroidogenesis

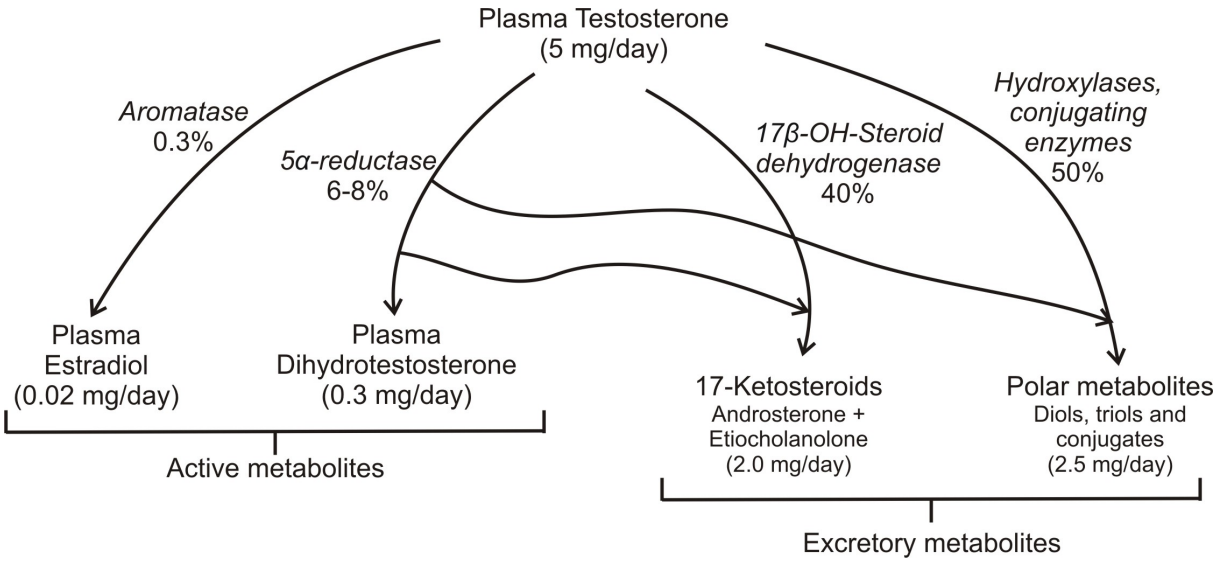


Metabolism

Circulating testosterone serves as a precursor in the formation of two types of active metabolites. 6 – 8 % of testosterone is converted to dihydrotestosterone (DHT) by 5α-reductase; approximately 0.3 %) is converted to estrogens by aromatase (as is androstenedione). DHT is formed in androgen target tissues, such as the skin, prostate, hair follicles and adrenal glands. Whereas aromatisation occurs in many tissue, peripheral aromatisation occurs primarily in adipose tissue in both men and women. Other tissues involved are those of the liver, muscles, hair follicles, Sertoli and Leydig cells and brain[2].
Other testosterone metabolites exhibit low androgenic effect. Testosterone degradation occurs mainly in the liver, where androsterone and eticholanolone are formed. These two metabolites belong to the 17-ketosteroids (17 KSs) and

are excreted primarily (over 90 %) via urine in the form of glucuronidates or sulfates (Fig.3). Testosterone production rates in the blood have been estimated at 0.34 mg/day in adult females and more than 20 times this amount in males.

Fig.3: Testosterone conversion



Total and free testosterone

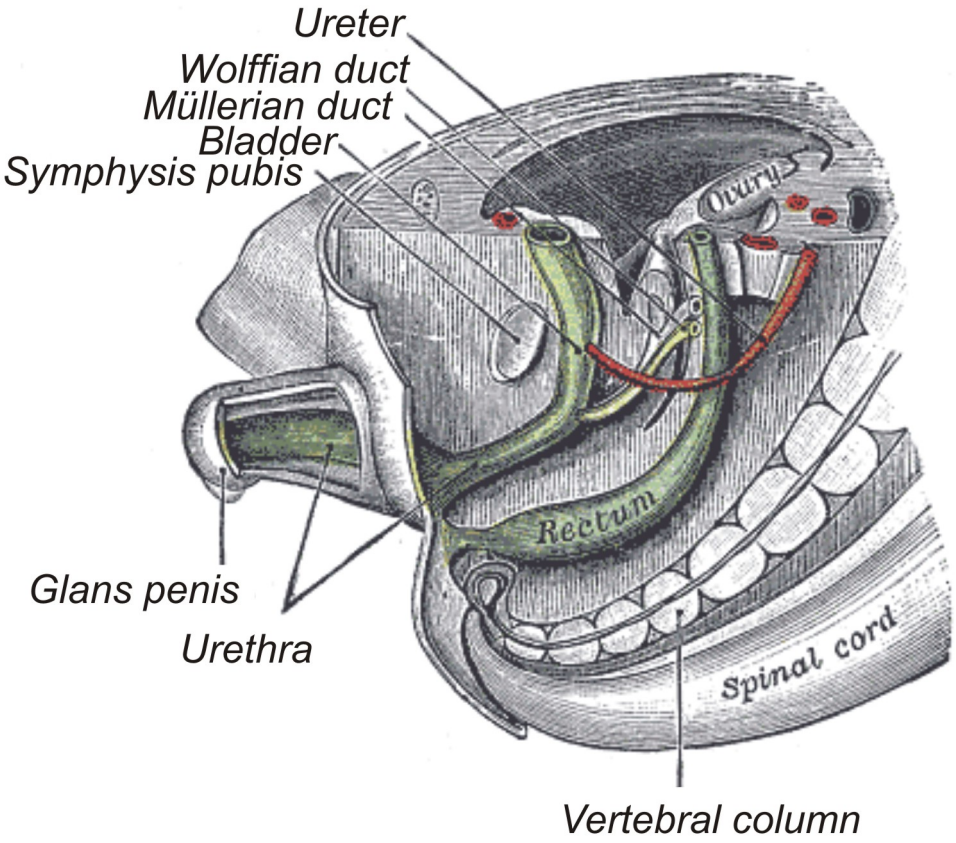
Testosterone (and DHT) circulates in plasma either in a free state (2 – 3 %)[4] or bound to plasma proteins. The binding proteins include sex hormone-binding globulin (SHBG) and a nonspecific protein – albumin. SHBG is α-globulin that has a low capacity for steroids but binds to them with very high affinity (K_a = $1x10^8$ to $1x10^9$), whereas albumin has high capacity but low affinity (K_a = $1x10^4$ to $1x10^6$)[4, 5]. SHBG has the highest affinity for DHT and the lowest for estradiol. In men, 44 – 65 % of all protein-bound testosterone is bound to SHBG, while 33 – 50 % is bound to albumin. In women, these figures are 66 – 78 % (SHBG-bound) and 20 – 30 % (albumin-bound).
It was previously believed that only free testosterone was biologically active. However, it has been confirmed in various studies that nearly all of albumin-bound testosterone is available for tissue uptake[6, 7]. Therefore, the bioavailable testosterone is equal to about 35 % of the total (free plus albumin-bound). This albumin-bound portion is referred to as "non-SHBG bound" or "weakly bound."

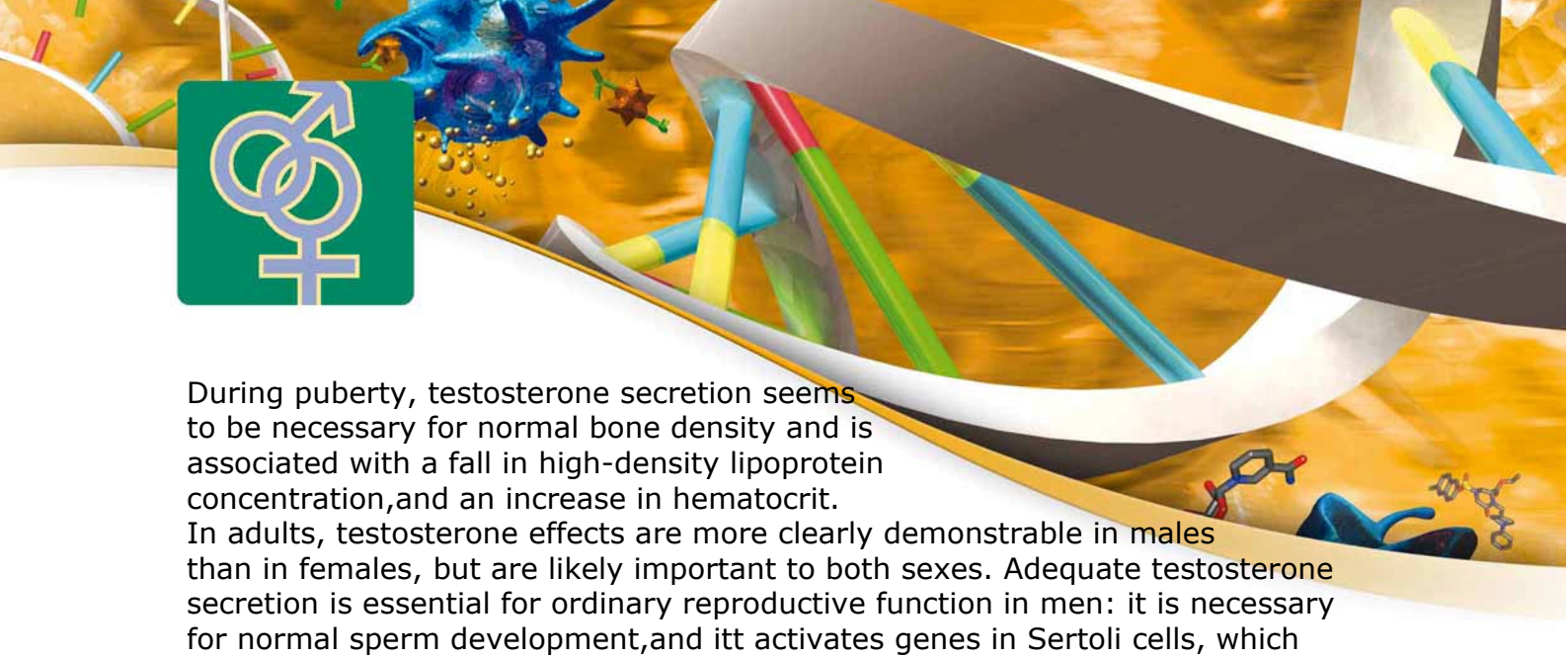
Physiological Function

As testosterone is the major androgenic hormone, adequate testosterone secretion is essential for normal male reproductive function.

During early embryogenesis, the fetus possesses both Müllerian and Wolffian genital ducts. Müllerian ducts have the potential to develop into fallopian tubes, uterus and upper part of the vagina; Wolffian ducts can differentiate into vas deferens, epididymis and seminal vesicles of the male reproductive tract. In the male fetus, testosterone is responsible for maintaining the Wolffian ducts and for virilisation of the urogenital sinus and external genitalia, while Müllerian inhibiting substance (MIS, also known as AMH) is responsible for the regression of the Müllerian ducts. The structure of Müllerian and Wolffian ducts is shown in the Fig.4.
The production of testosterone and the conversion of testosterone to DHT, functioning androgen receptors and AMH are all essential for normal male sexual development. A microphallus (less than 2.5 cm fully stretched)[2] at birth can be caused by defect of testosterone or DHT, or defective androgen receptors.

Fig.4: Müllerian and Wolffian ducts (eight and a half to nine weeks old human fetus)





During puberty, testosterone secretion seems to be necessary for normal bone density and is associated with a fall in high-density lipoprotein concentration,and an increase in hematocrit.
In adults, testosterone effects are more clearly demonstrable in males than in females, but are likely important to both sexes. Adequate testosterone secretion is essential for ordinary reproductive function in men: it is necessary for normal sperm development,and itt activates genes in Sertoli cells, which promote differentiation of spermatogonia, prostate function and potency. In females, its main role is as a precursor of estrogen. In both sexes, testosterone stimulates libido, and affects hair and beard growth and voice. Testosterone plays a role in increase of muscle mass and in prevention of osteoporosis.

Levels

In the male embryo, both the production of testosterone by the testes and plasma concentration start to rise by the second month of gestation, with a peak in the 12th week. A high concentration is maintained until it decreases late in gestation (Fig.5).
At birth, the concentration of testosterone is only slightly higher in boys than in girls. In boys, testosterone concentration increases shortly after birth, remains elevated for about 3 months, and than falls to baseline again by the end of the first year. The concentration of testosterone remains low until puberty, a little higher in boys than in girls.
In boys, testosterone levels increase sharply during Tanner pubertal stages III and IV and remain relatively constant thereafter.
In adult men, serum testosterone levels show a circadian variation with peak levels in the early morning (4 – 8 a.m.), decreasing 25 – 30 % to the evening minimum (4 – 8 p.m.)[2]. Testosterone levels increase after exercise and decrease after immobility and high glucose load.
Testosterone production decreases rapidly around age 50 – 60. Age-related decreases in testicular androgen production are primarily caused by a decline in Leydig cell count. Mean testosterone concentration at age 80 is approximately 60 % of that at ages 20 to 50.
This aging process connected with a physiological decrease in androgen levels (including testosterone) has been referred to as andropause[2], because of parallels with the changes observed in female aging during menopause. Andropause may be clinically asymptomatic, or may be associated with decreased well-being, energy levels and sexual function.
In girls, the testosterone levels increase only slightly during puberty.
In women, testosterone values are much lower than in men and are elevated for 1 – 2 days at midcycle. Testosterone production decreases after menopause, as ovarian production is significantly diminished.
Testosterone levels in the blood are influenced by SHBG levels. Changes in SHBG concentration alter the distribution of testosterone in circulation. Thus, total testosterone levels may change in accordance with SHBG levels in order to

maintain constant concentrations of free testosterone. On the other hand, decreased SHBG levels in the presence of a normal or slightly elevated total testosterone levels result in a higher amount of bioactive testosterone, with increased peripheral androgen activity[6].

Testosterone levels may be influenced by its administration as well.

Fig.5: Schematic drawing of changes in male and female total serum testosterone concentration throughout life[2]

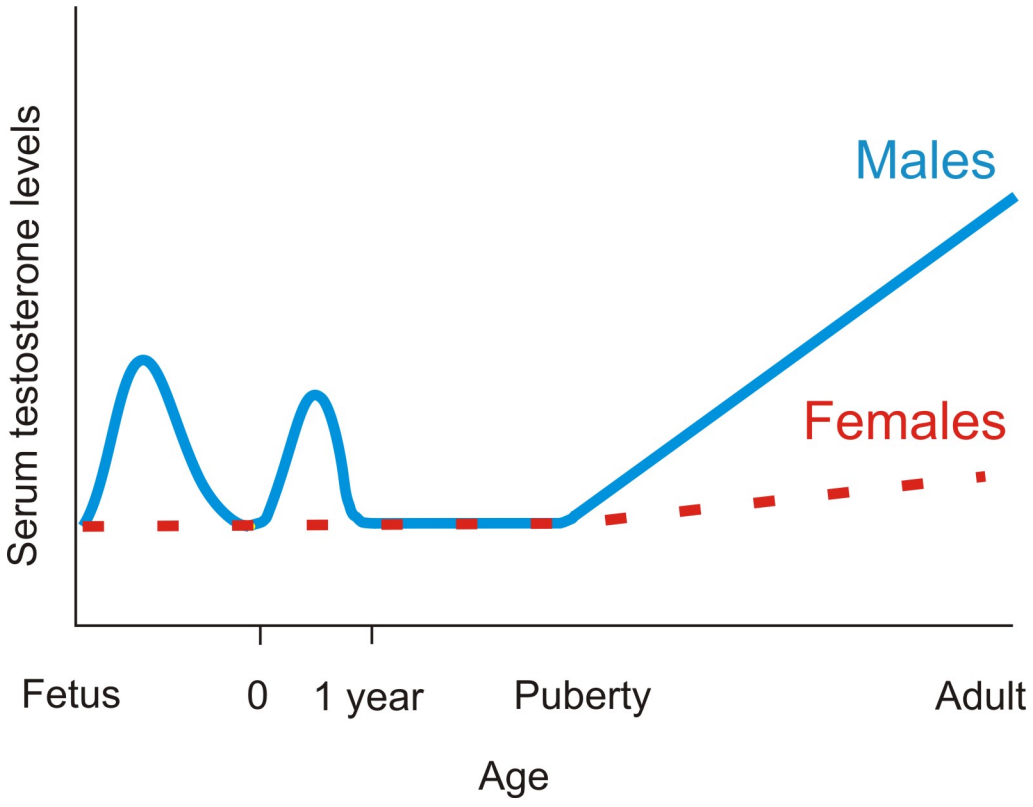


Fig.6: Changes of total testosterone levels during the life cycle

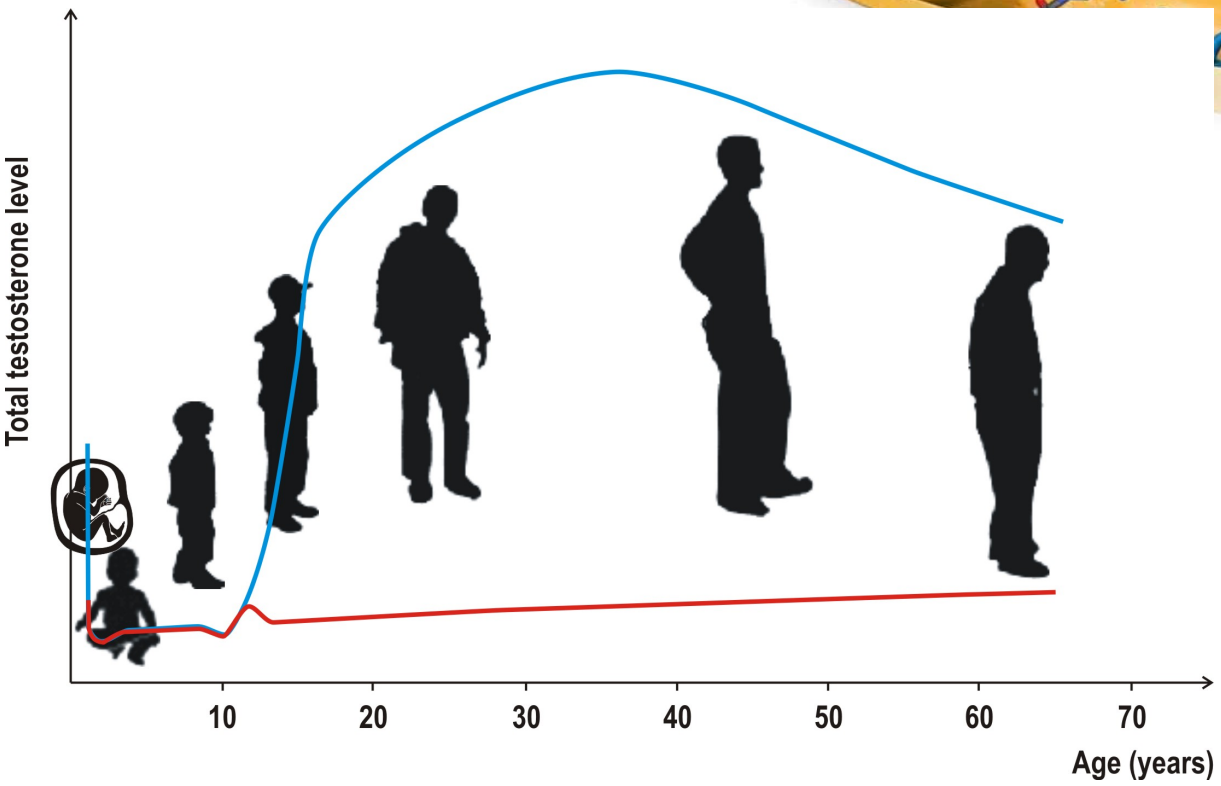
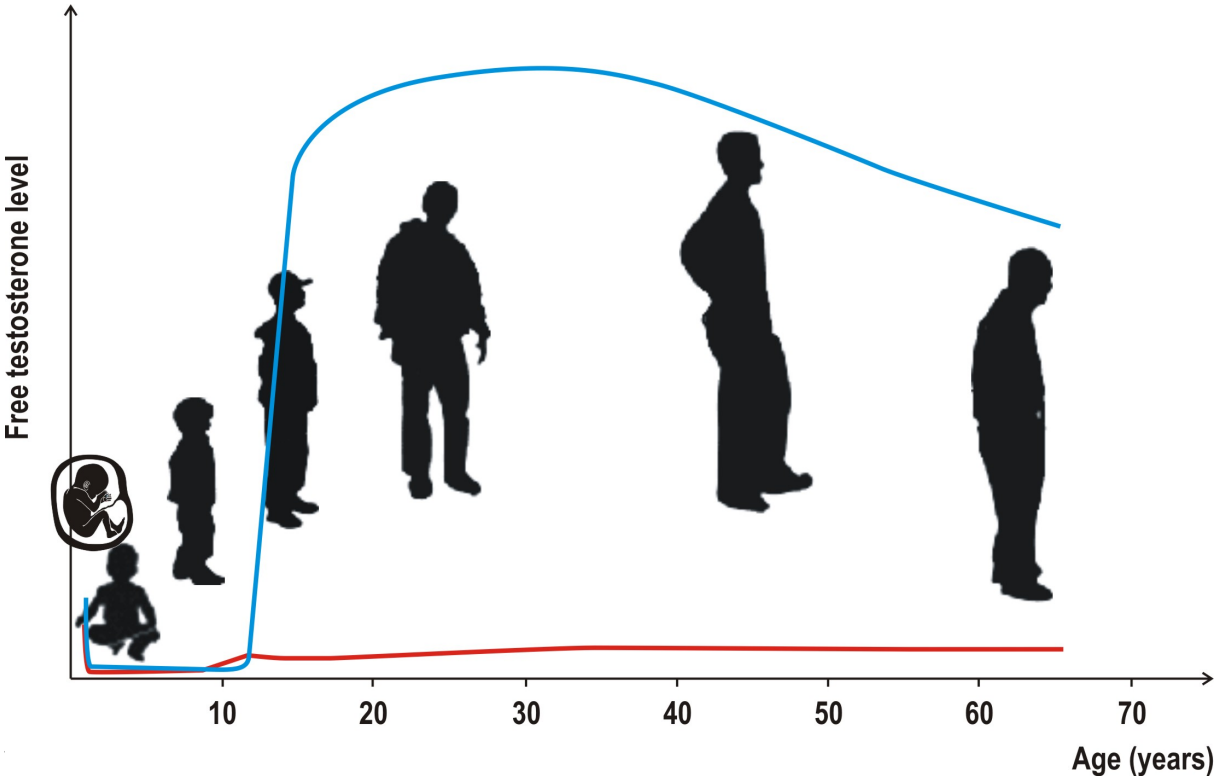
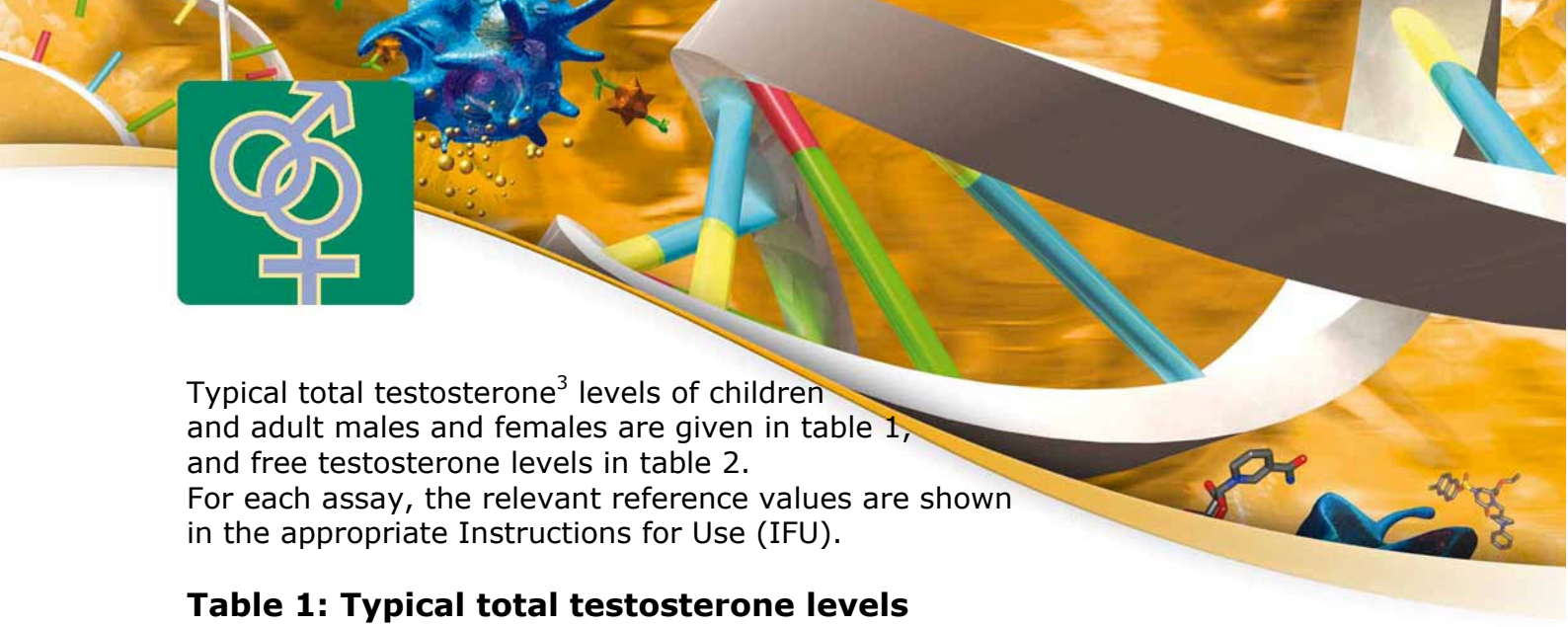


Fig.7: Changes of free testosterone levels during the life cycle

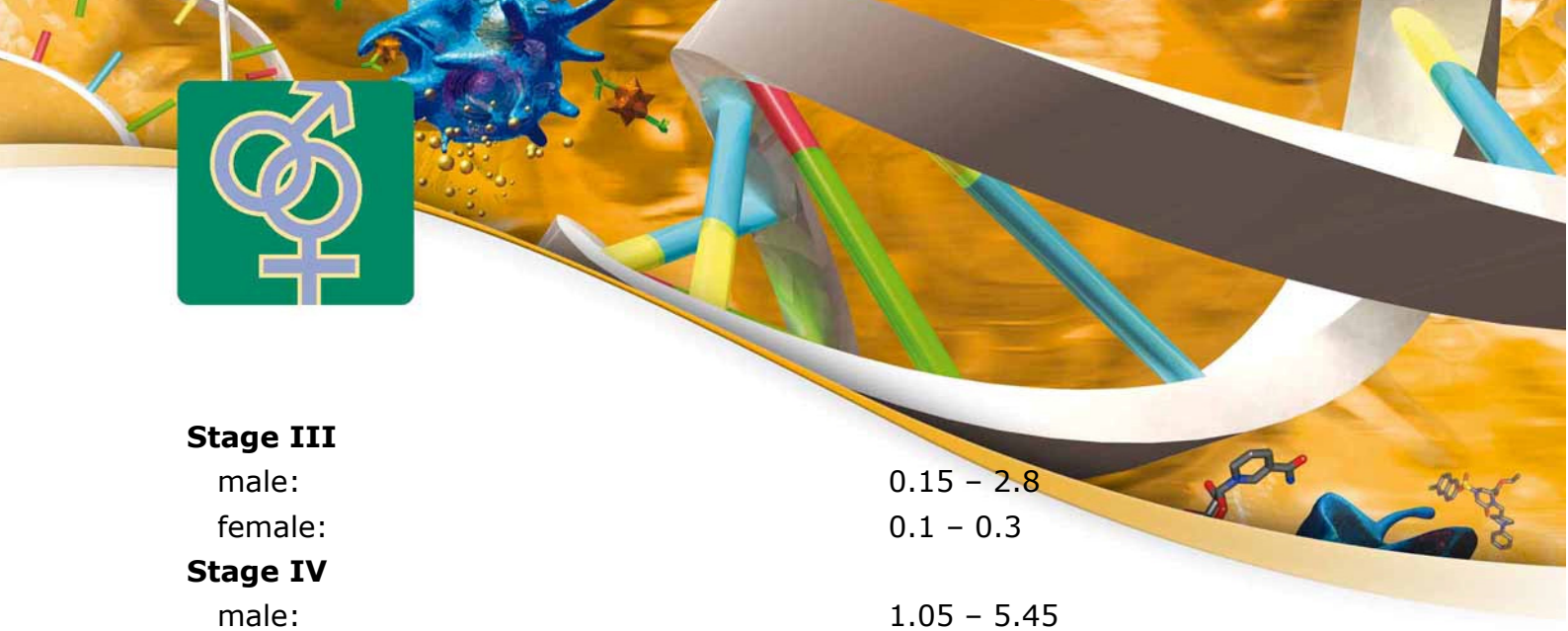




Typical total testosterone[3] levels of children and adult males and females are given in table 1, and free testosterone levels in table 2.
For each assay, the relevant reference values are shown in the appropriate Instructions for Use (IFU).

Table 1: Typical total testosterone levels

Specimen (serum)	**Reference interval** (ng/mL)
Cord	
male:	0.13 – 0.55
female:	0.05 – 0.45
Premature	
male:	0.37 – 1.98
female:	0.05 – 0.22
Newborn	
male:	0.75 – 4.0
female:	0.2 – 0.64
1 – 5 months	
male:	0.01 – 1.7
female:	0.01 – 0.5
6 – 11 months	
male:	0.02 – 0.07
female:	0.02 - 0.05
1 – 5 years	
male:	0.02 – 0.25
female:	0.02 – 0.1
6 - 9 years	
male:	0.03 – 0.3
female:	0.02 – 0.2
Puberty Tanner stage:	
Stage I	
male:	0.02 – 0.23
female:	0.02 – 0.1
Stage II	
male:	0.5 – 0.7
female:	0.5 – 0.3

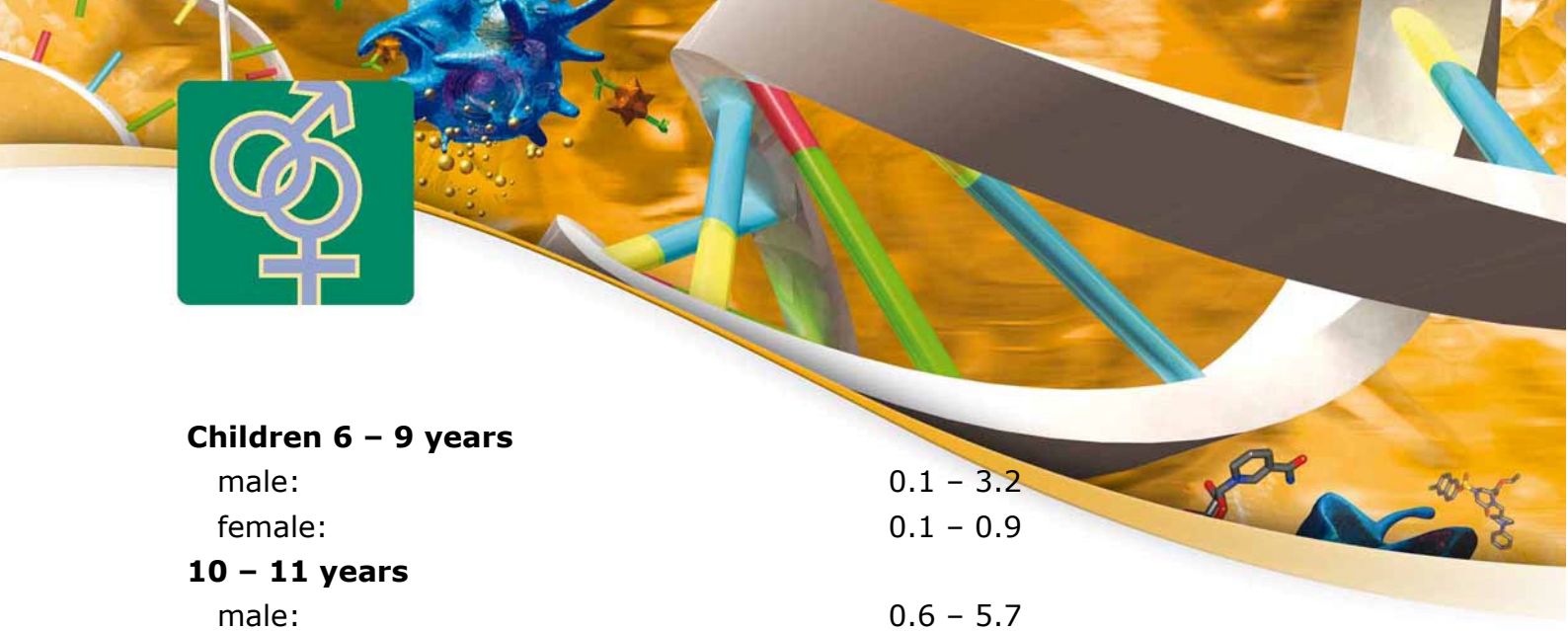


Stage III	
male:	0.15 – 2.8
female:	0.1 – 0.3
Stage IV	
male:	1.05 – 5.45
female:	0.15 – 0.4
Stage V	
male:	2.65 – 8.0
female:	0.1 – 0.4
Adult	
male:	2.8 – 11.0
female (higher at midcycle peak):	0.15 – 0.7
pregnancy:	3 - 4 x adult level
postmenopausal female:	0.8 – 0.35

Equation for the conversion of units: 1 ng/mLx3.47 = nmol/L

Table 2: Typical free testosterone levels

Specimen (serum)	**Reference interval** (pg/mL)
Cord	
male:	5.0 - 22
female:	4.0 - 16
Newborn (1 – 15 days)	
male:	1.5 - 31
female:	0.5 – 2.5
1 – 3 months	
male	3.3 – 8.0
female	0.1 – 1.3
3 - 5 months	
male:	0.7 – 14
female:	0.3 – 1.1
5 – 7 months	
male:	0.4 – 4.8
female:	0.2 – 0.6

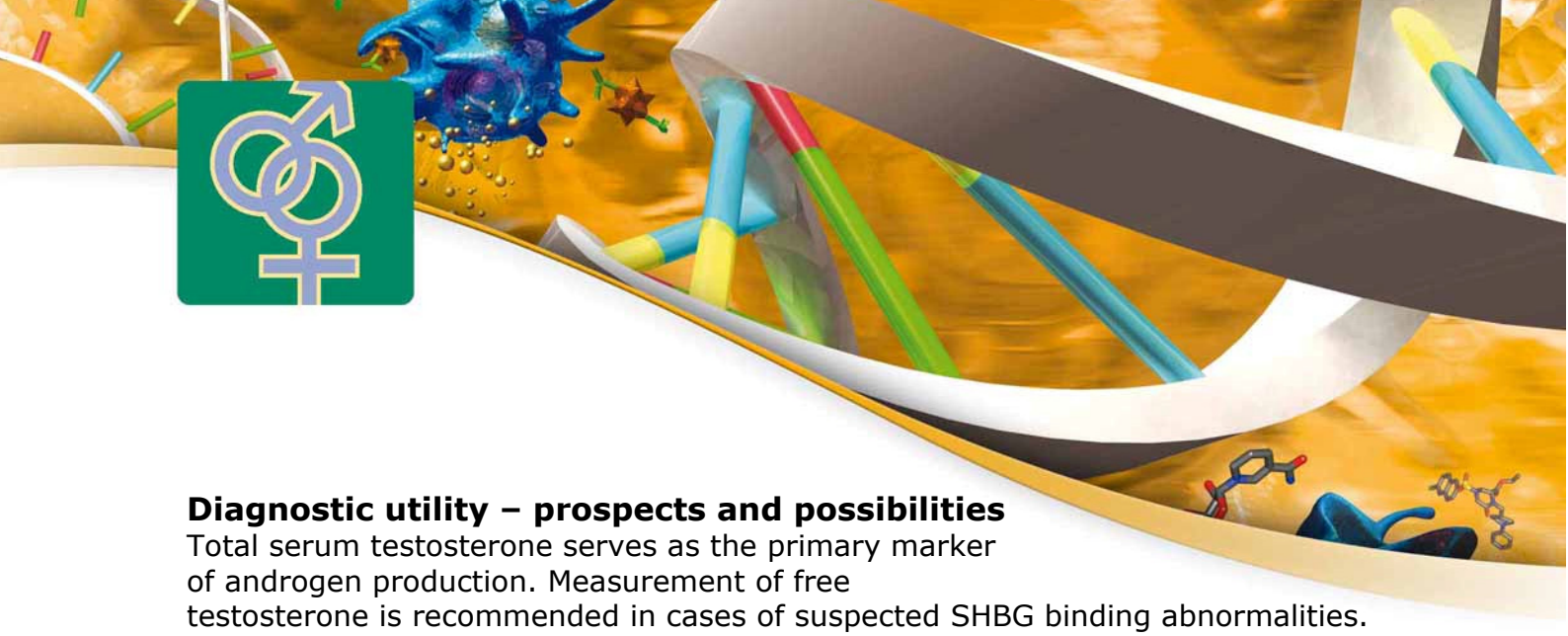


Children 6 – 9 years	
male:	0.1 – 3.2
female:	0.1 – 0.9
10 – 11 years	
male:	0.6 – 5.7
female:	1.0 – 5.2
12 – 14 years	
male:	1.4 - 156
female:	1.5 – 5.2
15 – 17 years	
male:	80 - 159
female:	1.0 – 5.2
Adult	
male:	50 - 210
female:	1.0 – 8.5

Free Androgen Index[9]:

True androgen status can be determined either by measuring free testosterone or by calculating the ratio of total testosterone (TT) concentration to SHBG concentration. This ratio is known as the Free Androgen Index (FAI) or Testosterone Free Index (TFI).

FAI = TT (nmol/L)/SHBG (nmol/L) x 100



Diagnostic utility – prospects and possibilities

Total serum testosterone serves as the primary marker of androgen production. Measurement of free testosterone is recommended in cases of suspected SHBG binding abnormalities.

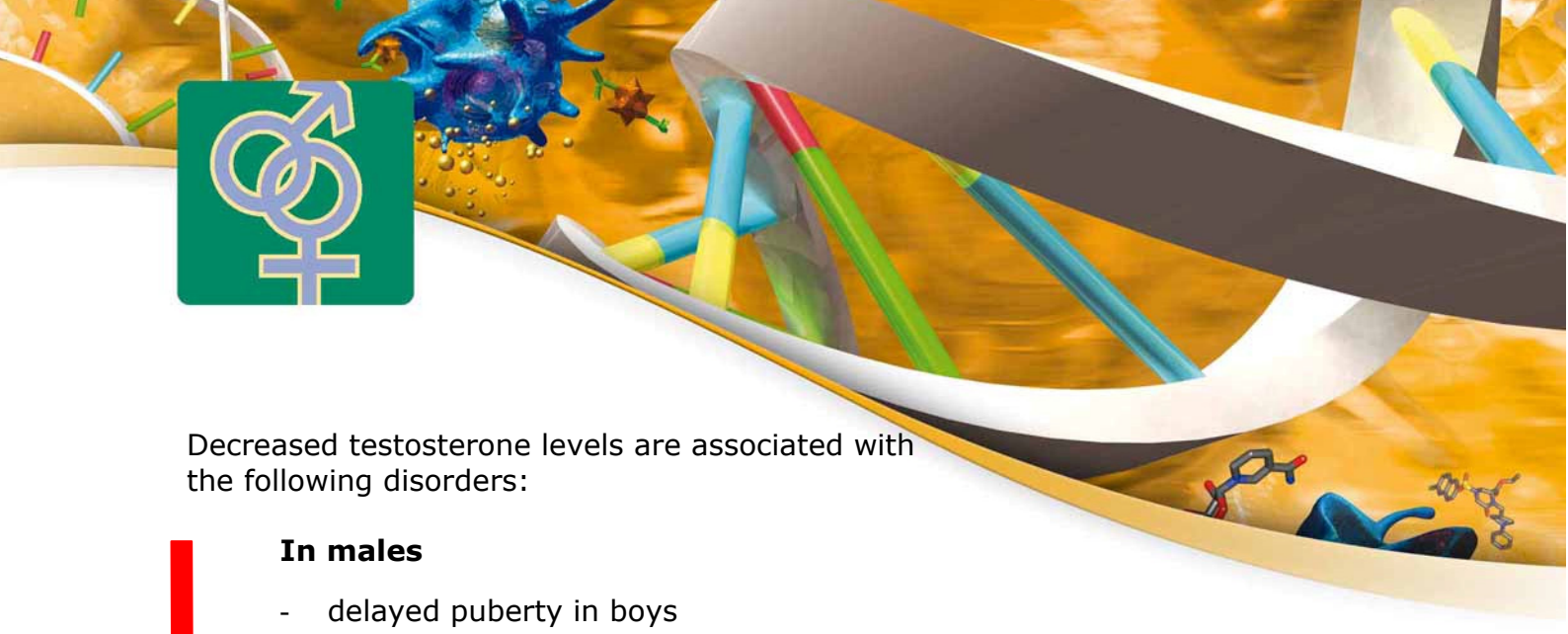
Elevated testosterone levels are associated with the following disorders:

In males

- precocious puberty in boys
- adrenal hyperplasia in boys
- testicular tumors in men
- adrenal tumors in men

In females

- precocious puberty in girls
- congenital adrenal hyperplasia
- ovarian or adrenal neoplasms
- polycystic ovary syndrome (PCOS)
- idiopathic hirsutism[10, 11]
- acne
- menstrual disturbances
- amenorrhea
- infertility
- insulin resistance
- trophoblastic disease during pregnancy
- testicular feminization



Decreased testosterone levels are associated with the following disorders:

In males

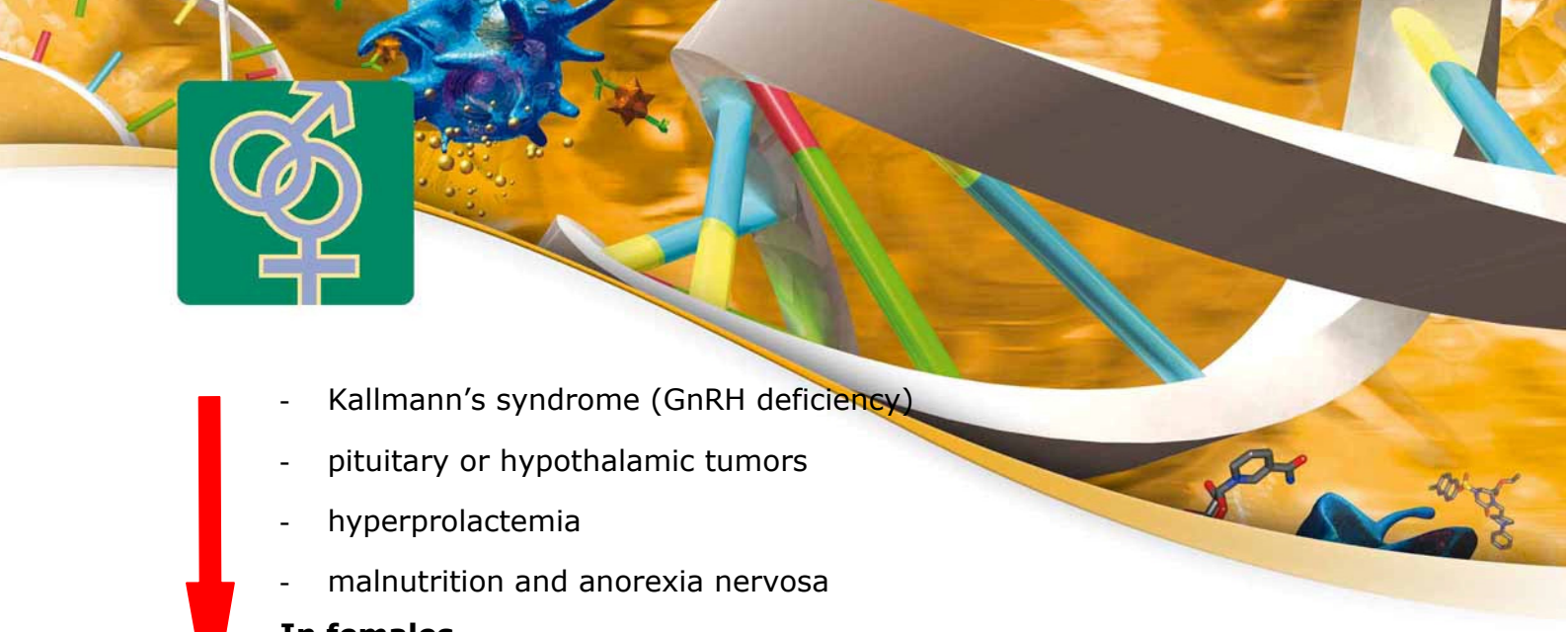
- delayed puberty in boys

hypergonadotropic hypogonadism -primary testicular failure:

- Klinefelter's syndrome (condition in which males have an extra X sex chromosome resulting in small testicles and reduced fertility)
- true hermaphroditism
- defective androgen biosynthesis:
  - 20α-hydroxylase deficiency
  - 17,20-lyase deficiency
  - 3β-hydroxysteroid dehydrogenase deficiency
  - 17α-hydroxylase deficiency
  - 17β-hydroxysteroid dehydrogenase deficiency
- testicular maldescent
- myotonic dystrophy
- testicular trauma or ischemia (testicular torsion)
- infections – mumps
- autoimmune diseases (autoimmune polyglandular endocrine failure)
- metabolic disorders (hemochromatosis, liver failure)
- orchidectomy

secondary/tertiary hypogonadism – hypogonadotrophic hypogonadism

- panhypopituitarism (congenital or acquired)
- hypothalamic syndrome (congenital or acquired):
  - Prader-Willi syndrome,
  - Laurence-Moon-Biedl syndrome



- Kallmann's syndrome (GnRH deficiency)
- pituitary or hypothalamic tumors
- hyperprolactemia
- malnutrition and anorexia nervosa

In females

- primary or secondary ovarian failure

Diagnostic utility – Practical application

Testosterone is often measured in conjunction with other androgens (DHEA/DHEA-S, androstenedione, 17α-hydroxyprogesterone), SHBG and gonadotropins (LH and FSH) to evaluate reproductive abnormalities in children, men and women.

Hyperandrogenism in children

Elevated androgen levels may be caused by ovarian disorders in girls, testicular disorders in boys, or adrenal disorders in both sexes. Measurement of testosterone and dehydroepiandrosterone sulphate (DHEA-S) levels determeine the source of elevated androgen levels. If the results indicate a disorder of gonadal origin, LH, FSH and hCG levels should also be examined.

Diagnosis and differential diagnosis of premature adrenarche

Testosterone and free testosterone is measured in conjunction with FSH, LH, DHEA-S, 17α-hydroxyprogesterone, estradiol (estrone), DHEA, androstenedione and SHBG.

Evaluation of infants with ambiguous genitalia or virilization

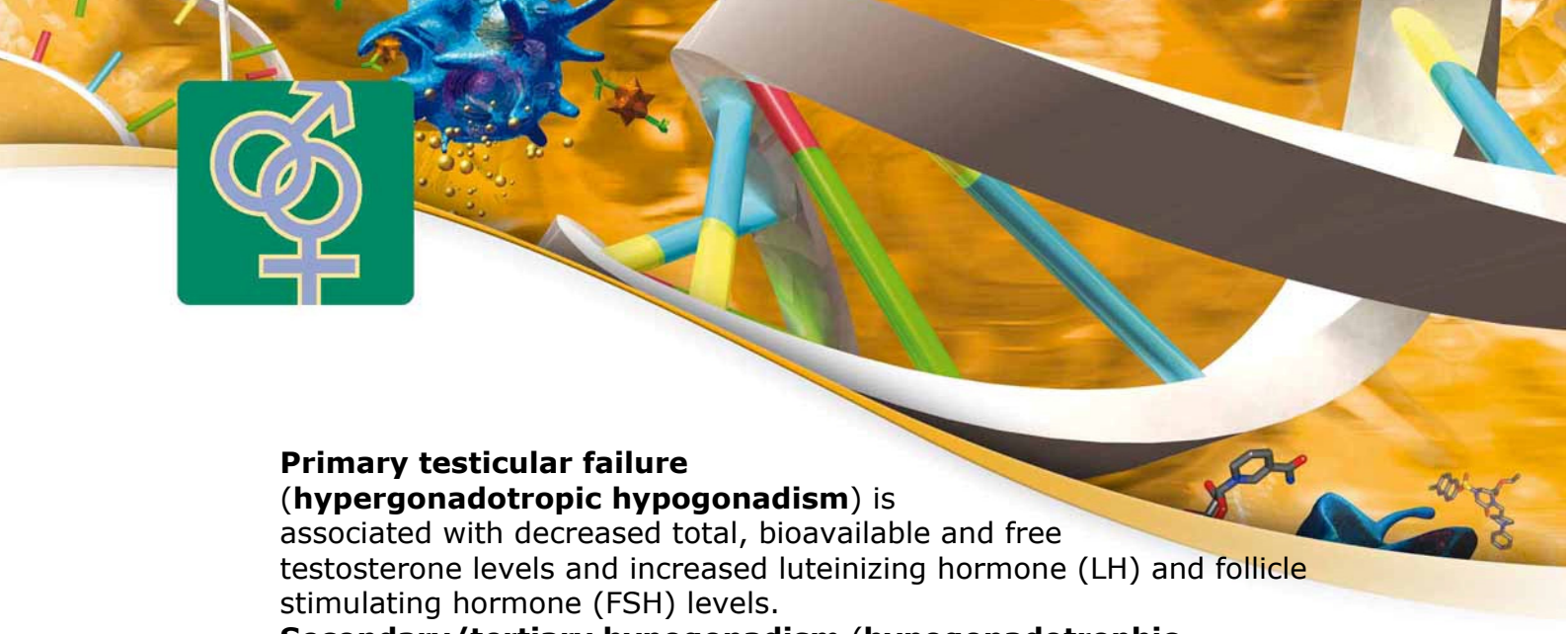
Monitoring of congenital adrenal hyperplasia (CAH) treatment

Testosterone is measured in conjunction with DHEA, 17-OHP, androstenedione and DHEA-S.

In men

Hypogonadism

Testosterone levels are measured in men with symptoms of hypogonadism, such as loss of libido, erectile dysfunction, gynecomastia, osteoporosis and infertility. Decreased testosterone levels indicate partial or complete hypogonadism. The cause may be primary, secondary (pituitary) or tertiary (hypothalamic) testicular failure.



Primary testicular failure (**hypergonadotropic hypogonadism**) is associated with decreased total, bioavailable and free testosterone levels and increased luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels.
Secondary/tertiary hypogonadism (**hypogonadotrophic hypogonadism**, e.g. Kallmann's syndrome) exhibits low testosterone and low to normal LH and FSH levels. The GnRH stimulatory test may be subsequently applied.

Male infertility

Testosterone as well as gonadotropins, LH, FSH and Inhibin B are measured.

Monitoring antiandrogen therapy

This therapy may be used in cases of prostate cancer. The aim is usually to reduce testosterone levels to those associated with castration or below (no more than 25 % of the lower reference range value).

Diagnosis of testicular or adrenal tumors

Testosterone levels exceeding the normal upper limit by more than 50% may indicate testicular or adrenal tumors. DHEA-S/DHEA levels are elevated in more than 90% of patients with adrenal tumors.

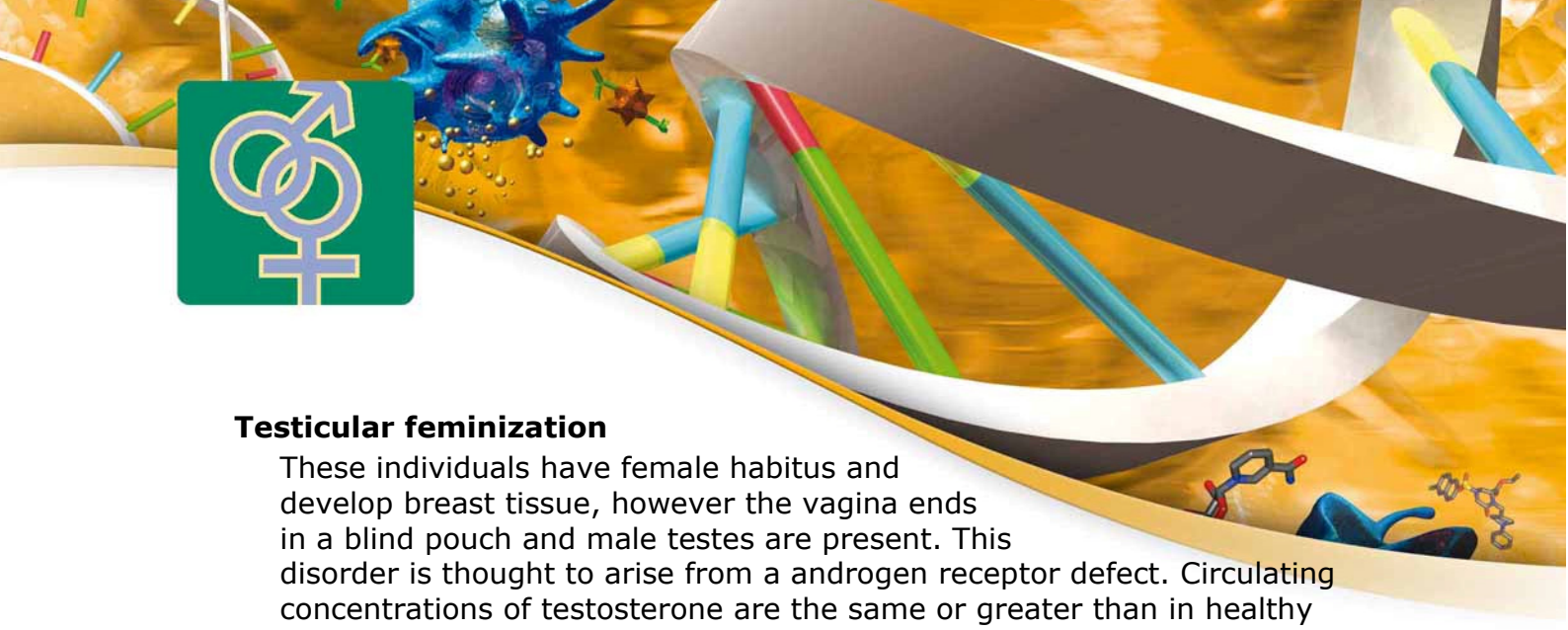
In women

- **Polycystic ovary syndrome (PCOS)**

  PCOS is associated with androgen hyperproduction and with decreased synthesis of ovarian estrogens, which is compensated for by increased synthesis in the periphery, such that the serum estrogen levels remain normal. Testosterone (total, bioavailable and free), androstanedione and AMH concentrations are usually elevated. LH levels are elevated as well, but FSH levels are normal or low. The ratio of LH to FSH is higher than 2.5. Other measureable parameters include 17α-hydroxyprogesterone to evaluate 21-hydroxylase deficiency.

Evaluation of women with hirsutism, virilisation or oligomenorrhea

Hyperproduction of androgens in women may result in hirsutism with virilism of various degrees, frequently connected with oligomenorrhea and infertility. Hyperproduction of androgens may originate in ovaries or adrenal glands. Determination of testosterone and DHEA-S levels should be carried out.



Testicular feminization

These individuals have female habitus and develop breast tissue, however the vagina ends in a blind pouch and male testes are present. This disorder is thought to arise from a androgen receptor defect. Circulating concentrations of testosterone are the same or greater than in healthy men. Testosterone serves as a substrate for further estrogen formation, so estrogen levels are also elevated. Concentrations of LH are also increased, presumably because of the resistance of the hypothalamic-pituitary system to androgen inhibition.

Evaluation of women with symptoms of possible testosterone deficiency

Why to determine free testosterone levels

Free testosterone levels are less susceptible to changes in SHBG concentration (the principal testosterone transport protein). In situations in which SHBG is continuously increased (e.g., hyperthyroidism, hyperestrogenic states such as pregnancy or oral contraceptive use, administration of antiepileptic drugs) or decreased (e.g., hypothyroidism, androgen excess, obesity), measurement of free testosterone may be more appropriate than total testosterone. The above-mentioned conditions may cause changes in total testosterone concentrations without necessarily influencing free testosterone levels.

Conditions in which it is preferable to determine free testosterone

Conditions involving treatment with corticosteroids and sex steroids

These conditions may result in changes of SHBG levels and availability of sex-steroid binding sites on SHBG. This may make diagnosis of subtle testosterone abnormalities difficult.

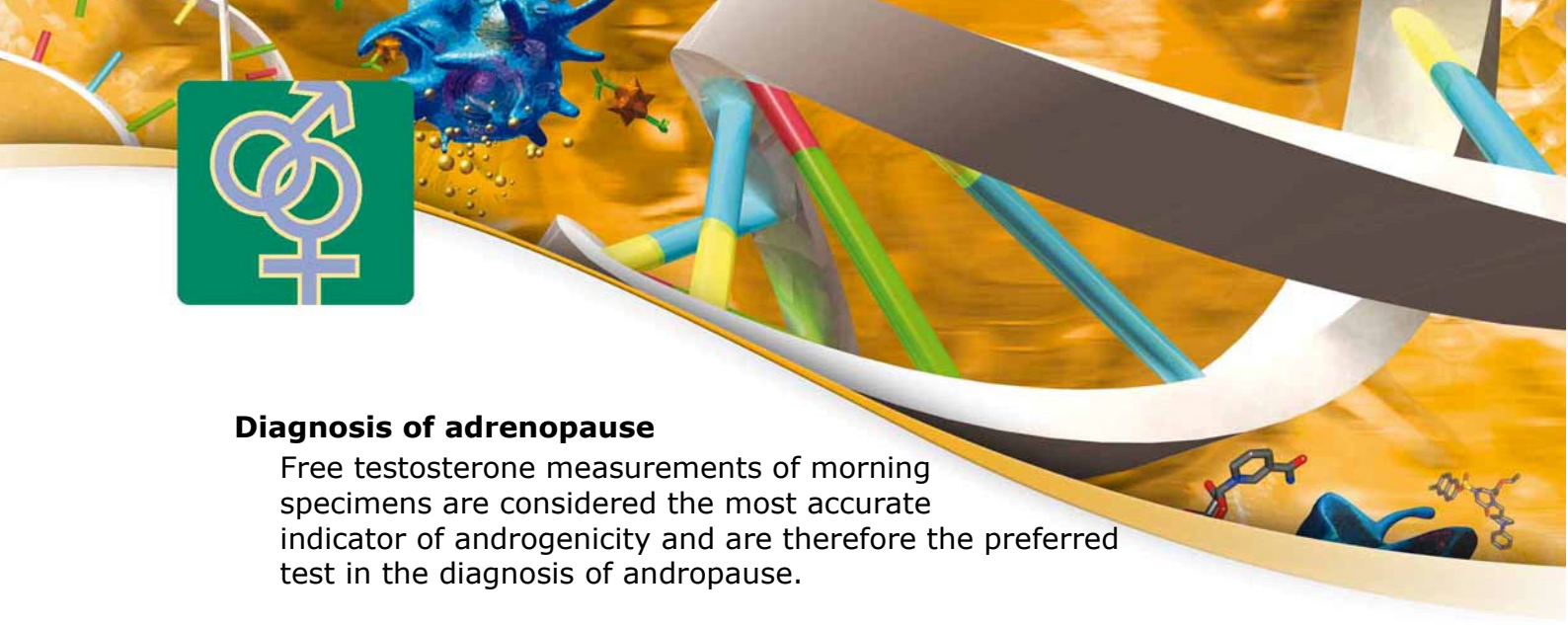
Inherited SHBG binding abnormalities

Determination of the etiology of hyperandrogenism in women and evaluation of infertility in men

Liver disease and severe systemic illness

Delayed puberty in boys and mild hypogonadism in men

Mild decreases in total testosterone without LH abnormalities can be associated with these conditions. Measurement of free testosterone gives better information than that of total testosterone.



Diagnosis of adrenopause

Free testosterone measurements of morning specimens are considered the most accurate indicator of androgenicity and are therefore the preferred test in the diagnosis of andropause.

Polycystic ovary syndrome (PCOS)

PCOS and related conditions are often associated with insulin resistance, which in turn is associated with low SHBG levels. Consequently, free testosterone levels may be further elevated in these cases.

Hirsute women

There is a discrepancy between testosterone production rate and circulating levels of total testosterone in hirsute women. In many hirsute women, total testosterone levels are within the normal range. In contrast, free testosterone is mostly elevated[10,11,12,13]. SHBG levels are usually decreased.

Hirsutism may be connected with other disorders such as hypothyroidism, obesity and hyperinsulinemia.

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