

Testosterone and the Androgen Receptor



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KEYWORDS

• Testosterone • Androgen receptor • Dihydrotestosterone • Structure • Function

KEY POINTS

- Testosterone is a critical steroid hormone involved in the development of male sexual characteristics and function as well as regulation of many homeostatic mechanisms throughout life.
- The production and metabolism of testosterone is tightly regulated by the hypothalamic-pituitary (HPG) axis in order to maintain strict homeostasis among the involved physiologic processes.
- Additional steroid hormones involved in testosterone steroidogenesis and metabolism, including dihydrotestosterone and estradiol, occupy an important position in exerting effects on target tissues and in regulating the HPG axis.
- When activated by androgens, the androgen receptor is involved in genomic and nongenomic signal transduction to exert the intended effect on the target tissue.

INTRODUCTION

The steroid hormone testosterone is among the most widely studied in the endocrine system. Testosterone is the major sex hormone in men and critically influences male development and maintenance of physiologic functions of multiple organ systems across all ages, including in sexual differentiation, development of male secondary sexual organs, sperm production, libido, muscle size and strength, and bone growth and strength. The male phenotype strongly depends on the expression of testosterone and its byproducts. Adolescent boys with too little testosterone may not experience normal masculinization. In contrast, athletes who use anabolic steroids, testosterone, or related hormones to increase muscle mass and athletic performance have abnormally high testosterone levels.

Testosterone levels are carefully regulated by an elegant multitier feedback system with positive

and negative feedback mechanisms that tightly regulate hormonal levels and expected physiologic changes. Testosterone is the ligand for the androgen receptor (AR), which produces its effects through regulation of both gene transcription and translation of proteins as well as through second messenger systems for both prolonged and rapid effects.

HISTORICAL EFFECTS OF TESTOSTERONE

The biological effects of the testes and testosterone have been known since antiquity. In Greek mythology, the titan Chronos castrates his father Uranos. Aristotle knew the effects of castration on reproductive biology. Castration has been performed as punishment and to produce obedient slaves but also to preserve the soprano voices of prepubertal boys. Imperial courts used castrated men as overseers in harems. In multiple cultures, testicles from various animals were consumed to

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improve virility.¹ In 1786, John Hunter transplanted testes into capons in London, without understanding the implications of the endocrine aspects. Arnold Berthold in Göttingen, Germany in 1849 observed that castrated roosters failed to develop expected secondary sexual characteristics, including development of the comb, aggressive behavior, crowing, muscle development, and sexual interest in hens. Berthold then showed that transplantation of testes back into two roosters resulted in remasculinization and appearance of secondary sexual characteristics.^{2,3} From these systematic studies, Berthold established the role of internal secretion from the testicular transplantations as regulators of sexual characteristics and is considered as the father of modern endocrinology.

In 1889, Charles E. Brown-Séquard famously reported on 10 subcutaneous self-injections of the combination of testicular vein blood, semen, and testicular extract from dogs and guinea pigs. He reported an astonishing self-described recovery of endurance, strength, cognition, and even urinary and bowel function.⁴ An analysis finding that the small amount of testosterone extracted by this method has pointed authors to conclude that Brown-Séquard likely experienced a placebo effect.⁵ However, the explosion of interest in the Western world in the use of animal organ extracts that subsequently occurred resulted in the springing up of factories in Europe and America for its production and thousands of physicians engaging in their prescription. This perfectly illustrates the fascination in the medical community and popular culture alike with restoration of male function and virility.^{2,3,5} Indeed, the twentieth century witnessed a steady progression in the understanding of male androgen function with the crystalline isolation of testosterone by Ernst Laqueur from bovine testes in 1935. Laqueur was the first to call the male androgenic hormone “testosterone.” In the same year testosterone was chemically synthesized independently by Adolf Butenandt in Göttingen and Leopold Ruzicka in Basel, a feat that was recognized by their joint Nobel prize in chemistry in 1939. The development of synthetic, longer acting, injectable testosterone enanthate preparations in the 1950s enabled widespread usage of synthetic testosterone.^{1–3} Currently, there continues to be increasing interest and the use of testosterone replacement among men of all age groups, with more than 4-fold increases in the number of men using testosterone replacement during the first decade of this century.^{6,7}

ANDROGEN STEROIDOGENESIS FROM CHOLESTEROL

The primary site of testosterone production is the Leydig cell within the testis, resulting in 95% of circulating testosterone being produced by the testes.⁸ Cholesterol is used as a precursor and is produced *de novo* within the cell or acquired via lipoprotein receptors. Cholesterol can either then be stored as an ester or transferred to the mitochondrial membrane by steroidogenic acute regulatory protein for steroid synthesis. A series of synthetic steps occurs ultimately resulting in testosterone (Fig. 1).^{8,9} Leydig cells preferentially express a specific isoform of 17 β -hydroxysteroid dehydrogenase (HSD), which favors the conversion of androstenedione to testosterone.¹⁰ Additionally, Leydig cells produce small amounts of other steroid hormones in this pathway including dihydrotestosterone (DHT), dehydroepiandrosterone, androstenedione, estradiol, estrone, and progesterone.¹¹

TESTOSTERONE REGULATION, PRODUCTION, AND TRANSPORT

Testosterone is under the regulation of the hypothalamic-pituitary-gonadal (HPG) axis (Fig. 2). In this classic positive and negative feedback system, gonadotropin-releasing hormone (GnRH) is released into the hypophyseal portal system by hypothalamic neurons responding kisspeptin stimulation from neighboring neurons.^{12,13} GnRH stimulates production and release of luteinizing hormone (LH) and follicle-stimulating hormone from the anterior pituitary. Leydig cells express the LH receptor, and through this, LH exerts a steroidogenic effect for testosterone production and trophic effect promoting Leydig cell growth and proliferation. Testosterone and estradiol then provide negative feedback at the level of the hypothalamus and pituitary to limit GnRH and LH production.^{14,15}

Testosterone produced within the Leydig cell has 2 ultimate fates: remain within the seminiferous tubule to optimize spermatogenesis or enter circulation to exert its effect on distant tissues.^{8,9,16} Local diffusion within the seminiferous tubule is mediated by androgen-binding protein, resulting in a significantly higher testosterone levels within the luminal compartment when compared with circulating levels.¹⁷ These levels optimize spermatogenesis by Sertoli cells and sperm function through independent and estradiol-mediated mechanisms. Within the peripheral circulation, testosterone reaches equilibrium with the serum proteins: 60% bound tightly

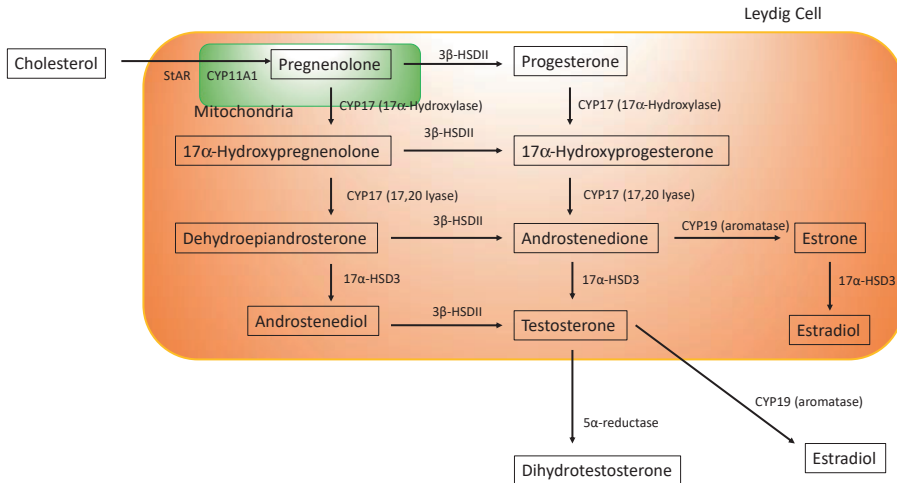


Fig. 1. Androgen steroidogenesis. Cholesterol is the precursor for androgen synthesis and is transported into the cell by lipoprotein receptors. It is transported to the mitochondrial membrane by steroidogenic acute regulatory protein. CYP17, 3 β -HSDII, and 17 α -HSD3 are involved with subsequent synthesis steps for testosterone. Testosterone may then enter the circulation and act primarily on target tissues or be converted to DHT or estradiol peripherally by 5AR and aromatase, respectively.

bound to sex hormone binding globulin (SHBG), 38% weakly bound to albumin, and 2% unbound. The proportion of testosterone that is bound to albumin and free is considered the bioavailable component.¹⁸ SHBG is produced in the liver, and its circulating levels can be influenced by medications and disease states. Estrogen, tamoxifen, antiepileptic drugs, age, and hyperthyroidism can increase SHBG levels, although exogenous androgen, glucocorticoid, or growth hormone therapy, untreated hypothyroidism, obesity, and hyperinsulinemic states may decrease SHBG. Alterations in SHBG levels in turn cause corresponding effects in the bioavailability of testosterone to target tissues, and polymorphisms and expression levels of SHBG may be associated with reduced fertility, erectile dysfunction, and late-onset hypogonadism as well as prostate cancer incidence and prognosis.^{19,20}

TESTOSTERONE VARIATION AND FUNCTION

Androgens drive a variety of biologic effects throughout an individual's lifetime. There are 3 peaks of testosterone activity: during fetal life, as a neonate, and during puberty (Fig. 3).¹⁶ During the fetal peak, testosterone production is under the control of maternal hCG and fetal LH. Testosterone and DHT during this phase are critical for the formation of male internal and external genitalia structures.²¹ Three to 6 months after birth, an LH surge drives a second peak in testosterone levels. This phase facilitates growth of a normal

phallus, testicular descent, Sertoli cell proliferation and spermatogonial development.^{22–24} However, the low level of expression of AR on Sertoli cells during the fetal and infantile testosterone surges may serve to induce a period of relative androgen insensitivity and preserve Sertoli cell proliferation to allow for pubertal spermatogenic development later in life.²⁵ During adolescence, hypothalamic GnRH stimulates a third peak in testosterone, which results in the development of secondary sexual characteristics of the male and other changes in body, behavioral and sexual function. These changes include growth of the phallus and scrotum, male hair growth pattern, bone growth and mineralization and closure of epiphyseal plate, increase in skeletal muscle mass, erythropoiesis, increase in very low density lipoprotein (VLDL) and low density lipoprotein (LDL) and decrease in high density lipoprotein (HDL), spermatogenesis, and acquisition of fertility potential and spontaneous erections. In addition, testosterone mediates brain function changes including libido, motivation, aggressiveness, and cognitive function.²⁶ Through adult life, testosterone levels remain elevated to maintain many of these changes; however, with aging, a gradual decline in testosterone may contribute to alterations in muscle mass, bone mineral density, cardiovascular risk, libido, cognition, fertility, and erections.^{27,28}

Testosterone levels demonstrate a predictable circadian rhythm that is dependent on sleep–wake cycles. Plasma levels begin to increase

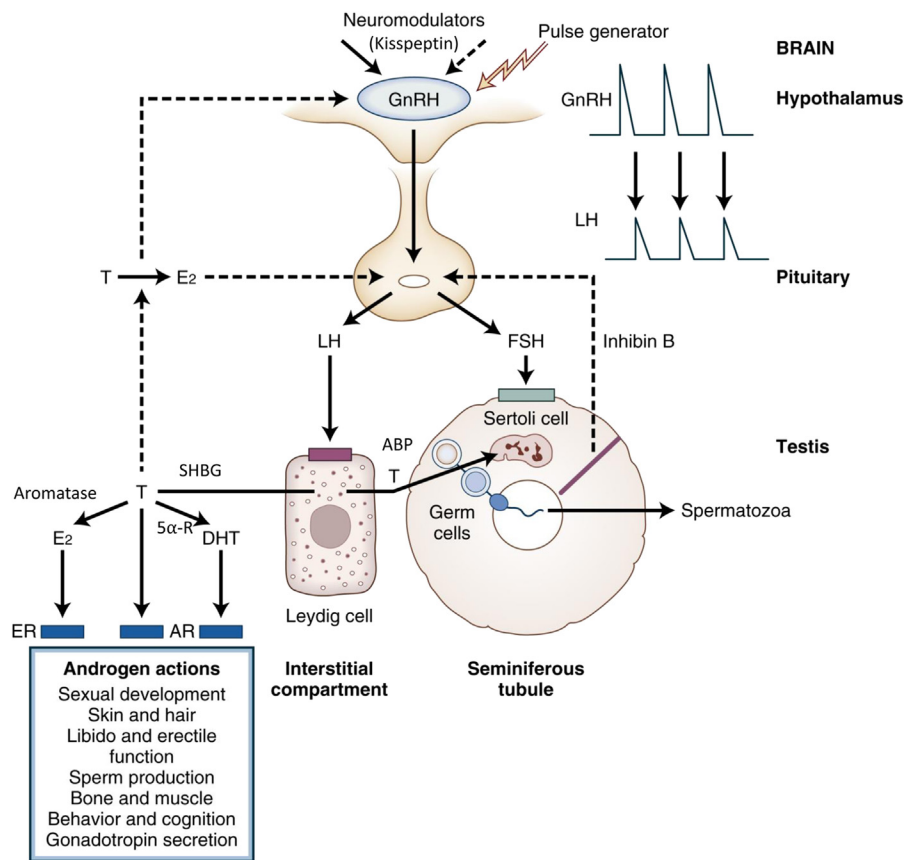


Fig. 2. Hypothalamic-pituitary-gonadal axis. Hypothalamic GnRH release into the hypophyseal portal system is driven by central neurotransmitter release in a pulsatile manner. The anterior pituitary is stimulated to produce LH and follicle-stimulating hormone. LH primarily acts on the Leydig cells in the testis to produce testosterone. Testosterone may then act on the Sertoli cells via androgen-binding protein within the seminiferous tubule to facilitate sperm production and maturation or enter the circulation bound to SHBG to affect distant target tissues. Peripherally, testosterone may be converted to DHT by 5α-reductase or estradiol by aromatase, which exert their own effects on target tissues. Testosterone and estradiol function as negative hypothalamic and pituitary feedback inhibitors. (Adapted from: Matsumoto AM, Anawalt BD. Testicular Disorders. In: Melmed S, ed. Williams Textbook of Endocrinology. 14th ed. Philadelphia: Elsevier; 2020:668-755; with permission.)

under the direction of LH at the onset of sleep and peak during the first 3 hours of sleep at the first rapid eye movement (REM) cycle. Testosterone remains at this level until waking, at which time it gradually declines while awake. There is also a superimposed ultradian rhythm with pulse increases every 90 minute throughout the day that reflects the pulsatile nature of LH release from the pituitary.²⁹ Overall, circadian rhythm disruptions do not seem to affect testosterone secretion and morning testosterone levels unless there is complete disruption of the sleep architecture preventing the initial increase in testosterone during the first REM cycle.^{30,31} There has been substantial interest in assessing circannual variations in serum testosterone levels in men. The data are overall conflicting, with studies showing peak

levels in winter months and nadir levels occurring during warm summer months, whereas other studies describe an opposite seasonal pattern or no variation at all. These differential findings may be attributed to differences in environments, temperatures, day–night patterns, timing of blood draws, and patient-specific factors.³²

TESTOSTERONE REPLACEMENT THERAPY

Testosterone replacement therapy (TRT) is indicated for patients with primary hypogonadism, in which the condition originates in the testes, and secondary/hypogonadotropic hypogonadism, a disease of the hypothalamus or pituitary gland. The primary goal of TRT is to restore serum testosterone levels to within the mid-normal physiologic

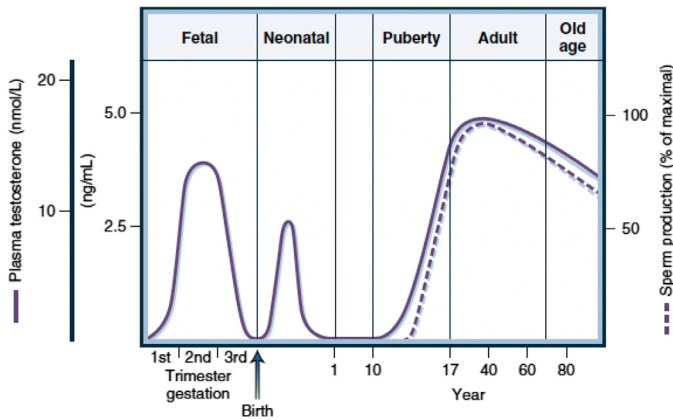


Fig. 3. Testosterone peaks in fetal, infant, and adolescent life. There are 3 peaks of serum testosterone in the lifetime of a man. The first is during fetal development and driven by maternal hCG and later by pituitary LH. The second occurs at 3 to 6 months of life and is responsible for penile growth, testicular descent, and Sertoli cell proliferation and development. Adolescence marks the third peak and gives rise secondary sexual characteristics and homeostatic mechanisms. (From Griffin JE, Wilson JD. The testis. In: Bondy PK, Rosenberg LE, eds. *Metabolic Control and Disease*, 8th ed. Philadelphia, PA: WB Saunders; 1980:1535–1578.)

range associated with the patient's age group, generally considered to be between 400 and 700 ng/dL and to improve symptoms in hypogonadal men. Because an estimated 2.4 million US men aged 40 to 69 years suffer from hypogonadism, the potential market for TRT is significant.³³ TRT efficacy and side effect profile are specific to each formulation, dose, and activity.

Soon after its synthesis in 1935, it became clear that, in reasonable doses, testosterone was not effective orally. We now know that the lack of oral effectiveness is due to the inactivation of testosterone by the first-pass effects in the liver and oral T administration would require extremely high doses. Currently, oral testosterone preparations are not available in the US due to low bioavailability along with gastrointestinal and liver adverse effects.³⁴ Fortunately, there are currently more than 30 different testosterone preparations to consider when choosing one for a patient, with different routes of delivery (topical gel, transdermal patch, buccal system [applied to upper gum or inner cheek], and injection), concentrations, and branded or generic choices.³³ Delivery and dosing information for formulations currently approved by the Food and Drug Administration (FDA) are highlighted in [Table 1](#).

Subdermal testosterone pellets were the first effective formulation for androgen replacement therapy, developed in the 1940s, and pellets are still in use today.³⁵ Testosterone pellets are designed for consistent and prolonged release, and their application requires a small operation. Dosing varies on patient age and diagnosis, and generally vary from 75 to 450 mg implanted subdermally at 3 to 6 month intervals. Potential advantages of pellet usage include the guaranteed compliance, the steady levels of testosterone and lack of transference. Potential disadvantages include the risks of implantation, infection, and extrusion.³⁶

In the 1980s, the first transdermal scrotal patches were found to be efficacious. This application allowed for maximal absorption through a thin-skinned area. Disadvantages included smaller skin surface area and application challenges (hair clipping). These scrotal patches have fallen out of favor and are no longer available.³⁷ Transdermal patches applied to the back, abdomen, upper arms, or thigh are effective and available but their efficacy is often limited by the lack of adherence or discontinuation due to skin blistering, pruritus, or irritation.³⁶

In 2004, the intramuscular testosterone undecanoate preparation entered the market and led to widespread usage of TRT. Importantly, the intramuscular (IM) administration is associated with significant fluctuation in serum testosterone levels: following injection, the testosterone level peaks at supraphysiologic doses at 4 to 5 days and followed by slow decline to levels less than the lower limit of normal. These large fluctuations in serum testosterone levels over the cycle of IM TRT can result in significant mood swings or changes in libido. Although the United States FDA recommended starting dose for male hypogonadism is 50 to 400 mg IM every 2 to 4 weeks, many patients opt for shorter durations between IM TRT administration to minimize the testosterone fluctuation and to overcome the less than ideal kinetics of IM TRT. One additional disadvantage of IM TRT is the necessity for IM injection, which can be painful.³⁸

Since 2002, several testosterone gels and liquids have been developed for transdermal TRT. The potential advantages of testosterone transdermal gels and liquids include ease of application, less skin irritation than patches, and more consistent serum testosterone levels than with IM TRT. However, due to the concern for testosterone gel or liquid being transferred to women and children who come into contact with a patient's skin after use, these

Table 1
Testosterone replacement therapies with Food and Drug Administration approval

Brand Name	Delivery System	Dose Delivery	Starting Dose	Dose Range
Androderm	Transdermal patch	2 or 4 mg patch	4 mg patch	2–6 mg
Androgel 1%	Transdermal gel	50 mg/packet 12.5 mg/pump	50 mg packet 4 pumps	50–100 mg
Androgel 1.62%		40.5 mg/packet 20.25 mg/pump	40.5 mg packet 2 pumps	20.25–81 mg
Testim		50 mg/tube	50 mg tube	50–100 mg
Fortesta		10 mg/pump	4 pumps	10–70 mg
Vogelxo		50 mg/tube 50 mg/packet 12.25 mg/pump	50 mg tube 50 mg packet 4 pumps	50–100 mg
Axiron	Transdermal lotion	30 mg/pump	2 pumps	30–120 mg
Striant	Buccal	30 mg/patch	1 patch Q12H	30 mg
Natesto	Nasal gel	11 mg/pump	1 pump per nostril Q8H	11 mg
Multiple	IM testosterone cypionate	IM injection	100 mg	50–200 mg every 7–14 d
Multiple	IM testosterone enanthate		100 mg	50–200 mg every 7–14 d
Aveed	IM testosterone undecanoate		750 mg at 0, 4, and then every 10 wk	750 mg
Testopel	Subcutaneous pellet	75 mg/pellet	10 pellets	6–12 pellets every 3–4 mo

formulations have received an FDA Boxed Warning. Patients should be reminded to wash their hands after application and to avoid skin contact with others. Recommended sites of application for these agents are areas that will be covered by clothing to minimize transfer.^{36–38}

Additional FDA-approved TRT formulations include buccal administration (side effects gum irritation, inflammation, or gingivitis), nasal gel (side effects headache, rhinorrhea, nosebleed, nasal discomfort, upper respiratory tract infection, sinusitis, bronchitis, and nasal scab).^{36,38}

The decision on the best product choice should include patient preference, treatment burden, cost, and insurance coverage. Products may also need to be switched throughout TRT based on patient response, preference, and adverse effects. In all circumstances, the decisions should be an open dialog between the patient and clinician to allow for the most successful TRT regimen. In addition, testosterone levels in patients should be considered.³³

ANDROGEN DEPRIVATION THERAPY

In 1941, Charles Huggins at the University of Chicago reported that testosterone was a growth factor and the driver of prostate cancer and that prostate cancer was sensitive to surgical castration. This study subsequently led to a Nobel Prize

in Medicine in 1966 for Huggins and established prostate cancer as a hormonally sensitive disease.³⁹ Surgical castration with bilateral orchiectomy rapidly results in castrate levels of testosterone (within 48 hours); however, the adrenal synthesis of testosterone is not affected. Chemical castration has become the mainstay of androgen deprivation therapy (ADT), due to the potential reversibility and avoidance of body dysmorphism with surgical castration.

ADT can be achieved through either GnRH agonists or antagonists. GnRH agonists are associated with an initial LH (and testosterone) surge and rely on a negative feedback loop. Within 4 weeks of initiation of therapy, the testosterone levels become castrate but concerns about the initial testosterone flare and its consequences for patients with metastatic prostate cancer must be considered. To avoid the effects of the flare, GnRH agonists are often preceded by direct AR antagonists, which block the downstream target of the androgens. In contrast, GnRH antagonists block LH and consequently testosterone production resulting in castrate levels of testosterone within 3 days of treatment.^{40–42} For more detailed understanding of ADT, please refer the review by Saad and Fizazi.⁴⁰

Although GnRH agonists and antagonists can stop the testicles from making androgens, cells in other parts of the body, such as the adrenal

glands, and prostate cancer cells themselves, can still make testosterone. Some drugs can block the formation of androgens made by these cells, including inhibitors of steps in cholesterol synthesis, such as cytochrome P450 (CYP-17) inhibitors and nonsteroidal azoles. In addition, competitive antagonists of AR effectively block testosterone binding and activation of its target receptor.⁴⁰

Because androgens have significant roles in multiple physiologic processes, the side effect profile of ADT is significant. Common adverse effects include “hot flashes,” development of gynecomastia, sexual dysfunction, bone loss and skeletal morbidity, anemia, cognitive effects, metabolic alterations (sarcopenia, weight gain, fat deposition, insulin resistance, and increased triglycerides).⁴³

THE ANDROGEN RECEPTOR

In 1989, Tilley, Wilson, and McPhaul cloned the human AR at the University of Texas Southwestern and provided the first evidence that the AR was a transcriptional factor that could regulate its own expression in prostate cancer.⁴⁴ The AR shares homology within the steroid hormone receptors superfamily, including the receptors for progesterone, mineralocorticoids, and glucocorticoids. It is composed of 920 amino acids and includes 4 key domains: the ligand-binding domain (LBD), the DNA-binding domain (DBD), the hinge domain, and an N-terminal transcription regulation domain (NTD) (Fig. 4). The NTD is the most variable among steroid hormone receptors, whereas the DBD is the most highly conserved.^{45,46}

Structurally, the transcriptional prowess of the AR is attributed to the AR NTD. The NTD comprises more than half of the protein (amino acids 1–537) and includes polyglutamine and polyglycine stretches that vary and regulate the activity of the AR. Indeed, the length of the polyglutamine stretch in the AR correlates inversely with AR transcriptional activity: the longer the polyglutamine stretch, the less potent the AR as a transcription factor. In Kennedy disease, the polyglutamine stretch includes more than 36 glutamines resulting in altered function of the AR. In contrast, in normal men the polyglutamine stretch includes 10 to 35 glutamines.⁴⁷ Finally, although the NTD of AR is

60% of the protein, it is intrinsically disordered, and no structural information is available for this domain, which increases the difficulty of potential therapeutic targeting of the AR NTD.⁴⁸

The AR DBD (residues 559–624) is highly conserved and encodes for 2 zinc fingers that enable interaction of AR with specific motifs on the DNA called AR responsive elements (AREs). Genes with AREs in their promoter are classically regulated by AR DNA binding and transcriptional regulation.⁴⁹ Mutations in this domain—which consist mainly of single nucleotide substitutions—cause the DNA-binding/dimerization activity of the protein to be defective, leading to impaired or absent transcriptional activation by the AR and can lead to androgen insensitivity syndromes.⁴⁹

The hinge region (amino acids 625–669) enables androgen-dependent conformational changes in the AR.⁵⁰ Finally, the LBD of AR (residues 670–920) encodes helical motifs that enable androgen binding. The liganded LBD undergoes a conformational change, enabling AR dimerization, cofactor recruitment, and nuclear translocation. Cross talk between the liganded AR LBD and AR-NTD is critical for AR activity.⁵¹ AR LBD mutations explaining the molecular basis of androgen resistance syndromes were first described by Wilson and McPhaul in the early 1990s. AR LBD mutations are commonly seen in complete androgen insensitivity syndromes, where genotypic XY males exhibit various female phenotypic characteristics including female external genitalia, underdeveloped vagina, absence of prostate, epididymis, vas deferentia, seminal vesicles, absence of sexual hair growth, and gynecomastia development.⁵²

The AR is a potent transcription factor. The classic pathway (Fig. 5) describes the process in which androgens exert their effect on protein synthesis, a mechanism that results in physiologic changes over hours to days. When circulating bioavailable testosterone reaches its target tissue, it diffuses through the cellular membrane and binds to the cytoplasmic AR. Chaperone proteins bound to the AR dissociate with this interaction and allow the testosterone–AR complex to translocate to the nucleus, where it binds to the AREs within the regulatory sites of target genes. Recruitment of coregulatory proteins to the promoters of hundreds of AR-regulated genes then results in androgen-directed gene transcription and protein synthesis. In prostate cancer cells, the AR is thought to regulate up to 5% of the transcriptional output of the cell. A secreted serine protease, prostate-specific antigen (PSA), is one of the canonical AR-regulated genes, and its expression level is virtually pathognomonic of active AR



Fig. 4. AR. The AR is composed of a ligand-binding domain (LBD) at the C-terminus, which is separated from the DBD and N-terminal transcription domain by the Hinge region.

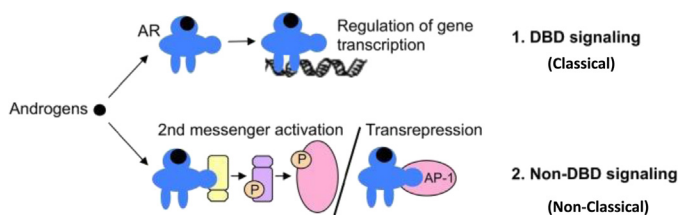


Fig. 5. Classical and nonclassical AR signal transduction. Androgens may affect changes in a target cell via the AR via classical and nonclassical mechanisms. In the classical mechanism, the AR ultimately regulates gene transcription and protein expression, producing an effect over hours to days. The nonclassical mechanism uses a second messenger signal transduction mechanism and produces an effect over seconds to minutes.

(Rana Kesha, Davey Rachel A, Zajac Jeffrey D, Human androgen deficiency: insights gained from androgen receptor knockout mouse models, 2014, 16 (2), 169–177.)

signaling within the prostate. PSA expression also serves as an important biomarker of prostate cancer development and progression. Analyses of AR-mediated regulation of the PSA gene have revealed that ARE-bound AR recruits a plethora of coregulatory proteins, which play important roles in the initiation of transcription, as well as the fine-tuning of overall transcriptional output.^{53,54} Large-scale gene expression profiling studies have indicated that approximately 1.5% to 5% of genes expressed in prostate cancer cells are directly or indirectly regulated by androgens. Similar to PSA, these AR-regulated genes have a profound impact on diverse cellular processes.^{53,55}

Changes in expression levels (through AR amplification), point mutations, splice variants, and polymorphisms in the AR and alterations of coregulators or chaperone proteins may enable the AR to be active even without ligand binding and become independent of testosterone levels. Thus, AR signaling is active even when testosterone levels are castrate, leading to the development of castration-resistance when patients are being treated with ADT for advanced prostate cancer.^{56–58}

A nonclassical pathway exists in which androgens produce a rapid effect on target tissues in seconds to minutes. These mechanisms involve both cell surface receptors and the activation of coregulators that lead to the activation of conventional signal transduction cascades using calcium, mitogen-activated protein kinase (MAPK), tyrosine kinases, and cyclic adenosine monophosphate (AMP). This nongenomic pathway can be found in brain, muscle, cardiovascular, prostate, immune, and Sertoli cells.^{59,60} There is considerable debate about the relative contribution of the nongenomic and genomic pathways to differentiation and growth responses.

FDA-approved agents targeting the AR target the AR LBD. These include bicalutamide, enzalutamide, darolutamide, and apalutamide (Table 2). These agents bind to AR in the cytoplasm,

compete for testosterone binding to AR, and inhibit AR nuclear translocation, DNA binding, and transcriptional programming. Darolutamide is unique in that it seems to inhibit wild type and clinically relevant AR mutations that cause other anti-androgens to switch from antagonist to agonist. It also has lower penetration of the blood–brain barrier, resulting in reduced rates of CNS side effects.^{61,62} Cells that are addicted to AR such as prostate cancer cells circumvent these agents by several methods, including AR gene amplification, AR LBD point mutations (that convert the AR antagonists to agonists), AR splice variants, and altered cofactor recruitment. It remains to be seen if agents that cause AR degradation can effectively overcome the cancer cell addiction to the AR.^{63–65}

METABOLISM OF TESTOSTERONE (AROMATASE, 5 α -REDUCTASE)

Circulating testosterone may exert its effects directly on target tissues and can also be converted to different steroid hormones. Aromatase adenosine monophosphate (CYP19) is an enzyme located especially in adipose tissue that converts testosterone to estradiol, and approximately 15% to 25% of circulating estradiol is produced in the testes by Leydig cells.⁶⁶ Estradiol promotes bone mineralization and epiphyseal plate closure, improves lipoprotein profiles and insulin sensitivity, and provides negative hypothalamic and pituitary feedback.^{67,68}

DHT is formed from testosterone by the enzyme 5 α -reductase (5AR), primarily within the skin and liver, and is 2.5 to 3.0 times more potent than testosterone. In prostate tissue, DHT is the primary ligand for the AR. There are 3 isoforms of 5AR. Type 1 is expressed most highly during puberty in the nongenital skin, contributing to sebum production. Type 2 is located in the genitourinary tract and other locations and is responsible for the development of the prostate and male external genitalia as well as the many changes that occur

Table 2 Agents targeting the androgen receptor				
Agent	Brand Name	Class	Mechanism of Action	Side Effects
Biclutamide	Casodex	First-generation nonsteroidal antiandrogen	Competitive AR inhibition	Gynecomastia, breast pain, hot flushes, diarrhea
Enzlutamide	Xtandi	Second-generation nonsteroidal antiandrogen	Bind AR with high affinity	Fatigue, hypertension, hot flushes, falls, dizziness, nausea, risk of seizures
Apalutamide	Erleada		Inhibit AR nuclear translocation	
			Inhibit AR DNA binding	
Darolutamide	Nubeqa		Inhibit coactivator recruitment	Fatigue, hypertension, rash, diarrhea, nausea, falls, hot flush, peripheral edema
				Fatigue, nausea, extremity pain, rash, cardiovascular events

during the testosterone peak of puberty.⁶⁹ Overexpression of isoform 2 may be associated with the progression of benign prostatic hypertrophy (BPH) and lower urinary tract symptoms, whereas overexpression of isoform 1 within the prostate may be associated with prostatic adenocarcinoma.⁷⁰ Type 3 has been shown to be overexpressed in hormone-resistant prostate cancer cells.⁷¹ Conversion of testosterone to DHT within the prostate results in intraprostatic levels of DHT that are 10 times higher than that of plasma, highlighting the autocrine and paracrine function of DHT within the prostate.^{72,73} The key discovery was made at the University of Texas Southwestern in the 1960s by Bruchovsky and Wilson who discovered 5AR and its importance in converting testosterone to 5 α -DHT as the primary hormone associated with prostatic growth.⁷⁴ Wilson went on to show that androgens are involved in every aspect of prostate development, growth, and function. Wenderoth and Wilson then showed that a 5AR inhibitor blocked the prostatic growth. These findings have been translated to widespread use of 5AR inhibitors in patients with BPH.⁷⁵

SUMMARY

Testosterone and its steroid metabolite hormones play an exceedingly important role in the development of male sexual characteristics and function and in the homeostasis of numerous organ systems throughout the life cycle. Although the endocrine products of the testis created curiosity and excitement among early physiologists and physicians as a wondrous curative agent for everything from loss of strength, cognition and vitality to

bowel and bladder habits, little was known regarding its production, mechanism of action and metabolism at that time. Present-day investigators have established the HPG axis as a tightly controlled feedback mechanism designed to strictly control homeostasis and have made great strides in characterization of the AR and its implications in normal physiologic as well as pathologic processes.

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CLINICS CARE POINTS

- Testosterone is a critical hormone in the development and maintenance of male characteristics and health.
- Testosterone levels predictably follow a normal lifetime and circadian pattern that should be considered during evaluation and management of hypogonadism.
- There are many options and delivery modalities for testosterone replacement therapy, each with their own profile and strengths and weaknesses.

- Androgen-deprivation therapy is a mainstay in the management of advanced prostate cancer. Clinicians treating patients in this setting should be familiar with the indications and side effects of each agent, particularly as the number of options and their applications expand.

DISCLOSURE

Nothing to disclose.

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