Abbreviations

- AAWD: antiandrogen withdrawal
- ACTH: Adrenocorticotropic hormone
- ADT: androgen deprivation therapy
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- AF: activation function
- AKT: serine/threonine-protein kinase
- APCs: antigen presenting cells
- AR: androgen receptor
- ATAD2: ATPase family, AAA domain containing 2
- ATP: adenosine triphosphate
- AUC: area under the concentration-time curve
- BAG: BCL2-associated athanogene
- bcl-2: B-cell lymphoma 2
- BID: twice-daily
- BMD: bone mineral density
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- C max: maximum concentration
- CI: confidence interval
- CRPC: castration-resistant prostate cancer
- CTC: circulating tumor cells
- CYP17A1: Cytochrome17A1
- DBD: DNA–binding domain
- DCs: dendrite cells
- DHEA : dehydroepiandrosterone
- DHEAS: dehydroepiandrosterone sulfate
- DHT: dihydrotesterone
- DNA : deoxyribonucleic acid
- ECOG: *Eastern Cooperative Oncology Group*
- EGFR: epidermal growth factor receptor
- ERG: v-ets avian erythroblastosis virus E26 oncogene homolog
- EUA: European Association of Urology
- FDA: Food and Drug Administration

- FlnA: Filamin-A (A cytoskeletal protein)
- GM-CSF: granulocyte-macrophage colony-stimulating factor
- GnRH: gonadotropin releasing hormone
- GTP: Guanosine triphosphate
- HSD3B1:3beta-hydroxysteroid dehydrogenase
- IL: interleukin
- kDa: kilodalton
- KDM1A: lysine (K)-specific demethylase 1A
- LBD: ligand-binding domain
- LH: luteinizing hormone
- LH: luteinizing hormone
- LHRH: Luteinizing-hormone-releasing hormone
- mCRPC: metastatic castration-resistant prostate cancer
- MDR: multidrug resistance
- NCOA1: nuclear receptor coactivator 1
- NLS: nuclear localization signal
- NTD: N-terminal domain
- NTx: N-telopeptide
- OPG: Osteoprotegerin
- PAP: prostatic acid phosphate
- PC: prostate cancer
- PFS: progression-free survival
- PI3-K: phosphatidylinositol 3-kinase
- PSMA: prostate-specific membrane antigen
- PTEN: phosphatase and tensin homolog
- PTH: Parathyroid hormone
- QD: 4 times daily
- RANK: *Receptor* Activator of Nuclear Factor κ B
- RANKL: RANK ligand
- RB: Relative benefit
- RECIST: Response Evaluation Criteria in Solid Tumors
- SEER: Surveillance, Epidemiology, and End Results
- SHBG: sex hormone-binding globulin
- Src: Schmidt-Ruppin A-2

- T max: time to maximum drug concentration
- TAU: transcriptional activation units
- TMPRSS2: transmembrane protease, serine 2
- TNF: tumor necrosis factor
- TTPP: time to PSA progression
- TURP: transurethral resections of prostate

ANDROGEN

An androgen, or male sex hormone, is defined as a substance capable of developing and maintaining masculine sexual characteristics (including the genital tract, secondary sexual characteristics, and fertility). Testosterone is the principal androgen in the circulation of mature male mammals. It has a characteristic four ring steroid structure and is synthesized and secreted mainly by Leydig cells, located in the interstitium of the testis between the seminiferous tubules. The classical biological effects of androgens are primarily mediated by binding to the androgen receptor which leads to a characteristic pattern of gene expression by regulating the transcription of an array of androgen responsive genes. This physiological definition of an androgen in the whole animal is now complemented by a biochemical and pharmacological definition that an androgen is a chemical that effectively competes with testosterone binding to the androgen receptor to stimulate post-receptor functions in isolated cells or cell-free systems. In addition, non-genomic mechanisms of androgen action involving rapid, membrane-mediated non-transcriptional processes in the cytoplasm have been described but not yet fully characterized **(Michels , Hoppe , 2008).**

Biosynthesis

Testosterone is synthesized by an enzymatic sequence of steps from cholesterol within the 500 million Leydig cells located in the interstitial compartment of the testis between the seminiferous tubules, which constitutes approximately 5% of mature testis volume. The cholesterol is predominantly formed by de novo synthesis from acetate, although preformed cholesterol either from intracellular cholesterol ester stores or extracellular supply from circulating low density lipoproteins also contributes. Testosterone biosynthesis involves two

multifunctional cytochrome P-450 complexes involving hydroxylations and side-chain splitting. The highly tissue-selective regulation of the 17, 20 lyase activity (active in gonads but inactive in adrenals) independently of 17-hydroxylase activity (active in all steroidogenic tissues) when both activities reside in a single, multifunctional protein remains to be fully explained. In addition, some extragonadal biosynthesis of testosterone and dihydrotestosterone from circulating weak adrenal androgen precursor dehydroepiandrosterone (DHEA) within specific tissues has been described **(Labrie , 2004)**. Although the net contribution of adrenal androgens to circulating testosterone is small.

Testicular testosterone secretion is principally governed by luteinizing hormone (LH), through its regulation of the rate-limiting conversion of cholesterol to pregnenolone within Leydig cell mitochondria, this happened by the cytochrome P-450 cholesterol side-chain cleavage enzyme complex located on the inner mitochondrial membrane. Cholesterol supply to mitochondrial steroidogenic enzymes is governed by proteins including sterol carrier protein. This facilitates cytoplasmic transfer of cholesterol to mitochondria as well as steroidogenic acute regulatory protein and peripheral benzodiazepine receptor which govern cholesterol transport across the mitochondrial membrane **(Miller, 2007)**. All subsequent enzymatic steps are located in the Leydig cell endoplasmic reticulum. The high testicular production rate of testosterone creates both high local concentrations (up to 1¼g/g tissue, ~100 times higher than blood concentrations) and rapid turnover (200 times per day) of intra-testicular Testosterone **(Jaro , Zirkin, 2005)**

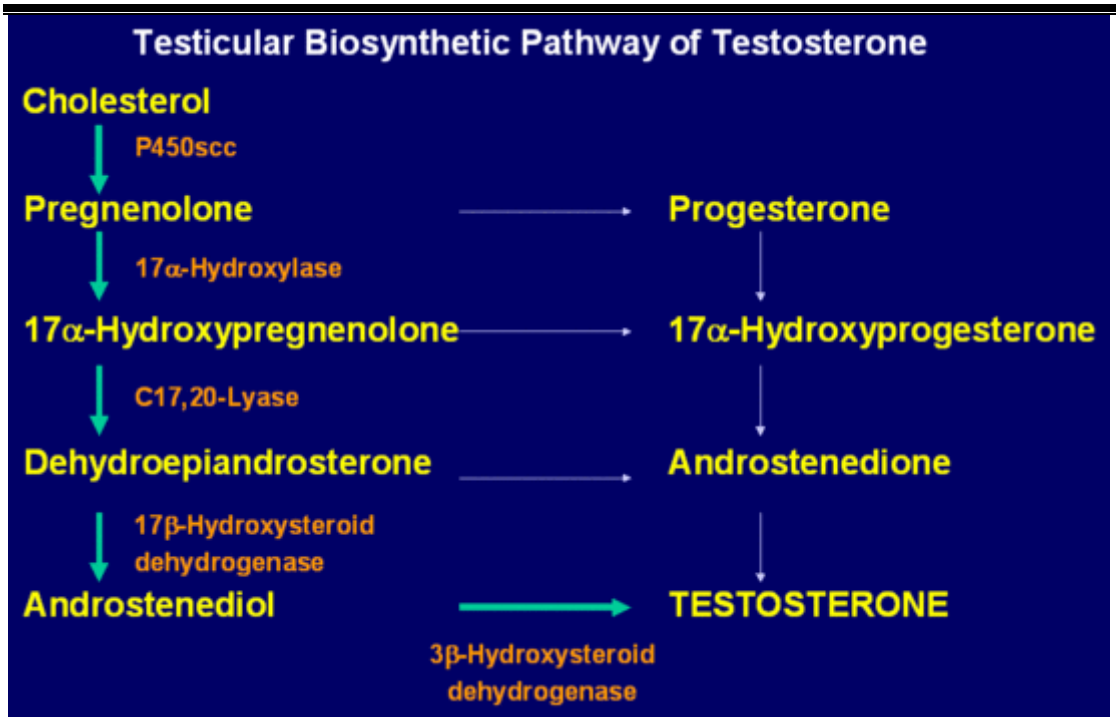

Fig. (1): Biosynthetic pathway of testicular Testosterone synthesis ***(Albert et al., 2009)***

Secretion

Testosterone is secreted during three epochs of male life: transiently during the first trimester of intrauterine life (coinciding with masculine genital tract differentiation), during early neonatal life as the perinatal androgen surge (with still undefined physiologic significance), and continually after puberty to maintain virilization. The dramatic somatic changes of male puberty are the consequence of striking increases in testicular secretion of testosterone rising ~30 fold over levels which prevail prior to puberty and in women or castrate men originating from extra-testicular sources. After middle age, there are gradual decreases in circulating testosterone as well as increases in gonadotrophin and sex hormone-binding globulin (SHBG) levels **(Travison et al., 2008)** with these trends being exaggerated by the coexistence of chronic illness. These age-related

changes, including the effects of concomitant accumulation of chronic disease states, are functionally attributable to impaired hypothalamic regulation of testicular function, as well as Leydig cell attrition and dysfunction and atherosclerosis of testicular vessels. As a result, the ageing hypothalamic-pituitary-testicular axis progressively increasingly operates with multi-level functional defects that lead to reduced circulating testosterone levels during male ageing.

Testosterone, like other lipophilic steroids secreted from steroidogenic tissues, leaves the testis by diffusing down a concentration gradient across cell membranes into the bloodstream, with smaller amounts appearing in the lymphatics and tubule fluid. After puberty, over 95% of circulating testosterone is derived from testicular secretion with the remainder arising from extragonadal conversion of precursors with low intrinsic androgenic potency such as dehydroepiandrosterone and androstenedione. These weak androgens, predominantly originating from the adrenal cortex, constitute a large circulating reservoir of precursors for conversion to bioactive sex steroids in extragonadal tissues including the liver, kidney, muscle, and adipose tissue.

Hormone production rates can be calculated from either estimating metabolic clearance rate (from bolus injection or steady-state isotope infusion using high specific-activity tracers) or mean circulating testosterone levels **(Gurpide, 1975)** or by estimation of testicular arteriovenous differences and testicular blood flow rate**.**

Transport

Testosterone circulates in blood at concentrations greater than its aqueous solubility by binding to circulating plasma proteins. The most important is SHBG (Sex hormone binding globulin), a high affinity but low capacity binding protein, and other low affinity binding proteins include albumin,

corticosteroid binding globulin and α1 acid glycoprotein. Testosterone binds avidly to circulating SHBG, a homodimer of two glycoprotein subunits each comprising 373 amino acids, containing a single high-affinity steroid binding site. The affinity of SHBG for binding testosterone does not change in liver disease. SHBG is secreted into the circulation by human liver and by placenta where it may contribute to the rise in blood SHBG during pregnancy. Circulating SHBG levels are particularly influenced by first-pass effects on the liver of oral drugs including sex steroids. Circulating SHBG (and thereby total testosterone) concentrations are characteristically decreased (androgens, glucocorticoids) or increased (estrogens, thyroxine) by supraphysiologic hormone concentrations at the liver such as produced by oral administration or by high-dose parenteral injections of androgens. In contrast, endogenous sex steroids and parenteral administration, which maintain physiologic hormone concentrations (transdermal, depot implants), have minimal effects on blood SHBG levels. Other modifiers of circulating SHBG levels include up-regulation by acute or chronic liver disease and androgen deficiency and down-regulation by obesity, protein-losing states, and genetic SHBG deficiency **(Kahn et al., 2002).**

Under physiologic conditions, 60% to 70% of circulating testosterone is SHBG bound with the remainder bound to lower affinity, high-capacity binding sites (albumin, α1 acid glycoprotein, corticosteroid binding protein) and 1% to 2% remaining non-protein bound.

Transfer of hydrophobic steroids into tissues is presumed to occur passively according to physicochemical partitioning between the hydrophobic protein binding sites on circulating binding proteins, the hydrophilic aqueous extracellular fluid and the lipophilic cellular plasma membranes. According to the free hormone hypothesis, the free (non-protein bound) fraction of testosterone is the most biologically active with the loosely protein-bound testosterone

constituting a less accessible but mobilizer fraction, with the largest part tightly bound to SHBG constituting only an inactive reservoir.

Metabolism

After testicular secretion, a small proportion of testosterone undergoes activation to two bioactive metabolites, estradiol and DHT, whereas the bulk of secreted testosterone undergoes inactivation by hepatic phase I and II metabolism to inactive oxidized and conjugated metabolites for urinary and/or biliary excretion **(Van Eenoo et al, 2006).**

The amplification pathway converts about 4% of circulating testosterone to the more potent, pure androgen, DHT. DHT has higher binding affinity 3-10 time greater molar potency in transactivation of the androgen receptor relative to testosterone, which is converted to DHT by the 5α-reductase enzyme that originates from two distinct genes (I and II) **(Russell et al., 1994)**. Type 1 5α-reductase is expressed in the liver, kidney, skin, and brain, whereas type 2 5α-reductase is characteristically expressed strongly in the prostate but also at lower levels in the skin (hair follicles) and liver. DHT circulates at ~10% of blood testosterone concentrations, due to spillover from the prostate and non-prostatic sources. An important issue is whether eliminating intra-prostatic androgen amplification by inhibition of 5α-reductase can prevent prostate disease. A major 10year chemoprevention study randomizing nearly 19,000 men over 55 years of age without known prostate disease to daily treatment with an oral 5α-reductase inhibitor, finasteride, or placebo observed a cumulative 25% reduction after 7 years of treatment in early stage, organ-confined, low-grade prostate cancer **(Thompson et al., 2003)**.

Although the study was not designed to determine survival benefit, there was an apparent shift toward higher grade, but still organ-confined, cancers, possibly a medication effect on prostate cancer cell structure **(Lucia et al., 2007).**

A further placebo-controlled study of 8000 men over 50 years of age at high risk of prostate cancer using a dual (type 1 & 2) 5α reductase inhibitor, dutasteride, underway will clarify these findings that highlight the importance of androgen amplification within the prostate in the origin of cancer during the long latent premalignant phase (**Gomella LG .,2005)**.

Testosterone is metabolized to inactive metabolites in the liver, kidney, gut, muscle, and adipose tissue. Inactivation is predominantly by hepatic oxidases (phase I metabolism), notably cytochrome P450 3A family followed by hepatic conjugation to glucuronides (phase II metabolism).

Regulation

During sexual differentiation in early intrauterine life, the testosterone required for masculine sexual differentiation is secreted by fetal Leydig cells. The regulation of this fetal Leydig cell testosterone secretion appears to differ between species.

Higher primate placenta secrete a chorionic gonadotropin during early fetal life **(Bousfield, et al., 1996)**, that may drive fetal human Leydig cell steroidogenesis at the relevant time

After birth, testicular testosterone output is primarily regulated by the pulsatile pattern of pituitary LH secretion. This is driven by the episodic secretion of GnRH from hypothalamic neurons into the pituitary portal bloodstream providing a direct short circuit route to pituitary gonadotropes. Under this regular but intermittent GnRH stimulation, pituitary gonadotrophs secrete LH in high amplitude pulses at ~60-90 min intervals with minimal intervening LH secretion between pulses with the net effect that circulating LH levels are pulsatile . This pulsatile pattern of trophic hormone exposure maintains Leydig cell sensitivity to LH to maintain mature male patterns of testicular testosterone secretion

Puberty is initiated by a still mysterious suprahypothalamic process involving a developmental clock and multiple permissive processes **(Veldhuis., 2003)** that lift the central neuroendocrine restraint on the final common pathway that drives reproductive function in the mature male, episodic secretion of gonadotropin-releasing hormone (GnRH) from hypothalamic neurons. Hypothalamic GnRH neurons are functional at birth but, after the perinatal androgen surge, remain suppressed during infantile life. Puberty is initiated by a maturation process that awakens the dormant hypothalamic GnRH neurons to mature patterns of pulsatile GnRH secretion which in turn entrains pulsatile LH secretion from pituitary gonadotropes. Initially this resurgence of pulsatile GnRH and LH secretion occurs mainly during sleep but eventually extends throughout the day with a persisting underlying diurnal rhythm. The timing and tempo of male puberty is under tight genetic control, encompassing nutrition influences on body weight and composition **(Silventoinen et al., 2008)**, with a correspondingly growing number of genetic causes of delayed puberty identified **(Pedersen-White et al., 2008).**

LH stimulates Leydig cell steroidogenesis via increasing substrate (cholesterol) availability and activating rate-limiting steroidogenic enzyme and cholesterol transport proteins

Additional fine tuning of Leydig cell testosterone secretion is provided by paracrine factors originating within the testis. These include cytokines, inhibin, activin, follistatin, prostaglandins E2 and F2α, insulin-like and other growth factors. LH also influences testicular vascular physiology by stimulating Leydig cell secretion of vasoactive and vascular growth factors.

Testosterone is a key element in the negative testicular feedback cycle through its inhibition of hypothalamic GnRH and, consequently, pituitary

gonadotropin secretion. Such negative feedback involves both testosterone effects via androgen receptors as well as aromatization to estradiol within the hypothalamus **(Pitteloud, et al, 2008).**

These culminate in reduction of GnRH pulse frequency in the hypothalamus together with reductions in amplitude of LH pulses. By contrast, the small proportion of estradiol in the bloodstream that is directly secreted from the testes (~20%) means that cirulating estradiol is under minimal physiological regulation and unlikely to be a major influence on negative feedback regulation of physiological gonadotropin secretion in men.

ANDROGEN RECEPTOR

Structure

The human androgen receptor (AR) gene is a nuclear transcription factor and a member of the steroid hormone receptor superfamily of genes. It is located on the X chromosome (q11-12) and consists of 8 exons. It codes for a protein of 919 amino acids with a mass of 110 kDa. The AR consists of four structurally and functionally distinct domains:

1. a poorly conserved N-terminal domain (NTD),
2. a highly conserved deoxyribonucleic acid (DNA) –binding domain (DBD)
3. And a moderately conserved ligand-binding domain (LBD).
4. A short amino acid sequence called the 'hinge region' separates the LBD from the DBD and also contains part of a bipartite ligand-dependent nuclear localization signal (NLS) for AR nuclear transport **(Claessens et al., 2008)**.

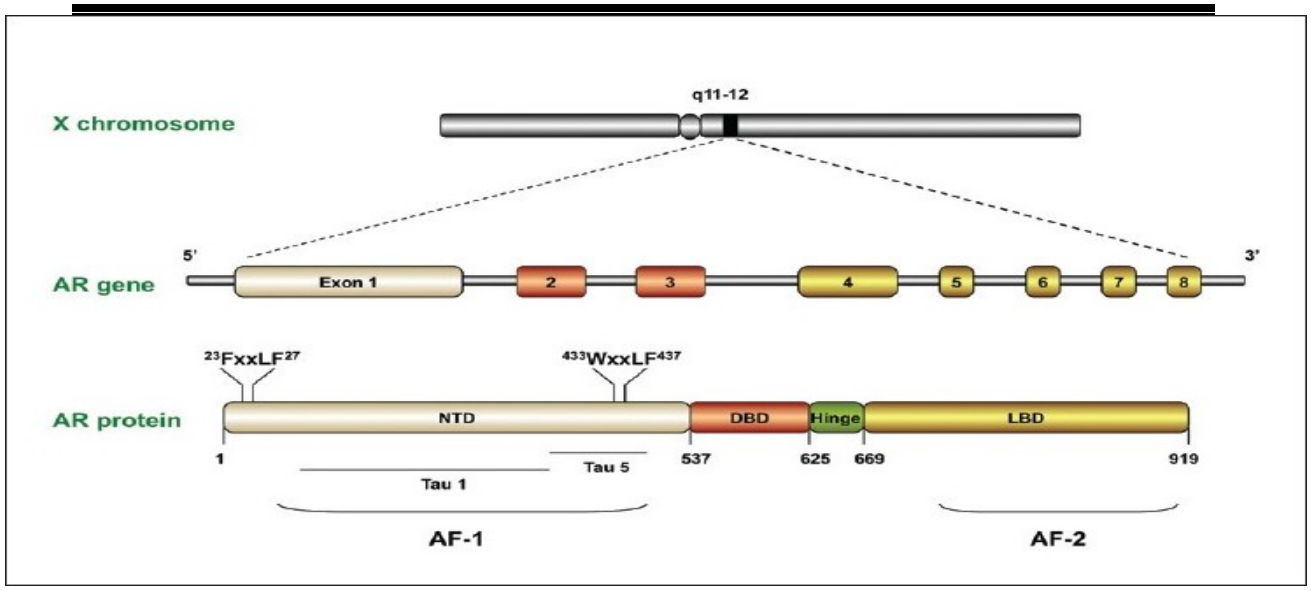

Fig. (2): *Schematic representation of the androgen receptor gene and protein, with indications of its specific motifs and domains* **(Peter et al., 2011)**

- **The N-terminal domain**

The NTD (amino acids 1-537 coded by exon 1) is considered to be mainly active, and can activate transcription independently of androgenic stimulus in LBD-deletion mutants **(Simental et al, .1991).**

The NTD also harbors transcriptional activation function (AF)-1, which encompasses two transcriptional activation units (TAU): TAU-1 and TAU-5. TAU5 is responsible for the majority of constitutive transcriptional activity within the NTD, and is mediated through the core sequence 433 WHTLF 437, accounting for approximately 50% aberrant AR activity and mutation in CRPC cells.