

# Testosterone: An Insight into Its Clinical and Biochemical Assessments

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## ABSTRACT

Testosterone levels are very important to be maintained in males, but the level reduces as age advances. If symptoms of severe or chronic low testosterone are present, hormone deficiency is amenable, it may be caused by an active disease or condition. In this review, we have tried to summarize how testosterone biosynthesis, regulation, as well as metabolism occurs in the human body. How it plays numerous roles: starting from intrauterine life to an advanced age, various factors such as age, weight, sleep, food habits, etc., affecting testosterone levels in the body are also discussed. These factors are very important in understanding the clinical implications of testosterone on the health of an individual. We also summarize various assays that are currently done for testosterone evaluation.

**Keywords:** Immunoassay, Mass spectrometry, Sexual dysfunction, Testosterone, Type 2 diabetes mellitus.

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## INTRODUCTION

In 1935, David and colleagues extracted hormone from testicular material which was known as "Testosterone" (Fig. 1). It is important in the development of reproductive organs (prostate and testes) in males. It also facilitates secondary sexual characters.<sup>1</sup> Apart from this, it is associated with well-being, health, and the prevention of osteoporosis.<sup>2,3</sup> Its deficiency in males leads to protean manifestations including sexual dysfunction, bone loss, and frailty.<sup>4</sup>

It is secreted mainly by testicles and by ovaries (to less extent). Normal males secrete 4–12 mg of testosterone per day. Adult males have approximately 7–8 times higher levels of testosterone.<sup>5</sup> This is probably due to the greater production of testosterone in males than in females. Adult females are sensitive to testosterone.

Testosterone is a hormone that is also used as an intervention to treat many conditions such as low levels of this hormone in males.<sup>6</sup>

Testosterone in males is necessary for maintaining and developing reproductive tissues like the prostate, testis, seminal vesicles, epididymis, as well as penis. Testosterone is important in maintaining muscle strength and hair growth. To maintain balanced levels of testosterone, the rate of production should be balanced with metabolism and utilization.<sup>7</sup>

Symptoms of testosterone deficiency in adult males include:

- Reduced body and facial hair
- Loss of muscle mass
- Low libido, impotence, small testicles, reduced sperm count, and infertility
- Increased breast size
- Hot flashes
- Irritability, poor concentration, and depression
- Loss of body hair
- Brittle bones and an increased risk of fracture.

In some males having testosterone deficiency in addition to symptoms/conditions related to low testosterone could be improved by testosterone replacement therapy. For example, a man with osteoporosis and low testosterone can increase bone strength and reduce his fracture risk with testosterone replacement therapy.<sup>8</sup>

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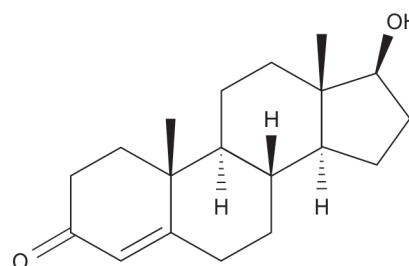
**Conflict of interest:** None

Surprisingly, low-dose testosterone restores libido in females affected by hypotestosteronemia. Disease in the pituitary gland may lead to reduced testosterone production from adrenal glands. They may experience low libido, reduced bone strength, poor concentration, or depression.<sup>9</sup>

## Biosynthesis of Testosterone

Leydig cells are the key source for the synthesis of testosterone. It is also secreted in trace amounts in the adrenal cortex as well as by seminiferous tubule (Sertoli cells).<sup>10</sup>

Testosterone's *de novo* biosynthesis occurs continuously as Leydig cells are incapable of storing androgens such as testosterone.



**Fig. 1:** Structure of testosterone molecule

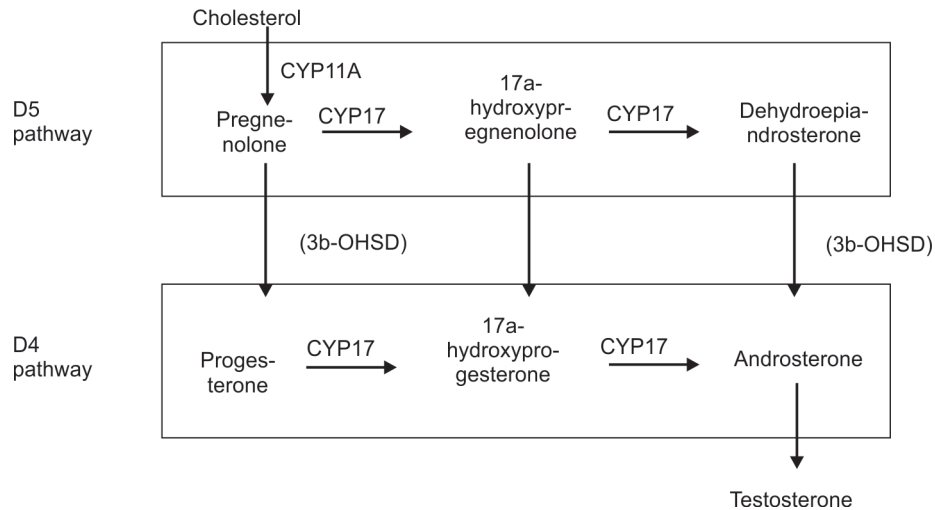


Fig. 2: Biosynthesis of testosterone

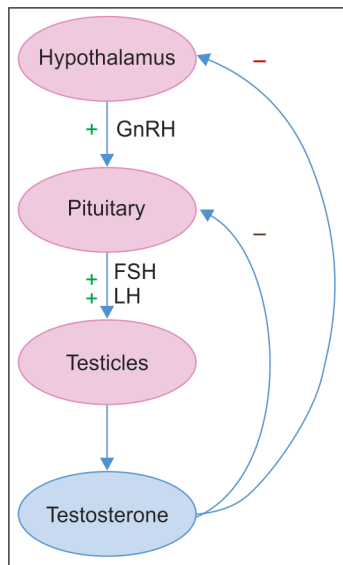


Fig. 3: Hypothalamus–pituitary–testicular axis

The synthesis of testosterone is initiated from a biomolecule known as cholesterol (Fig. 2). Cholesterol homeostasis in Leydig cell *in vivo* is maintained by luteinizing hormone (LH) which acts as a key regulatory factor. Cholesterol can be synthesized from acetyl-coenzyme A. It can also be regulated by incorporation from low-density lipoproteins (LDLs) through receptor-mediated endocytosis. In addition, lipid homeostasis in Leydig cells is regulated by testosterone signaling. Storage of cholesterol is done as lipid droplets in the cytoplasm. The amount of testosterone synthesis is contrariwise to lipid droplets formed.

Testosterone biosynthesis occurs in differential enzymatic steps:

**Step 1:** Cholesterol is acted upon by hydroxylases leading to shortening of side-chain by cleaving the bond between C20 and C22 that ultimately leading to pregnenolone production.

After the formation of pregnenolone, the remaining steps take place in the endoplasmic reticulum (ER) via either the D4 or D5 pathway (D4 or D5 denotes double bond localization in steroid molecule). In humans, the D5 pathway is dominant than

D4. Conversion of pregnenolone to testosterone involves five enzymes for action.

- 3β-hydroxysteroid dehydrogenase (3β-OHSD).
- $\Delta^{5-4}$  isomerase.
- 17α-hydroxylase.
- C<sub>17,20</sub> Lyase.
- 17β-hydroxysteroid dehydrogenase (17β-OHSD).

**Step 2:** In the D4 pathway, progesterone which is an essential biomolecule is formed as a result of dehydration. This leads to the formation of an intermediate, i.e., 17α-hydroxyprogesterone. At this step, if removal of side-chain occurs then an intermediate androstene-3,17-dione is formed. Again reduction occurs at position C17, which is then converted into testosterone.

**Step 3:** In the D5 synthesis pathway, intermediates 17α-hydroxypregnenolone lead to the synthesis of testosterone via dehydroepiandrosterone (DHEA).<sup>11</sup>

### Regulation

In males, testosterone is produced basically in Leydig cells. The quantity of Leydig cells is directed by LH and follicle-stimulating hormone (FSH). Additionally, a measure of testosterone delivered by existing Leydig cells is heavily influenced by LH, which alters the functioning of 17β-hydroxysteroid dehydrogenase.<sup>10</sup>

Production of testosterone is under the control of the hypothalamo-pituitary testicular axis.<sup>12</sup> When testosterone levels are low, gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus stimulating the pituitary to release FSH and LH. These hormones stimulate the testis to produce testosterone. A testosterone level via a negative feedback loop (from the hypothalamus and pituitary) leads to decreased production of LH and FSH (Fig. 3).

### Testosterone Levels vs Testosterone Action, Does Sex Hormone-binding Globulin (SHBG) Matter?

Sex hormone-binding globulin is a glycoprotein principally produced in the liver. It binds to any of 17 sex hormones, including testosterone and estrogen, and transports these synthetic mixtures throughout the body. Testosterone is referred to as “bound” when attached to sex hormone-binding globulin (SHBG) and albumin.

Free testosterone and the fraction bound to albumin are referred to as “bioavailable testosterone”, and are biologically active. The total bound and free testosterone is referred to as total testosterone (Fig. 4).

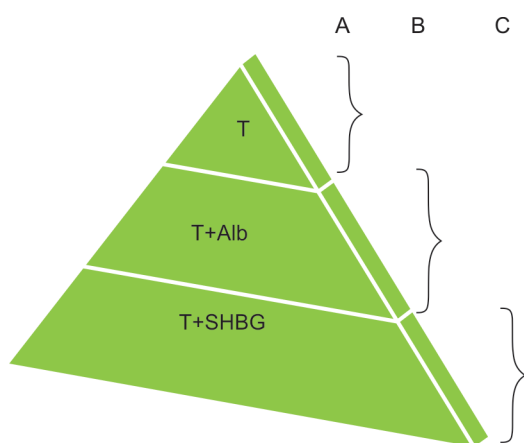
Low SHBG is associated with high concentrations of triglycerides and LDL.<sup>13</sup> Also, low degrees of SHBG are related to various cardiovascular diseases in both genders, including coronary disease, type 2 diabetes, and hypertension.<sup>14</sup>

High SHBG is hazardous particularly for males since it reduces the level of free testosterone. Significant levels of SHBG are related to sterility, a diminished sex drive, and erectile dysfunction (ED), particularly when testosterone levels are low.<sup>15</sup> In both males and females, low degrees of free testosterone can lead to diminished muscle strength. It is found in disease states like hyperthyroidism and pregnancy and other estrogenic excess conditions.

#### Variables Influencing Testosterone Levels

- Age: Testosterone levels decrease with increasing age in men.<sup>16</sup>
- Exercise: Resistance training enhances testosterone levels.<sup>17,18</sup> Endurance training in men may reduce testosterone levels.<sup>19</sup>
- Supplements: Deficiency of vitamin A leads to altered plasma testosterone levels.<sup>20</sup> Secosteroid, vitamin D when taken as 400–1,000 IU/day (10–25 µg/day) elevates testosterone levels.<sup>21</sup> Testosterone levels decrease in deficiency of zinc (Zn).<sup>22</sup> However, there is no significant impact of zinc on serum testosterone on supplementation if one is already getting enough of the mineral from the diet. The study participants included men whose daily diets included the recommended amounts of zinc. Giving these men zinc supplements did not increase their testosterone levels.<sup>22</sup>

Moreover, Zn acts as a toxic repercussion against heavy metals and cigarette inflammatory agents. Zinc as a hormone balancer helps hormones such as sexual health, testosterone, and prostate functions. It acts as an antibacterial agent in men’s urea system. It plays a role in epithelial integrity, showing that Zn is essential for maintaining the lining of reproductive organs. It may have a regulative role in the progress of capacitation and acrosome reaction. In contrast, Zn deficiency impedes spermatogenesis and is a reason for sperm abnormalities, and has a negative effect on serum testosterone concentration.



**Fig. 4:** Testosterone in circulation T = Testosterone. A = Free testosterone (1.5–2.0%). B = Testosterone bound to albumin (33%). C = Testosterone bound to sex hormone-binding globulin (65%). Bioavailable testosterone is A + B, and total testosterone is A + B + C

- Weight reduction: Testosterone levels increase on reduction in weight. Fat cells aromatase converts testosterone into estradiol (female sex hormone).<sup>23</sup> However, there is no relationship between body mass index and testosterone levels.<sup>24</sup>

#### Other Factors

- Sleep: Night-time testosterone levels increase during rapid eye movement (REM) sleep.<sup>25</sup>
- Behavior: Dominant nature in males can stimulate excessive testosterone release.<sup>26</sup>
- Drugs: Testosterone levels can be decreased by natural or man-made antiandrogens (e.g., spearmint tea).<sup>20,21</sup>
- Licorice: The formation of testosterone is reduced by licorice use.<sup>27</sup>

#### Distribution

The plasma protein binding of testosterone is 98.0–98.5%, with 1.5–2.0% free or unbound.<sup>28</sup> It is bound 65% to SHBG and 33% bound weakly to albumin<sup>29–31</sup> (Fig. 4).

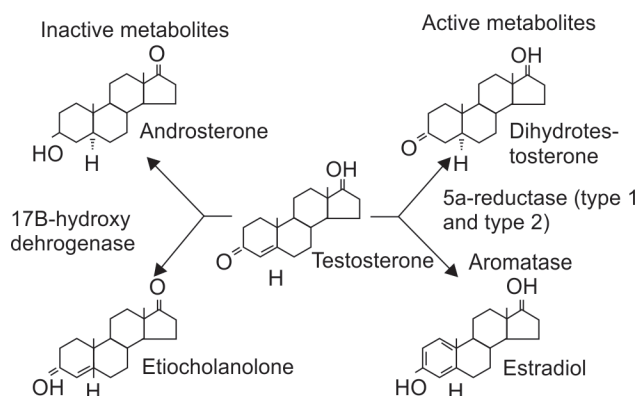
#### Metabolism of Testosterone

Testis mainly secretes testosterone, and also secretes 5 $\alpha$ -dihydrotestosterone (5 $\alpha$ -DHT), androstenedione, androsterone, 17-hydroxyprogesterone, pregnenolone, and progesterone (Fig. 5). Testosterone transforms to dihydrotestosterone in the prostate.<sup>10</sup>

5 $\alpha$ -dihydrotestosterone produced by testosterone through 5 $\alpha$ -reductase, is more potent than testosterone (3–5 times). The half-life of testosterone is approximately 12 minutes.<sup>15</sup>

Testosterone is converted to  $\alpha$ -DHT in ER by an enzyme 5 $\alpha$ -reductase, i.e., located in microsome. Testosterone and  $\alpha$ -DHT binds to the same intracellular androgen receptor to regulate gene expression in target tissues producing different biological effects. 5 $\alpha$ -reductase has two isoforms in humans. 5 $\alpha$ -reductase type 1 and type 2 genes are located on chromosomes 5 and 2, respectively. They encode a protein of 259 and 254 amino acids, respectively. Instead of similarity, both isoforms show dissimilar biochemical properties. Working optimal pH for type 1 reductase is alkaline, while for type 2 reductase it is acidic. The tissue distribution of both isoforms is also different. Type 1, 5 $\alpha$ -reductase is localized in non-genital organs like skin, liver, brain, prostate, testis, and ovaries while 5 $\alpha$ -reductase type 2 is found in genital skin, epididymis, testis, prostate, and seminal vesicle, it is also found in breast, uterus, as well as the placenta.

5 $\alpha$ -dihydrotestosterone is very important in cell growth and differentiation and also for normal sexual development as well as



**Fig. 5:** Metabolism of testosterone

virilization in men. It is mainly responsible for the deepening of the voice and the development of muscle mass. Largely, testosterone has effects due to its metabolites, i.e., estradiol and 5 $\alpha$ -DHT (Table 1).

Partial androgen insensitivity syndrome (PAIS) or complete androgen insensitivity syndrome (CAIS) results due to altered properties of 5 $\alpha$ -reductase type 2 as a result of mutation. Type 3 $\alpha$ -HSD (hydroxysteroid dehydrogenase) eliminates dihydrotestosterone to form 3 $\alpha$ -androstenediol by reducing 5 $\alpha$ -DHT. Only Aldo-keto reductase family 1 member C2 (AKR1C2) activity can convert back 3 $\alpha$ -androstenediol to 5 $\alpha$ -DHT, for prostate growth.<sup>16</sup> Varying reductase activity on different ketosteroid positions, mainly converts 5 $\alpha$ -DHT to 3 $\alpha$ -diol (inactive) and is mainly excreted in the urine. Certain metabolites are excreted in their free form while others are glucuronidated in the liver before excreting out. The glucuronidation of  $\alpha$ -DHT metabolite is correlated with numerous metabolic risk factors such as disturbed lipid profile, total fat mass, intrahepatic fat, diabetes mellitus, and insulin resistance.<sup>17,32</sup>

### Biological Effects of Testosterone

Testosterone plays numerous roles starting from intrauterine life to advanced age (Table 2).

- Intrauterine life: A fetus having 46 XY chromosomes is fated to develop into a male as chromosome Y has a testicular determining gene that transforms undifferentiated gonad to testes. The testes consequently produce testosterone for development as well as the growth of the Wolffian system that includes vas deferens, seminal vesicle. DHT too is the main androgen causing external genitalia androgenization.<sup>33</sup>
- Puberty: Hypothalamus activation leads to increased release of GnRH to release LH and FSH. Stimulation of these hormones elevates the production of testosterone in testes to initiate secondary sexual characteristics related to puberty like spermatogenesis, libido, growth spurt, acne, erectile function, growth of body hair, and muscle mass, deepening of the voice.
- Bones: There are a few main effects of testosterone on bones.
  - Via alteration by the aromatase enzyme, testosterone works opposite to osteoclastic activity. Therefore, enhancing bone resorption.<sup>34</sup>
  - Via alteration by the 5 $\alpha$ -reductase enzyme to DHT. It stimulates osteoblastic activity and hence, accelerates bone formation.
  - Males having the hypogonadal condition are at risk of developing osteoporosis or osteopenia. This ultimately results in a fracture.<sup>18</sup>
- Libido: Testosterone is associated with increased libido.
- Erythropoiesis and anemia: Hematopoiesis is stimulated by testosterone through increased production of erythropoietin.
- Low testosterone levels and cardiovascular risk: Testosterone levels decrease gradually with age in males. Many observational and epidemiological studies have revealed, decreased levels

of testosterone are related to enhanced risk of cardiovascular disease.

- Obesity, diabetes mellitus, metabolic syndrome: Metabolic syndrome has been termed as central adiposity (waist circumference is >94 cm) with hypertension, decreased HDL cholesterol and increased triglycerides, insulin resistance, and diabetes mellitus.

Testosterone is converted to estradiol in adipose tissue that contains high concentrations of aromatase. Reduced testosterone levels are associated with diabetes mellitus.

A study was done to evaluate the effect of testosterone therapy on glycemic control and insulin resistance in males having decreased levels of testosterone in addition to diabetes mellitus type 2. It was concluded that males who were undergoing testosterone therapy had reduced fasting blood sugar, glycated hemoglobin, insulin resistance, waist/hip ratio, waist circumference, and total cholesterol.

- Testosterone and aging: Testosterone levels decrease as age increases. This drop-in availability of testosterone may start early but in the 50s and 60s, it becomes clinically obvious. Reduction in testosterone levels affects both physiological functions (muscle mass, bone metabolism, cognitive function, erectile function, and libido) and pathophysiological functions such as obesity, insulin resistance, diabetes mellitus, metabolic syndrome, autoimmune disease, etc.

Sufficient availability of testosterone is cardioprotective. Obesity, diabetes, and metabolic syndrome are risk factors for coronary heart diseases also related to decreased levels of testosterone. It has been proposed that with reducing levels of testosterone in aging males, fat mass, as well as lean body mass, decreases.<sup>35</sup>

### Forms of Testosterone Supplements

Various forms of testosterone replacement therapy are important in improving testosterone levels.<sup>36</sup>

#### Skin Patch (i.e., Transdermal)

Androderm, skin patch worn on the arm or upper body which is applied once a day.

#### Gels

Testim and AndroGel come in a pack of testosterone gel. Testosterone is directly absorbed *via* the skin when applied gel once a day. AndroGel, Axiron, and Fortesta also come in a pump. Natesto is a gel that is applied inside the nose.

#### Mouth Patch

Striant is a tablet that sticks to the upper gums above the incisor, the tooth just to the right or left of two front teeth. It is applied twice a day; it continuously releases testosterone into blood through oral tissues.

**Table 1:** Percentage of plasma protein binding of testosterone

Compound	Group	Plasma protein binding of testosterone				
		Level (nM)	Free (%)	SHBG (%)	CBG (%)	Albumin (%)
Testosterone	Adult men	23.0	2.23	44.3	3.56	49.9
	Adult women					
	Follicular phase	1.3	1.36	66.0	2.26	30.4
	Luteal phase	1.3	1.37	65.7	2.20	30.7
	Pregnancy	4.7	0.23	95.4	0.82	3.6

**Table 2:** Biological effects of testosterone

S. no.	Effect	Mechanism	Outcome
1	Endocrine system	Hypothalamus regulates the production of testosterone	It has a negative feedback regulation
2	Reproductive system	Testosterone is required for spermatogenesis	Low levels of it result in infertility in males
3	Sexuality	Growth of testicles, penis, and pubic hair	Decreased levels of testosterone result in the development of female characters in males
4	Central nervous system	Develops aggression and dominance	Decreased levels affect behavioral traits in males
5	Skin and hair	Growth of hair on the face, in armpits, and around genitals	Low levels result in alteration of male pattern of hair distribution
6	Muscle, fat, bone	Development of muscle bulk and strength	Decreases muscular strength
7	Circulatory system	Spurs bone marrow to produce red blood cells affect cholesterol, blood pressure, and clot-busting ability	Low levels of testosterone decrease hematopoiesis
8	Cardiovascular system	Testosterone acts <i>via</i> binding to androgen receptor altering myocardial and vascular cell behavior	Low levels of testosterone are associated with an increased risk of CAD
9	Blood pressure	Testosterone acts on mean atrial pressure	With an increased level of testosterone, blood pressure increases
10	Diabetes mellitus, obesity, and metabolic syndrome	Testosterone acts <i>via</i> regulating insulin resistance and glycemic control	Low levels of testosterone result in metabolic syndrome
11	Aging	The level of testosterone decreases with increasing age	Results in loss of physiological functions

### Injections and Implants

Testosterone can also be injected directly into muscles, or implanted as pellets in soft tissues. The body slowly absorbs the testosterone into the bloodstream.

Why not a simple testosterone pill?

Oral testosterone is available. However, their pharmacodynamics is unreliable and there are peaks and troughs in 24 hours duration, they are associated with hepatotoxicity. Using other methods, like gels, skin patches, injections, or orally disintegrating tablets, bypasses the liver and gets testosterone directly into the blood.

### Pros and Cons of Testosterone Treatment Modalities

Worldwide, intramuscular injections are generally used formulations of testosterone which is not only the oldest one but also the most time-tested formulation. "For injections, the advantages include predictable on-treatment levels of testosterone. If a patient uses weekly injections, that will result in physiologic levels of testosterone. But, to avoid the weekly needle stick, patients and clinicians usually opt for a higher dose, which is given twice a month".

The cons, however, is that this less frequent dosing results in peaks and troughs in serum testosterone concentrations. The weekly smaller dose, despite the inconvenience, yields more even levels. "It is also inexpensive, for many patients who do not have insurance or have limited coverage, injections are one formulation that they can afford".<sup>36</sup>

### Who should be Screened for Testosterone Deficiency?

Screen for TD in:

- Adult men with consistent and multiple signs of TD.
- All men presenting with ED, loss of spontaneous erections, or low sexual desire.
- All men with type 2 diabetes mellitus, BMI >30 kg/m<sup>2</sup> or waist circumference >102 cm.
- All men are on long-term opiate, antipsychotic, or anticonvulsant medication.

TD = Testosterone deficiency; ED = Erectile dysfunction; BMI = Body mass index

### Assays for Testosterone Measurement<sup>37</sup>

Diagnosing testosterone deficiency is quite challenging. Variations in serum testosterone levels may be due to seasonal or age-related reasons. Some medications such as opiates and glucocorticoids in addition to disease can temporarily alter testosterone concentration. Change in level of total testosterone is due to altered SHBG. This ultimately affects age and results in medical comorbidities. Different assays are available for measuring testosterone levels as well as performance characteristics (Table 3).

#### Immunoassays

In the clinical laboratory, immunoassays are commonly used to measure total testosterone levels. Currently, there are two widely used assays for it. These include radioimmunoassay (RIA) and chemiluminescent immunoassays. Both of these assays can be done on plasma or serum. This is done after extraction or chromatography. These additional steps are more laborious, can remove cross-reacting hormones as well as interfering proteins.

**RIAs:** It measures testosterone level when serum is incubated with anti-testosterone antibodies linked to a radioactive antigen. Total testosterone concentration can be measured when antigen present in serum detaches radioactive antigen from its antigen-binding site, releasing particles.

**Chemiluminescent assays:** This assay uses an antibody that is linked to an enzyme, usually horseradish peroxidase (HRP). Serum testosterone is incubated in a well coated with antibodies specific to this hormone. After complex formation, a secondary antibody is added followed by adding a chemiluminescent substrate. This produces a detectable luminescent signal.<sup>37</sup>

**Benefits of immunoassays:** Precisely rapid, simple, and comparatively affordable, cost-effective with high output.

**Drawbacks of immunoassays:** Concentrations of testosterone were overestimated, susceptible to matrix effects, limited accuracy for testosterone levels (not >300 ng/dL) when performed.

Radioimmunoassay also has a drawback as this immunoassay creates radioactive waste. Essentially, due to the use of antibodies



**Table 3:** Assays for testosterone measurement

Method of assay	Minimum level measured	Advantages	Disadvantages
Radioimmunoassay	300 ng/dL	Precisely rapid, simple, and comparatively affordable, cost-effective with high throughput	Create radioactive waste
Chemiluminescent assays	300 ng/dL	Precisely rapid, simple, and comparatively affordable, cost-effective with high throughput	Concentrations of testosterone are overestimated, susceptible to matrix effects, limited accuracy
Mass spectrometry	144 ng/dL	In a single run, able to detect many analytes, accuracy over a wide range of concentrations, specificity to detect compounds, and the error rate is very low antibody cross-reactivity reactions	Require high technical expertise, operating cost is high, lacking standardization, special disposal is required for solvents used

**Table 4:** Comparison between gas and liquid chromatography

S. no.	Gas chromatography	Liquid chromatography
1	Requires large sample volume	Requires low sample volume
2	Provides a more comprehensive screen of an individual's steroid profile	Open to automation
3	Does require chemical steroid derivatization	Does not require chemical steroid derivatization
4	More time-consuming	Less time-consuming
5	Suitable for athlete/bodybuilder using anabolic androgenic steroid	Can be used for any patient's sample

targeting specific chemical moieties on steroid molecules and high specificity for testosterone or DHT, immunoassays do not detect anabolic steroids that do not have this type of structure. As a result, immunoassay will show low levels of circulating testosterone in an anabolic steroid user, despite looking highly masculinized.<sup>38</sup>

#### Mass Spectrometry (MS)

As immunoassays have limitations such as in specificity and precision mainly at low to very low levels of testosterone. Mass spectrometry when used in combination with chromatography has gradually been translated from a laboratory research technique to clinical application. This is mainly due to its ability to identify many analytes, its high-performance accuracy over a wide variety of concentrations. It is specific to detect compounds of interest and is immune to antibody cross-reactivity reactions. It involves preparation of sample to remove interfering matrix (such as salts and proteins), chromatographic separation, ionization of analyte, and analysis with a spectrometer.

Chromatographic separation can be performed in a gas (GC) or a liquid (LC). Some of the characteristics of GC and LC are discussed below (Table 4):

Mass spectrometry has increased the specificity and accuracy of performance of tandem (MS/MS) by applying the second round of ionization. These further fragment analyte ions are of interest to increase the differentiating ability of this assay.<sup>39</sup>

The amplified sensitivity and specificity of LC-MS/MS have permitted quantifying free testosterone as low as 5 pmol/L.

**Drawbacks of MS:** It requires high technical expertise. Therefore, not available in all laboratories due to the high cost of operation, lacking of standardization, special disposal is required for solvents used.

## CONCLUSION

For men, testosterone levels are important to maintaining, but they naturally decrease over time. If symptoms of severe or chronic low testosterone are showing, hormone deficiency may be caused by an active disease or condition. Treatment can lead to unwanted side effects, so it is crucial to balance the expected benefits of testosterone with the risks of treatment.

In this review, we have tried to summarize how testosterone biosynthesis, regulation as well as metabolism occur in the body. How it plays numerous roles starting from intrauterine life to an advanced age, various factors such as age, weight, sleep, food habits, etc., affecting testosterone levels in the body.<sup>40</sup> This is very important in understanding the clinical implications of testosterone on the health of an individual. At last, we have biochemically discussed various assays that are currently done for testosterone evaluation. Immunoassays are widely used to measure total testosterone levels but have limitations such as in specificity and precision mainly at low to very low levels of testosterone. Mass spectrometry when used in combination with chromatography has gradually been translated from a laboratory research technique to clinical application. It has increased specificity and accuracy of performance than immunoassays.

Still, there are several areas in the testosterone deficiency states, more specifically, epidemiology, diagnosis, treatment, and adverse events, which warrant more detailed investigation.

## AUTHOR'S STATEMENT

The manuscript has been read and approved by all the authors, and each author believes that the manuscript represents honest work.

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