ANDROGEN PHYSIOLOGY, PHARMACOLOGY AND ABUSE

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ABSTRACT

Testosterone, together with its bioactive metabolites dihydrotestosterone and estradiol, determines the development and maintenance of male sexual differentiation and the characteristic mature masculine features. Defects in androgen action at various epochs of life produce characteristic clinical features. From an outline of the biochemistry and physiology of androgen action, the pathophysiology of defects in androgen action are derived and defined. The pharmacology of testosterone and its applications to replacement therapy for pathological hypogonadism as well as for pharmacological androgen therapy based on using either testosterone or synthetic androgens is described. For complete coverage of all related areas of Endocrinology, please see our FREE on line web-book, www.endotext.org.

INTRODUCTION

An androgen, or male sex hormone, is defined as a substance capable of developing and maintaining masculine characteristics in reproductive tissues (notably the genital tract, secondary sexual characteristics, and fertility) and contributing to the anabolic status of somatic tissues. Testosterone together with its potent metabolite, dihydrotestosterone (DHT), are the principal androgens in the circulation of mature male mammals. Testosterone has a characteristic four ring C18 steroid structure and is synthesized mainly by Leydig cells, located in the interstitium of the testis between the seminiferous tubules. Leydig cell secretion creates a very high local concentration of testosterone in the testis as well as a steep downhill concentration gradient into the bloodstream maintaining circulating testosterone levels which exert characteristic androgenic effects on distant androgen sensitive target tissues. The classical biological effects of androgens are primarily mediated by binding to the androgen receptor, a member of the steroid nuclear receptor superfamily encoded by a single gene located on the X chromosome, which then leads to a characteristic patterns of gene expression by regulating the transcription of an array of androgen responsive target genes. This physiological definition of an androgen in the whole animal is now complemented by a biochemical and pharmacological definition of an androgen as a chemical that effectively competes with testosterone binding to the androgen receptor (1) to stimulate post-receptor functions in isolated cells or cell-free systems. In addition, non-genomic mechanisms of androgen action involving rapid, membrane-mediated

nontranscriptional processes in the cytoplasm have been described but not yet fully characterized (2-4).

Testosterone is used clinically at physiologic doses for androgen replacement therapy and, at typically higher doses, testosterone or synthetic androgens based on its structure are also used for pharmacologic androgen therapy. The principal goal of androgen replacement therapy is to restore a physiologic pattern of androgen exposure to all tissues. Such treatment is usually restricted to the major natural androgen, testosterone, and aims to replicate physiological circulating testosterone levels and the full spectrum (including pre-receptor androgen activation) of endogenous androgen effects on tissues and recapitulating the natural history of efficacy and safety. Pharmacologic androgen therapy exploits the anabolic or other effects of androgens on muscle, bone, and other tissues as hormonal drugs that aim to modify the natural history of the underlying disorder and are judged on their efficacy, safety, and relative cost effectiveness like other therapeutic agents. Insight into the physiology of testosterone is a prequisite for understanding and making most effective use of androgen pharmacology (5-6).

TESTOSTERONE PHYSIOLOGY
Androgen biosynthesis

Testosterone is synthesized by an enzymatic sequence of steps from cholesterol (7-8) (Figure 1) 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 (see Endotext, Endocrinology of Male Reproduction, Chapter 1, Endocrinology of the Male Reproductive System and Spermatogenesis, for details) (9). 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 (8). Testosterone biosynthesis involves two multifunctional cytochrome P-450 complexes involving hydroxylations and side-chain scissions (cholesterol side-chain cleavage [CYP11A1 or P450scc which produces C20 and C22 hydroxylation and C20,22 lyase activity] and 17-hydroxylase/17,20 lyase [CYP17A1 or P450c17 which hydroxylates the C17 and then excises two carbons (20 & 21) coverting a 21 to a 19 carbon structure]) together with 3 and 17β-hydroxysteroid dehydrogenases and $\Delta^{4,5}$ isomerase (8). 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) is a key branch-point in steroidogenic pathways. Both activities reside in a single, multifunctional protein with the directionality of pathway flux determined by enzyme co-factors, notably electron supply from NADPH via the P450 oxidoreductase (POR), a membrane-bound flavoprotein serving diverse roles as a reductase, and cytochrome b_5 (10-11). In addition, some extragonadal biosynthesis of testosterone and dihydrotestosterone from circulating weak adrenal androgen precursor DHEA within specific tissues has been described (12); however, the net contribution of adrenal androgens to circulating testosterone in men is minor (13-14) though it makes a much larger proportional contribution to circulating testosterone in women (15-16).
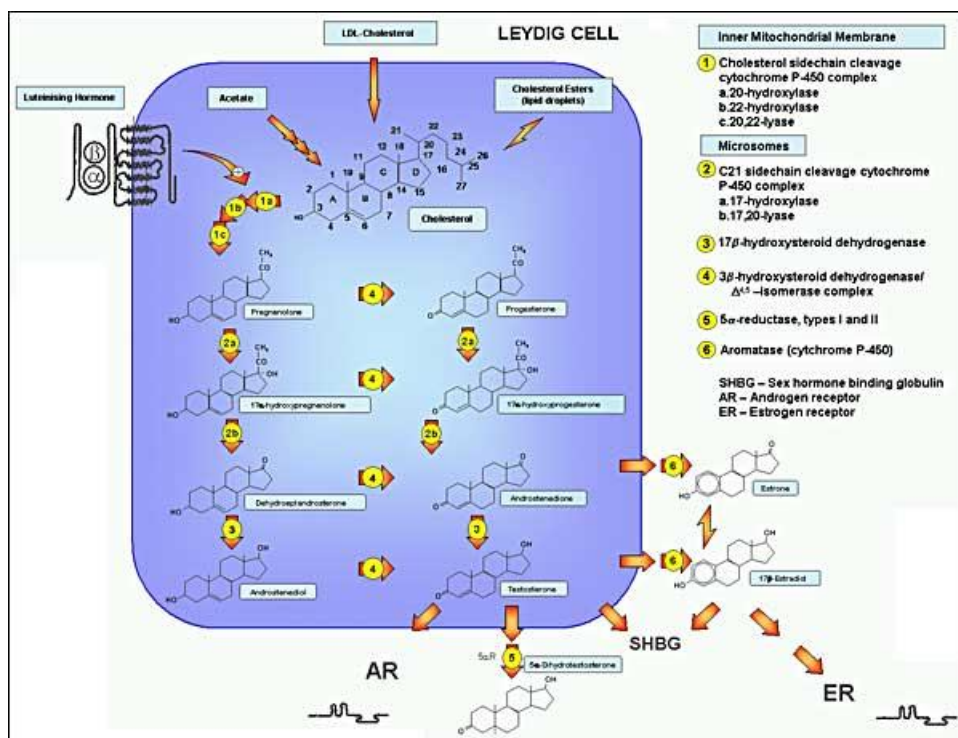
FIGURE 1. Pathways of testosterone biosynthesis and action. *In men, testosterone biosynthesis occurs almost exclusively in mature Leydig cells by the enzymatic sequences illustrated. Cholesterol originates predominantly by the de novo synthesis pathway from acetyl-CoA with luteinizing hormone regulating the rate-limiting step, the conversion of cholesterol to pregnenolone within mitochondria, while remaining enzymatic steps occur in smooth endoplasmic reticulum. The Δ^5 and Δ^4 steroidal pathways are on the left and right, respectively. Testosterone and its androgenic metabolite, dihydrotestosterone, exert biological effects directly through binding to the androgen receptor and indirectly through aromatization of testosterone to estradiol, which allows action via binding to the estrogen receptor (ER). The androgen and ERs are members of the steroid nuclear receptor superfamily with highly homologous structure differing mostly in the C-terminal ligand binding domain. The LH receptor has the structure of a G-protein linked receptor with its characteristic seven transmembrane spanning helical regions and a large extracellular domain which binds the LH molecule. LH is a dimeric glycoprotein hormone consisting of an α subunit common to other pituitary glycoprotein hormones and a β subunit specific to LH. Most sex steroids bind to sex hormone binding globulin (SHBG) which binds tightly and carries the majority of testosterone in the bloodstream.*

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 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 2 (17). This facilitates cytoplasmic transfer of cholesterol to mitochondria together with steroidogenic acute regulatory protein (18) and translocator protein (19), which govern cholesterol transport across the mitochondrial membrane. 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 1 μg/g tissue, ~100 times higher than blood concentrations) and rapid turnover (200 times per day) of intratesticular testosterone (20); however, the precise physical state in which such high concentrations of intratesticular testosterone and related steroids exist in the testis remains to be clarified.

Androgen Secretion

Testosterone is secreted at adult levels 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 triggered by the striking increases in testicular secretion of testosterone, rising ~30-fold over levels which prevail in pre-pubertal children 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 (21-22) with these trends being absent till late old age among men who remain in excellent health (23-24) but exaggerated by the coexistence of chronic illness (22, 25-27) as well as temporal trends including increasing prevalence of obesity (28-30) and immunoassay-derived artefacts from serial substitution of assay reagents (31-32). These age-related changes from the accumulation of chronic disease states are functionally attributable to impaired hypothalamic regulation of testicular function (33-36), as well as Leydig cell attrition (9) and dysfunction (37-39) and atherosclerosis of testicular vessels (40). As a result, the ageing hypothalamic-pituitary-testicular axis progressively increasingly operates with multi-level functional defects that, in concert, lead to reduced circulating testosterone levels during male ageing (41-42).

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 male puberty, over 95% of circulating testosterone is derived from testicular secretion with the remainder arising from extragonadal conversion of precursors with negligible 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. Unlike in women where adrenal androgens are the major source of biologically active androgen precursors, endogenous adrenal androgens contribute negligibly to direct virilization of men (13) and residual circulating and tissue androgens after medical or surgical castration have minimal biologic effect on androgen-sensitive prostate cancer (43). Conversely, however, adrenal androgens make a proportionately larger contribution to the much lower circulating testosterone concentrations in children and women (~5% of men) in whom blood testosterone is derived approximately equally from direct gonadal secretion and indirectly from peripheral interconversion of adrenal androgen precursors (15-16). Exogenous dehydroepiandrosterone at physiologic replacement doses of 50 mg/day orally (15) is incapable of providing adequate blood testosterone for androgen replacement in men but produces dose-dependent increases in circulating estradiol in men (44-45) and hyperandrogenism in women (14).

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) and mean circulating testosterone levels (46-47) or by estimation of testicular arteriovenous differences and testicular blood flow rate (48). These methods give consistent estimates of a testosterone production rate of 3 to 10 mg/day using tritiated (49-50) or nonradioactive deuterated (51) tracers with interconversion rates of approximately 4% to dihydrotestosterone (DHT) (50, 52) and 0.2% to estradiol (53) under the assumption of steady-state conditions (hours to days). These steady-state methods are a simplification that neglects diurnal rhythm (54-55), episodic fluctuation in circulating testosterone levels over shorter periods (minutes to hours) entrained by pulsatile LH secretion (56) and postural influence on hepatic blood flow (49). The major known determinants of testosterone metabolic clearance rate are circulating SHBG concentration (57), diurnal rhythmn (51) and postural effects on hepatic blood flow (49, 51). Major genetic influences on circulating testosterone levels mediated via changes in SHBG (58-61) and other mechanisms (50) have been described as well as environmental (28-29, 51) factors.

Transport

Testosterone circulates in blood at concentrations greater than its aqueous solubility by binding to circulating plasma proteins. The most important is SHBG, a high affinity but low capacity binding protein, and other low affinity binding proteins include albumin, corticosteroid binding globulin (62) and α_1 acid glycoprotein (63). Testosterone binds avidly to circulating SHBG, a homodimer of two glycoprotein subunits each comprising 373 amino acids with 3 glycosylation sites, 2 N-linked and 1 O-linked and containing a single high-affinity steroid binding site (64). The affinity of SHBG for binding testosterone is subject to genetic polymorphisms (65) but is not altered by acquired liver disease (66). It remains unknown as to whether it is influenced by other chronic diseases or pregnancy (when circulating levels increase). SHBG is secreted into the circulation by human, but not rodent, liver as well as into the seminiferous tubules of the testis by rodent, but not human, Sertoli cells where it is known as testicular androgen-binding protein (67), and by the placenta where it may contribute to the rise in blood SHBG during pregnancy (68). As a product of hepatic secretion, 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 (first pass effects) or by high-dose parenteral injections of androgens. In contrast, endogenous sex steroids and parenteral (non-oral) administration, which maintain predominantly physiologic circulating hormone concentrations (transdermal, depot implants), have minimal effects on blood SHBG levels (69-70) (71). 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 (64) and, rarely, genetic SHBG deficiency(72-74). Under physiologic conditions, 60% to 70% of circulating testosterone is SHBG bound with the remainder bound to lower affinity, high-capacity binding sites (albumin, α_1 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 (75-77), the free (non-protein bound) fraction of testosterone is the most biologically active with the loosely protein-bound testosterone constituting a less accessible but mobilizable fraction, with the largest moiety tightly bound to SHBG constituting only an inactive reservoir. The free hormone hypothesis derived from now outdated 1970's pharmacological theory on the mechanism of drug-drug interactions as due to mutual protein binding displacement; however, this theory is superseded by well-established physiological mechanisms such as cytochrome P450 enzyme induction, drug transporter activity and cognate mechanisms unrelated to binding to circulating proteins (78). As the free and/or bioavailable fractions would also have enhanced access to sites of testosterone inactivation by degradative metabolism that terminates androgen action, the free fractions may equally be considered the most evanescent and least active.

Testosterone Measurement
Measuring blood testosterone concentration is an important part of the clinical evaluation of androgen status and for confirming a clinical and pathological diagnosis of androgen deficiency. The circulating testosterone concentration is a surrogate measure for whole body testosterone production rate and the inferred impact of androgens on tissues. However, the reliance on a spot measurement of blood testosterone concentration neglects changes in the whole body metabolic clearance rate as well as other factors influencing net androgen effects at tissue levels. These include the efficiency of blood testosterone transfer into adjacent tissues during capillary transit as well as pre-receptor, receptor and post-receptor factors influencing the testosterone activation, inactivation and action in that tissue. Circulating testosterone levels are also dynamic and feature distinct circhoral and diurnal rhythms. Circhoral LH pulsatility entrains some pulsatility in blood testosterone levels (36) although the buffering effects of the circulating steroid-binding proteins dampens the pulsatility of blood testosterone concentrations. This is illustrated by comparison with the strikingly pulsatile patterns of circulating testosterone in rodents which lack hepatic SHBG gene expression thereby having no circulating SHBG to buffer testosterone fluctuations (88-89). Diurnal patterns of morning peak testosterone levels and nadir levels in the mid-afternoon are evident in younger and healthy older men (54) but lost in some ageing men (55). Consequently, it is conventional practice to standardize testosterone measurements to morning blood samples on at least 2 different days.

The advent of steroid radioimmunoassay in the 1970's made it feasible to measure blood testosterone concentrations affordably with speed and sensitivity. However, cross-reacting steroids and non-specific matrix effects are limitations on modern direct (non-extraction) testosterone immunoassays relative to the high specificity of mass spectrometry-based methods, the reference method (90). In the next decades, the steep rise in demand for testosterone measurements in clinical practice and research led to method simplications to integrate steroid immunoassays into automated immunoassay platforms. These changes, notably eliminating preparative solvent extraction and chromatography as well as introducing bulky non-authentic tracers, undermine the specificity of unextracted testosterone

immunoassays (91), particularly at the low circulating testosterone levels such as in women and children (92). Even at the higher testosterone concentrations in men, commercial testosterone immunoassays demonstrate wide discrepancies due to method-specific bias (32). New generation, bench-top mass spectrometers with higher sensitivity and throughput now overcome these limitations of testosterone immunoassays.

Assays to measure blood "free" testosterone levels directly in serum samples have been developed using tracer reference methods of equilibrium dialysis (93-94) or ultrafiltration (95-96) or calculated various formulae based on immunoassay measurement of total testosterone and SHBG (97-98). Similarly, another derived testosterone measure, bioavailable testosterone, is defined as the non-SHBG bound testosterone (in effect the combination of albumin-bound plus unbound testosterone) and can also be measured directly or calculated by a formula from total testosterone and SHBG and albumin measurements. Some estimates of free testosterone, notably the direct analog assay (99-100) and the free testosterone index (101) are invalid for use in men. As measurement of "free" or "bioavailable" testosterone is laborious, calculational formulae with limited validation (97-98, 102) have been widely used; however, these estimates for "free" (103-105) or "bioavailable"(106-107) testosterone are not accurate in large scale evaluation. Overall, the clinical utility of various derived ("free", "bioavailable") measures of testosterone arising from the unproven free hormone hypothesis remain to be established; consequently, they have minimal involvement in consensus clinical guidelines for diagnosis and management of androgen deficiency.

Testosterone 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 (108).
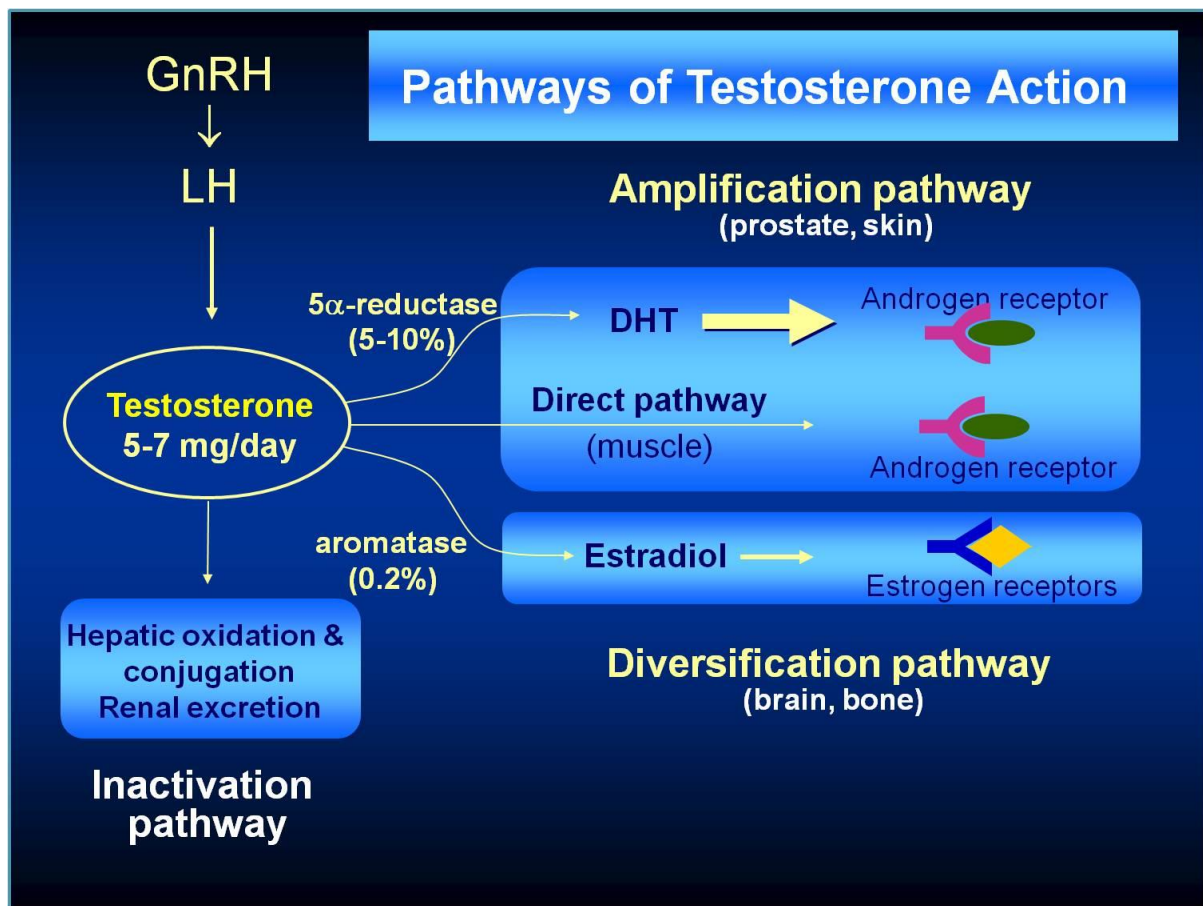
FIGURE 2. Pathways of Testosterone Action. *In men, most (>95%) testosterone is produced under LH stimulation through its specific receptor, a heptahelical G-protein coupled receptor located on the surface membrane of the steroidogenic Leydig cells. The daily production of testosterone (5-7 mg) is disposed along one of four major pathways. The direct pathway of testosterone action is characteristic of skeletal muscle in which testosterone itself binds to and activates the androgen receptor. In such tissues there is little metabolism of testosterone to biologically active metabolites. The amplification pathway is characteristic of the prostate and hair follicle in which testosterone is converted by the type 2 5α reductase enzyme into the more potent androgen, dihydrotestosterone. This pathway produces local tissue-based enhancement of androgen action in specific tissues according to where this pathway is operative. The local amplification mechanism was the basis for the development of prostate-selective inhibitors of androgen action via 5α reductase inhibition, the forerunner being finasteride. The diversification pathway of testosterone action allows testosterone to modulate its biological effects via estrogenic effects that often differ from androgen receptor mediated effects. The diversification pathway, characteristic of bone and brain, involves the conversion of testosterone to estradiol by the enzyme aromatase which then interacts with the ERs α and/or β. Finally the inactivation pathway occurs mainly in the liver with oxidation and conjugation to biologically inactive metabolites that are excreted by the liver into the bile and by the kidney into the urine.*

The amplification pathway converts ~4% of circulating testosterone to the more potent, pure androgen, DHT (50, 52). DHT has higher binding affinity to (109) and 3-10 time greater molar potency in transactivation (110-112) of the androgen receptor relative to testosterone.

Testosterone is converted to the most potent natural androgen DHT by the 5α-reductase enzyme that originates from two distinct genes (I and II) (113). 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 (113). Congenital 5α-reductase deficiency due to mutation of the type 2 enzyme protein (114) leads to a distinctive form of genital ambiguity causing undermasculinization of genetic males, who may be raised as females, but in whom puberty leads to marked virilization including phallic growth, normal testis development and spermatogenesis (115) and bone density (116) as well as, occasionally, masculine gender reorientation (117). Prostate development remains rudimentary (118) and sparse body hair without balding is characteristic (119). This remarkable natural history reflects the dependence of urogenital sinus derivative tissues on strong expression of 5α-reductase as a local androgen amplification mechanism for their full development. This amplification mechanism for androgen action was exploited in developing azasteroid 5α-reductase inhibitors (120). As the type 2 5α-reductase enzyme results in over 95% of testosterone entering the prostate being converted to the more potent androgen DHT (121), blockade of that isoenzyme (the expression of which is largely restricted to the prostate) confines the inhibition of testosterone action to the prostate (and other urogenital sinus tissue derivatives) without blocking extra-prostatic androgen action. DHT circulates at ~10% of blood testosterone concentrations, due to spillover from the prostate (122-123) and nonprostatic sources (124). Whereas genetic mutations disrupting type 2 5α-reductase produce disorders of urogenital sinus derived tissues in men and mice (125), genetic inactivation of type 1 5α-reductase has no male phenotype in mice and no mutations of the human type 1 enzyme have been reported. Whether this reflects the type I enzyme having an unexpected phenotype or an evolutionarily conserved vital function, remains unclear. A third 5α-reductase enzyme (type 3, SRD5A3) has been described (126) but is widely expressed in human tissues, lacks steroidal 5α-reductase activity and has other roles in fatty acid metabolism (127).

An important issue is whether eliminating intraprostatic androgen amplification by inhibition of 5α-reductase can prevent prostate disease. Two major randomized, placebo-controlled studies of men at high risk of (but without diagnosed) prostate cancer have both shown that oral 5α reductase inhibitors (finasteride, dutasteride) reduced the incidence of low-grade prostate cancer as well as prevalence of lower urinary tract symptoms from benign prostate hyperplasia(128-129). The Prostate Cancer Prevention Trial (PCPT) was a major 10-year chemoprevention study randomizing 18,882 men over 55 years of age without known prostate disease to daily treatment with 5 mg finasteride (inhibitor of type 2 5α reductase) or placebo observed a cumulative 25% reduction after 7 years of treatment in early stage, organ-confined, low-grade prostate cancer. Another study randomized over 8231 men aged 50-75 years with serum PSA <10 ng/mL and negative prostate biopsy to either daily treatment with 0.5 mg dutasteride (inhibitor of both type 1 and 2 5α reductases) or placebo for 4 years observed a 23% reduction in incidence of biopsy-proven prostate cancer. Although neither study was designed to determine mortality benefit, both showed no reduction in higher grade, but still organ-confined, cancers. Although this stage selectivity may be explained by diagnostic biases due to drug effects on prostate size and histology

(130-131), registration for chemoprevention of prostate cancer was refused by FDA (132). Whether or not preventive use of prostatic 5α-reductase inhibition in men with high prostate cancer risk proves warranted, novel synthetic androgens refractory to 5α-reductive amplification may have advantages for clinical development.

The diversification pathway of androgen action involves testosterone being converted by the enzyme aromatase to estradiol (133) to activate estrogen receptors (ERs) (also see Endotext, Endocrinology of Male Reproduction, Chapter 17, Estrogens and Male Reproduction). Although this involves only a small proportion (~0.2%) of testosterone output, the higher molar potency of estradiol (~100-fold higher vs testosterone) makes aromatization a potentially important mechanism to diversify androgen action via ER-mediated effects in tissues where aromatase is expressed. The diversification pathway is governed by the cytochrome P-450 enzyme (CYP19) aromatase (133-134). In eugonadal men, most (~80%) circulating estradiol is derived from extratesticular aromatization (53). The biological importance of aromatization in male physiology was first recognized in the early 1970's (135) when the local conversion of testosterone to estradiol within the neural tissues was identified and subseqeuntly shown to have an important role in mediating testosterone action, including negative feedback as well as activational and organisational effects, on the brain (136). More recently the importance of local aromatization in testosterone action has been reinforced by the striking developmental defects in bone and other tissues of men and mice with genetic inactivation of aromatase, leading to complete estrogen deficiency due to genetic inactivation of the aromatase (137). This phenotype is also strikingly similar to that of a man (138) and mice (138) with genetic mutations inactivating ERα. Furthermore, men with aromatase deficiency treated with exogenous estradiol or other estrogens also demonstrated significant bone maturation. By contrast, genetic inactivation of ERβ has no effect on male mice (139) and no human mutations have been reported. Aromatase expression in tissue such as bone (140) and brain (136) may influence development and function by variation in aromatization that modulates local tissue-specific androgen action. By contrast other tissues, like mature liver and muscle, express little or no aromatase. Nevertheless, despite the importance of aromatization for male bone physiology, other observations indicate that androgens acting via androgen receptors have important additional direct effects on bone. These include the greater mass of bone in men despite very low circulating estradiol concentrations compared with young women (141), the failure of androgen insensitive rats lacking functional androgen receptors but normal estradiol and ERs to maintain bone mass of normal males (142) and the ability of nonaromatizable androgens to increase bone mass in estrogen-deficient women (143-144). Testosterone action on bone and in the brain cannot be accounted for solely as a prohormone for local estradiol production (and action via estrogen receptors α and/or β) and androgen receptor mediated effects are required to manifest the full spectrum of testosterone effects on bone (145-146) and in the brain (147). Conflicting evidence is available about the need for aromatisation to mediate the effects of testosterone on male sexual function. One study using aromatase inhibitor-induced estrogen deficiency showed partial dependence (148) whereas another using DHT-induced estrogen deficiency showing no requirement of male sexual function for aromatization (149). Further studies are needed to fully understand the significance of aromatization in maintaining androgen action in mature male animals (150).

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 P-450 3A family (151) leading ultimately to oxidation of most oxygen moieties followed by hepatic conjugation to glucuronides (phase II metabolism), which are rendered sufficiently hydrophilic for renal excretion. Uridine diphospho (UDP) glucuronosyl transferase (UGT) enzymes UGT2B7, UGT2B15 and UGT2B17 catalyze most phase II metabolism (glucuronidation) of testosterone with 2B17 being quantitatively the most important (152). A functional polymorphism of UGT 2B17, a deletion mutation several times more frequent in Asian than European populations (153), explains the concordant population difference in testosterone to epitestosterone (T/E) ratio (153). A World AntiDoping Agency-approved urine screening test for testosterone doping in sport, constitutes an ethnic differential, false negative in surveillance for exogenous testosterone doping (154).

The metabolic clearance rate of testosterone (as fraction of the pool/day) is reduced by increases in circulating SHBG levels (57) or decreases in hepatic blood flow (e.g. posture) (49) or liver function. Theoretically, drugs that influence hepatic oxidase activity could alter metabolic inactivation of testosterone, but empirical examples of sufficient magnitude to influence clinical practice are rare. Rapid hepatic metabolic inactivation of testosterone leads to both low oral bioavailability (155-156) and short duration of action when injected parenterally (157). To achieve sustained androgen replacement, these limitations dictate the need for to deliver testosterone via parenteral depot products (e.g., injectable testosterone esters, testosterone implants, transdermal testosterone) or oral delivery systems that either bypass hepatic portal absorption (buccal (158-159), sublingual (158, 160), gut lymphatic (161)) or use synthetic androgens with substituents rendering them resistant to first pass hepatic inactivation (162).

Testosterone 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 and equine placenta secrete a chorionic gonadotropin during early fetal life (163) that may drive fetal human Leydig cell steroidogenesis (164) at the relevant time. By contrast, in subprimate mammals male sexual differentiation occurs without expression of any placental gonadotropin and prior to the time when pituitary gonadotropin secretion starts so that fetal Leydig cell testosterone secretion may be autonomous of gonadotropin stimulation during fetal development of most mammalian species (165).

Puberty is initiated by a still mysterious suprahypothalamic process involving a developmental clock and multiple permissive processes (166-167) that lifts the central neuroendocrine restraint on the final common pathway that drives reproductive function in the mature male – the episodic secretion of gonadotropin-releasing hormone (GnRH) from hypothalamic neurons (56). Various explanatory theories including the gonadostat, somatometer (168), neurally-driven changes in GABAergic inhibition and glutaminergic stimulation (169), triggering by kisspeptin-1 secretion and activating its receptor GPR54 (170-171) and epigenetic factors (172) are proposed to explain the restraint and resurgence of the hypothalamic GnRH pulse generator without a comprehensive picture having yet

emerged (167). Hypothalamic GnRH neurons are functional at birth but, after the perinatal androgen surge, remain tonically suppressed during infantile life. Puberty is initiated by a maturation process that awakens the dormant hypothalamic GnRH neurons to unleash mature cirhoral 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 (173) but eventually extends throughout the day with a persisting are associated with underlying diurnal rhythmn. The timing and tempo of male puberty is under tight genetic control, encompassing nutrition influences on body weight and composition (174), with a correspondingly growing number of genetic causes of delayed puberty identified (175). Environmental factors that optimize growth (eg high socio-economic status with better nutrition and health care) may explain secular trends to earlier puberty with increased statural growth (176-177) whereas claims that exposure to hormonally active chemical pollution contributes to earlier puberty (178) remain speculative (179) and not supported by available evidence in boys (180-181). Very large UK population-based studies, using voice breaking as a self-reported marker of male puberty, show that both early and late male puberty are associated with a wide-range of adverse health outcomes (182-183).

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 distinctly pulsatile. This pulsatile pattern of trophic hormone exposure maintains Leydig cell sensitivity to LH to maintain mature male patterns of testicular testosterone secretion (184).

LH stimulates Leydig cell steroidogenesis via increasing substrate (cholesterol) availability and activating rate-limiting steroidogenic enzyme and cholesterol transport proteins (8, 18). LH is a dimeric glycoprotein consisting of an α subunit common to the other glycoprotein hormones (human chorionic gonadotropin (hCG), follicle-stimulating hormone, and thyrotropin-stimulating hormone) and a β subunit providing distinctive biologic specificity for each dimeric glycoprotein hormone by dictating its specific binding to the LH/hCG rather than the folliclle-stimulating hormone or thyroid stimulating hormone receptors (185-186). These cell surface receptors are highly homologous members of the heptahelical, G protein–linked family of membrane receptors. LH receptors are located on Leydig cell surface membranes and use signal transduction mechanisms involving primarily cyclic adenosine monophosphate as well as calcium as second messengers to cause protein kinase–-dependent protein phosphorylation and DNA transcription, ultimately resulting in testosterone secretion (187). Functionally, hCG is a natural, long-acting analogue of LH because they both bind to the same LH/CG receptor and their β subunits are nearly identical except that hCG has a C-terminal extension of 31 amino acids containing four O-linked, sialic acid–capped carbohydrate side chains. These glycosylation differences confer greater resistance to degradation, which prolongs circulating residence time and biologic activity compared with LH (188-189), a feature that has been exploited to engineer longer acting analog of other circulating hormones such as FSH (190), TSH (191) and erythropoietin (192).

Additional fine tuning of Leydig cell testosterone secretion is provided by paracrine factors originating within the testis (193). These include cytokines, inhibin, activin, follistatin, prostaglandins E_2 and $F_{2\alpha}$, insulin-like and other growth factors as well as still uncharacterized factors secreted by Sertoli cells. LH also influences testicular vascular physiology by stimulating Leydig cell secretion of vasoactive and vascular growth factors (194).

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 (195-196). These culminate in reduction of GnRH pulse frequency in the hypothalamus together with reductions in amplitude of LH pulses due to both reduced GnRH quantal secretion as well as gonadotropin response to GnRH stimulation(184). 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 Action

Androgen action involves pre-receptor, receptor and post-receptor mechanisms that are centred on the binding of testosterone (or an analog) to the androgen receptor. Testosterone undergoes pre-receptor activation by conversion to potent bioactive metabolites, DHT and estradiol. The steroidogenic enzyme 5α-reductase has two isozymes, types 1 and 2, which form a local androgen amplification mechanism converting testosterone to the most potent natural androgen, DHT (197). The two isozymes have different chromosomal location and distinct biochemical features but are homologous genes (113). They are structurally and functionally unrelated to a third 5α-reductase (SRD5A3) which may have physiological role in fatty acid rather than steroidal biochemistry (126-127). This local androgen amplification mechanism is exemplified in urogenital sinus derived tissues, notably external and internal genitalia and the prostate, which characteristically express high levels of 5α-reductase type 2 (113). Other tissues such as nongenital skin and liver express 5α-reductase type 1. The other form of pre-receptor androgen activation is conversion of testosterone to estradiol by the enzyme aromatase (198) which diversifies androgen action by facilitating effects mediated via ERs. Consequently, while DHT may be considered a pure androgen because its bioactivity is solely mediated via AR, testosterone has a wider spectrum of action which includes diversification by aromatisation and ER mediated effects. These pre-receptor mechanisms provide testosterone with a versatile and subtle range of regulatory mechanisms prior to receptor mediated effects, depending on the balance between direct AR mediated vs indirect actiational and/or ER mediated mechanisms. In addition, tissues vary in their androgenic thresholds and dose-response characteristics to testosterone and its bioactive metabolites.

ANDROGEN RECEPTOR
The androgen receptor is required for masculine sexual differentiation and sexual maturation that ultimately leads to development of a mature testis capable of supporting

spermatogenesis and testosterone production that form the basis for male fertility. The human androgen receptor is specified by a single X chromosome encoded gene located at Xq11-12 that specifies a protein of 919 amino acids (1), a classical member of the large nuclear receptor superfamily (199) which includes receptors for the 5 mammalian steroid classes (androgen, estrogen, progesterone, glucocorticoid, mineralocorticoid) as well as for thyroid hormones, retinoic acid and vitamin D and numerous orphan receptors where the ligand was originally not identified (200). Androgen receptor expression is not confined to reproductive tissues and it is ubiquitously expressed, athough levels of expression and androgen sensitivity of non-reproductive tissues vary.

The androgen receptor gene has 8 exons specifying a protein of 919 amino acids with the characteristic structure of mammalian steroid receptors. It has an N-terminal domain (NTD) that specifies a long transactivating functional domain (exon 1), a middle region specifying a DNA-binding domain (DBD) consisting of two zinc fingers (exons 2 and 3) separated by a hinge region from the C-terminal ligand binding domain (LBD) which specifies the steroid binding pocket (exons 4 to 8).

The NTD (exon 1) is relatively long comprising over half (535/919) the overall length of the AR. It has the least conserved sequence compared with other steroid receptors with a flexible and mobile tertiary structure harbouring a transactivation domain (AF-1) that interacts with AR co-regulator proteins and target genes (201). Its loose, naturally disordered structure (202) also contains three homopolymeric repeat sequences (glutamine, glycine, proline) with the most important being the CAG triplet (glutamine) repeat polymorphism (203). The less variable glycine (usually 24 residues) and proline (9 residues) repeat polymorphisms have little apparent independent pathophysiological significance although linkage dysequilibrium between the glutamine and glycine repeat polymorphisms requires haplotype analysis for interpretation (203). Among healthy people, where the glutamine repeat polymorphism has alleles of lengths between 5 and 35 (population mean ~21), the length of the glutamine repeat is inversely proportional to AR transcriptional efficiency so that this polymorphism dictates genetic differences between individuals in the androgen sensitivity of their target tissues (204). This genetic variation in in vivo tissue androgen sensitivity is proven experimentally (205) but, although modest in magnitude, influences physiological responses to endogenous testosterone in prostate size (206) and erythropoeisis (207) in carefully controlled studies. Wider epidemiological implications of population variation in the genetic androgen sensitivity as specified by the polyglutamine repeat have been studied in a variety of potentially androgen sensitive disorders (reviewed in (203) including reproductive health disorders and hormone dependent cancers in men and women as well as non-gonadal disorders where there are significant gender disparities in prevalence. In men these include prostate (208) and other male preponderant cancers (liver, gastrointestinal, head & neck), prostate hypertrophy, cryptorchidism and hypospadias, male infertility (209) whereas in women they include reproductive health disorders (polycystic ovary syndrome, premature ovarian failure, endometriosis, uterine leiomyoma, preeclampsia) and hormone dependent cancers (breast, ovary, uterus). In addition, studies have also examined risks of obesity and cardiovascular disease, mental and behavioural disorders including dementia, psychosis, migraine, and personality disorders (203). However, as in many large-scale genetic association studies (210), the findings remain

mostly inconsistent reflecting methdological limitations notably in recruitment, participation and publication bias as well as multiple hypothesis testing all of which tend to inflate spurious associations.

Remarkably, the pathological elongation of the polygutamine (CAG triplet) repeat to lengths of over 37 cause a neurodegenerative disease, Spinal Bulbar Muscular Atrophy (SBMA, also known as Kennedy's syndrome), a form of late-onset, slow progressing but ultimately fatal motor neuron disease (211), one of several late-onset neurodegenerative polyglutamine repeat disorders (212). Although the extreme length of the polyglutamine repeat does determine mild androgen resistance, these men usually have normal reproductive function including fertility and virilization prior to diagnosis in mid-life (213). Furthermore, since complete androgen receptor inactivation in humans and other mammals does not cause motor neuron disease and female carriers are protected from symptomatic neurodegeneration, SBMA like other genetic polyglutamine repeat neurodegenerative diseases (214) represents a toxic gain-of-function involving pathological protein aggregates of the mutant AR (215). Transgenic mouse models of SBMA suggest that testosterone deprivation by medical castration using a GnRH agonist may slow progression of neuropathy (215) and that genetic (216) or pharmacological (217) administration of IGF-I may slow disease progression. However the first major clinical trial of leuprolide, a GnRH analog, failed to demonstrate neuromuscular benefit in swallowing (218) and further studies of selected subgroups and therapeutic targets are warranted (219-220).

The DBD (exons 2 and 3) consists of ~70 amino acids with a high proportion of basic amino acids including eight cysteines distributed as two sets of 4 cysteines, each forming a zinc cooordination center for a single zinc atom, thereby creating two zinc fingers. The DBD is highly conserved between steroid receptors reflecting its tightly defined function of forming the two zinc fingers that bind to DNA by intercalating between its grooves. The first zinc finger (exon 2) is directly involved with the major DNA groove of the androgen response element through a proximal (P-box) region whereas the second zinc finger (exon 3) is also responsible for enhancing receptor dimerization through its distal (D-box) region.

The hinge region (first half of exon 4) of ~40 amino acids between the DBD and LBD is considered a flexible linker region but may have additional functions involving interactions with DNA (nuclear localization, androgen response element) and protein (AR dimerization, co-regulators) which influence AR transcriptional activity.

The LBD (mid-exon 4 to 8) of AR comprises ~250 amino acids which specify a steroid binding pocket which creates the characteristic high affinity, stable and selective binding of testosterone, DHT and synthetic androgens. While the LBD's overall architecture is broadly conserved among nuclear receptors, the AR sequence diverges significantly to ensure the specificity of binding from other steroid classes and their different cognate ligands. Structural studies of the AR's LBD shows it has similar tertiary conformation as other steroid receptors (most closely resembling PR) with 12 stretches of α helix interspersed with short β pleated sheets. The most C terminal helix 12 seals the binding pocket and influences whether a bound ligand acts as an agonist or antagonist as well as forming a hydrophobic surface for binding of co-regulator proteins that modify transcriptional activity of the androgen target

genes. The LBD also participates in receptor dimerization, nuclear localization and transactivation via its activation function (AF-2) domain.

The AR has a predominantly nuclear location in androgen target cells regardless of whether bound to its ligand or not, unlike other steroid receptors which are more often evenly distributed between cytoplasm and nucleus when not bound to their cognate ligands. Androgen binding to the C-terminal LBD causes a conformational change in the androgen receptor protein and dimerization to facilitate binding of the ligand-loaded receptor to segments of DNA featuring a characteristic palindromic motif known as an androgen-response element, located in the promoter regions of androgen target genes. Ligand binding leads to shedding of heat shock proteins 70 and 90 that act as a molecular chaperone for the unliganded androgen receptor (221). Specific binding of the dimerized, ligand-bound androgen receptor complex to tandem androgen-response elements initiates gene transcription so that the androgen receptor acts as a ligand-activated transcription factor. Androgen receptor transcriptional activation is governed by a large number of coregulators (222-223) whose tissue distribution and modulation of androgen action remain incompletely understood.

ANDROGEN INSENSITIVITY

Mutations in the androgen receptor are relatively common with over 1000 different mutations recorded by 2012 (224) in the McGill database (http://androgendb.mcgill.ca/) making androgen insensitivity the most frequent form of genetic hormone resistance. As the androgen receptor is an X chromosomal gene, functionally significant AR mutations are effectively expressed in all affected males because they are hemizygous. By contrast, women bearing these mutations (including the obligate heterzygote mothers of affected males) are silent carriers without any overt phenotype because they have a balancing allele as well as their circulating testosterone levels never rise to post-pubertal male levels sufficient to activate AR mediated effects.

Germline AR mutations produce a very wide spectrum of effects from functionally silent polymorphisms to androgen insensitivity syndromes that display phenotypes proportionate to the impairment of AR function and, thereby, the degree of deficit in androgen action (1). These clinical manifestations extend from a complete androgen insensitivity syndrome (CAIS, formerly known as testicular feminization) which produces a well developed female external phenotype in a spectrum spanning across all grades of undervirilized male phenotype to, at the other extreme, a virtually normal male phenotype. The severity of androgen insensitivity can be categorized most simply as complete, partial and mild although a more detailed 7 stage Quigley classification based on degree of hypospadias, phallic development, labioscrotal fusion and public/axillary hair is also described (1, 203). The degree of urogenital sinus derivative development together with testis descent provide clinical clues to the degree of androgen sensitivity. In addition, somatic androgen receptor mutations, notably generated during androgen deprivation treatment of prostate cancer (225), result in generation and expression of mutations and splice variants of the androgen receptor in a form of accelerated molecular evolution which may result in resistance to androgen effects and/or efficacy of androgen deprivation treatment (226).

CAIS due to completely inactivating AR mutations results in a 46XY individual with a hormonally active testis that secretes abundant testosterone but which cannot activate AR-mediated action so no male internal or external genitalia or somatic features develop. However, testosterone aromatization to estradiol is unimpeded, leading to the development of normal female somatic features including breast and external genital development after puberty. The population prevalence of CAIS is estimated to be at least 1:20,000 male births or 1-2% among female infants with inguinal hernia (1). The typical presentation of CAIS is a relatively tall, normally developed girl with delayed puberty and/or primary amenorrhea. The clinical features usually include well developed breasts, hips and female fat pattern deposition, acne-free facial complexion with minimal axillary and pubic hair with testes located within an inguinal hernia or in the abdominal cavity. The uterus and fallopian tubes are absent and the vagina is short and blind ending reflecting unimpeded effects of testicular AMH secretion causing regression of Mullerian structures including the upper third of the vagina. Earlier diagnosis is increasingly possible where a prenatal 46XY karyotype is discrepant from a female phenotype on ultrasound or at birth or among female infants presenting with inguinal hernia (227). The family history may be informative with infertile maternal (but not paternal) aunts consistent with an X-linked inheritance. Laboratory investigations of post-pubertal individuals show elevated blood LH, SHBG (at adult female levels) and testosterone (at adult male levels) prior to gonadectomy. The androgen sensitivity index, the product of LH and testosterone concentrations, is elevated (228). These features reflect high amplitude and frequency LH pulses due to the absence of effective negative androgenic feedback on the hypothalamus as well as the increased LH drive to maintain high-normal male levels of testicular testosterone secretion. In untreated individuals, failure to suppress blood SHBG with short-term, high dose androgen administration may be useful confirmation of androgen resistance (229-230). After gonadectomy, blood LH and FSH increase to castrate levels but are partially suppressed by estradiol replacement therapy.

Long-term management includes (a) reinforcing female gender identity with counseling to cope with eventual infertility and acceptance of the genetic diagnosis, (b) post-pubertal gonadectomy to prevent the risk of gonadoblastoma (especially if the gonad is impalpable) but allowing the completion of puberty balanced against the low risks of tumour at that age and of unwanted virilization due to any residual AR function or mosaicism (231), and (c) post-gonadectomy estrogen replacement therapy to maintain bone density, breast development and quality of life. Long-term bone density is often subnormal for age due not only to the deficit in androgen action but also inadequate post-gonadectomy estrogen replacement, often resulting from suboptimal adherence to medication (116, 232-234). Although the long-term outcomes for AR mutations based on large prospective studies of a consistent management approach remain very limited, the clinical outcomes for individuals with CAIS reared as females are reported as successful (235-236) although some gender role and psychosexual functional outcomes remain suboptimal (237-239).

Partial androgen insensitivity syndrome (PAIS) is characterized by a full range of external genital virilization and breast development from female to male phenotype, reflecting the functional severity of the AR mutation. A simple clinical guide to the severity of the deficit in AR function is provided by the level of testis descent and phallic development. PAIS was

originally recognised under a variety of eponymously named syndromes (Reifenstein, Gilbert-Dreyfus, Lubs, Rosewater) and only more recently clearly distinguished from other developmental disorders of 46XY individuals with incomplete virilisation especially those due to steroidogenic enzyme defects. Severe forms of PAIS with minimal AR function produce a predominantly female phenotype with clitoromegaly whereas PAIS with mutations displaying more functional AR are characterized by a male phenotype with various grades of labioscrotal formation (varying from minimal posterior partial labial fusion to labioscrotal fusion and bifid, ruggose scrotum) and hypospadias (urinary orofice ranging from perineal aperture to hypospadia with meatus at locations along penile shaft to the corona), micropenis and gynecomastia, each in inverse proportion to the AR function. These features have been combined into a External Masculinization Score (EMS) ranging from 0 (female) to 12 (male) based on degree of scrotal fusion, phallic development, location of urethral meatus and testis descent each scored 0-3 (240). The biochemical finding in PAIS are similar to those of CAIS but with a wide spectrum of severity from mildly virilized, predominantly female to an undervirilized male phenotype. The increase in blood LH and testosterone are less severe and consistent but the androgen sensitivity index (228) may help confirm the diagnosis of androgen resistance. Unlike CAIS, which usually presents during adolescence with failure of puberty, PAIS usually presents at birth with ambiguous genitalia requiring a crucial and decisive clinical judgement on sex of rearing to be made rapidly. The expert pediatric endocrinologist must balance the need for early genital surgery and vicarious decision-making against the risk of possible subsequent regret by the affected individual as an adult. This makes for inevitably complex, difficult and contentious choices as the available systematic prospective evidence from long-term follow-up of sex or rearing is still limited. Most intersex individuals due to PAIS, especially those with an EMS of 4 or more (241), are raised as males (240). Genital surgery for hypospadias is often required and usually uncertainty remains about the adequacy of the potential for post-pubertal virilization due to either endogenous or exogenous testosterone. If pubertal progression is inadequate, exogenous testosterone may be useful but higher than usual dosage may be required to get satisfactory effects. Long-term follow-up of PAIS raised as males has shown apparently adequate psychosexual function despite phallic underdevelopment, limited somatic virilization and dissatisfaction with outcomes by some patients as adults (239, 242). For those to be reared as females, the management is similar to that for CAIS and involves early genital surgery and pre-pubertal gonadectomy to prevent unwanted virilization.

Mild androgen insensitivity (MAIS) is the most minor form of androgen insensitivity dsplaying near-normal male phenotype with only subtle changes in hair patterns relative to family norms (less body and facial hair, absence of temporal recession or balding) and/or minor defects restricted to spermatogenesis alone. The blood LH and testosterone concentrations are usually but not always elevated although the androgen sensitivity index, the product of serum LH and testosterone concentrations, is more consistently raised. In common with mutations in many other genes, making a clear distinction between the most minor grades of clinical pathology and a silent, functionally insignificant polymorphism is challenging and depends on reproducing experimentally the functional consequences of the mutation in an authentic biological system. Ideally such verification is performed in vivo (eg in genetically modified mouse models) but, as this is laborious and expensive, it is rarely undertaken. The functional verification of putative mutations is usually undertaken by either *in silico* prediction

of functional effects of structural protein changes from sequence data or *in vitro* studies of cultured cells or cell-free systems aiming to characterize protein functions. Nevertheless, although informative, the biological fidelity of these surrogate endpoints relative to the in vivo effects on androgen action may remain questionable.

All types of mutations have been reported in the AR gene including disruption of the reading frame by deletions, insertions, splice site interruption and frame-shift which usually produce major interference with function as well as the more common single base substitutions with effects ranging from nil to complete functional inactivation. In addition, mutation can produce less common mechanisms of interrupting AR function such as inefficient translation, unstable protein, or aberrant translational start sites all leading to reduced expression of functional AR protein. Mutations occur throughout the AR gene, probably at random; however, those reported are distributed unevenly because the most important functional regions of the gene are sensitive to even minor changes in sequence whereas the more variable regions may tolerate sequence changes without functional consequences. Over 90% of known mutations are single base substitutions which have pathophysiological consequences when they change the amino acid sequence in the functionally critical DBD or LBD regions whereas sequence changes in other regions may not alter AR function thereby constituting silent polymorphisms. For example, despite forming more than half the AR sequence, few functionally important mutations are reported in the NTD (exon1). Those described in exon 1 mostly represent major disruptions of the AR protein due to creation of a premature stop codon, a major deletion or frame shift mutation causing mistranslation onward from exon 1 whereas point mutations are more likely to constitute functionally insignificant (silent) polymorphisms. Mutations in the LBD, comprising ~25% of AR sequence, constitute the majority (~60%) of reported mutations whereas mutations in the DBD, representing ~7% of AR sequence, constitute ~14% of cases (243). The functional effects of these two types of mutations generally differ in that LBD mutations demonstrate various degrees of reduced affinity and/or loosened specificity of ligand binding characteristics whereas DBD mutations demonstrate normal ligand binding but reduced or absent receptor binding to DNA. The profusion of AR mutations has created numerous experiments of Nature with multiple different mutations involving the same amino acid with the physiological consequences depending generally on how conservative is the amino acid substitution. Nevertheless, there are exceptions to such categorization with mutations in regions other than the DBD or LBD sometimes unexpectedly affecting DNA or ligand binding properties presumably through physical interaction effects in the tertiary structure of the AR in its 3 dimensional topography.

The familial occurrence of androgen insensitivity due to X-linked inheritance of mutated AR makes carrier detection and prenatal genetic diagnosis feasible. A carrier female has a 50% chance of having a child bearing the mutant AR allele so they would be either a carrier female or an affected male and 50% of her fertile daughters will also be carriers. A specific mutation detection test needs to be established usually involving PCR-based genotyping for point mutations athough other mutational mechanisms may require more complex genotyping methods. For prenatal genetic diagnosis now usually applied to chorionic villus samples, the genetic diagnosis must be rapid, reliable and efficient. However, accurate genetic counselling relies on the a consistent and predictable phenotype for any specific

genotype. This is usually, but not invariably, true for AR mutations as the clinical manifestations for the same mutation are usually consistent in CAIS with rare exceptions (244) whereas for PAIS the phenotype may vary even within a single family with significant implications for sex of rearing and/or need for genital surgery so that skilled genetic counselling is essential (245). Discrepancies in the fidelity of phenotype within families, or between unrelated individual bearing the identical mutation, is relatively common in PAIS and may be attributable to somatic mosaicism (246) or the effect of modifier genes that influence androgen action such as 5α reductase (247). An exotic, complex DNA breakage repair slippage mechanism has also been described to produce mutiple mutations within a single family (248). Wider population genetic screening for AR mutations is not currently cost-effective because, despite diminishing costs for increasingly facile genetic testing, the large number of different mutations featuring diverse mechanisms and variable phenotype which still mostly predict a normal life expectancy but a diminished quality of life that is difficult to cost or cure (249).

Acquired androgen insensitivity during life can arise either through postnatal somatic or germline AR mutations or by non-genetic, non-receptor mechanisms that hinder androgen action. Among overt cases of androgen insensitivity, ~30% are absent in the mother's germline so must arise as a de novo mutation in the postnatal maternal germline (246) or in the fetal germline soon after fertilization (250). Somatic AR mutations, arising de novo postnatally in the stem cell pool of repopulating cells, are theoretically possible but have not been reported. Somatic AR mutations are relatively common in prostate cancer usually arising in late stage disease palliatively treated by androgen deprivation when AR mutations and functional splice variants are reported (226). The switch of highly androgen dependent prostate cancer cells to an androgen deplete milieu may encourage clonal selection of androgen insensitive sublines to proliferate in the terminal stage of the disease. Genetic instability of prostate cancer cells may also contribute to this process although somatic AR mutations are rare in other cancers such as liver (251) or breast (252) cancer in the absence of androgen deprivation. Somatic AR mutation in prostate cancer cells are responsible for the paradoxical anti-androgen withdrawal syndromes observed with non-steroidal (flutamide, bicalutamide, nilutamide) or steroidal (cyproterone, megestrol) (253-254) treatment. In this state, anti-androgen withdrawal or switch-over (254) produces remission of worsening disease attributable to the occurrence of a de novo AR mutation in prostate cancer cells which alters ligand specificity turning the non-steroidal antiandrogens into AR agonists (226, 255). The LNCaP prostate cell line widely used in cancer cell biology research harbours a mutated AR (T877A) which occurs relatively frequently in prostate cancer metastases and can cause the flutamide withdrawal syndrome (256). Since the Nobel prize-winning discovery in the 1940's of androgen deprivation as palliative treament of advanced prostate cancer (257), targeting of AR fo treatment of prostate cancer has focused on surgical or medical castration to eliminate AR's cognate endogenous ligand, testosterone. After transient remission following castration, however, prostate cancers resume growth in the apparently androgen independent terminal, treatment resistant stage of the disease. Although castration eliminates the major (>95%) contribution to overall androgen synthesis, ongoing production of androgens from other tissues expressing steroidogenic enzymes, such as the adrenal (258) and prostate tumors (259), has been proposed to explain the late development of apparent androgen independence. Extensive clinical trials of maximum

androgen blockade which aims to more thoroughly ablate androgen action by adding anti-androgens to castration, however, have produced only minimal improvement in survival (260), possibly due to antiandrogens countering the deleterious initial "flare" effect of superactive GnRH analogs used for medical castration. A more effective approach has been the development of abiraterone, a rationally designed, mechanism-based inhibitor of CYP17A1 (17-hydroxylase/17,20 lyase) incorporating a 16-17 double bond to inhibit 17-hydroxylation. Abiraterone has proven effective and well-tolerated in treatment of late stage, apparently androgen independent prostate cancer (261) although the blockade of glucocorticoid and mineralocorticoid synthesis requires adrenal replacement therapy. In addition, newer androgen receptor blockers also provided promising new therapeutic approaches especially for castration-resistent advanced prostate cancer (262).

Acquired androgen insensitivity may occur without AR mutations by mechanisms such as drugs including non-steroidal (flutamide, bicalutamide, nilutamide) and steroidal (cyproterone acetate), drugs that block part of testosterone activation such as 5α reductase inhibitors (finasteride, dutasteride) or estrogen antagonists or aromatase inhibitors. In addition, drugs may have physiological effects or pharmacological actions that oppose various steps in androgen action such as LH and FSH suppression by estrogens or progestins or that cause an increase in circulating SHBG which may influence testosterone transfer from blood into tissues to produce a functional phenocopy of androgen insensitivity.

Acquired androgen insensitivity in various disease states is reported with hormonal findings reflecting impeded androgen action which may be reversible with alleviation of the underlying disease. The disease-related mechanisms that impede androgen action vary but the most frequent is increase in hepatic SHBG secretion due to the underlying disease and/or its drug treatments that impede androgen action by reducing testosterone transport from blood to tissues as part of its overall reduction in metabolic clearance rate of testosterone. For example, in hyperthyroidism, increased blood LH and testosterone concentrations with clinical features of androgen deficiency (263) are mediated by increased circulating SHBG due to thyroid hormone-induced hepatic SHBG secretion (264) whereas in hypothyroidism the reduced blood testosterone and SHBG are rapidly corrected by thyroid hormone repacement therapy (263). In epilepsy, anticonvulsant-induced increase in hepatic SHBG secretion appears to be a common denominator in the near ubiquitous reproductive endocrine abnormalities in men with epilepsy (265). The relative contributions of impaired tissue transfer of testosterone, reduced testosterone metabolic clearance rate (266) or direct anti-androgenic effects of valproate (267) remain to be clarified. A similar mechanism of disease- and/or drug-induced increases in hepatic SHBG secretion may explain apparent acquired androgen insensitivity, often reversible with alleviation of the underlying disease, in various other conditions such as gluten enteropathy (268-269), Wilson's disease (270), relapsed acute intermittent porphyria (271), acute alcoholism (272), chronic liver disease and transplantation (66, 273).

PHARMACOLOGY OF ANDROGENS

Indications for Androgen Therapy

Androgen therapy can be classified as physiologic replacement or pharmacologic therapy according to the dose, type of androgen and objectives of treatment. Androgen replacement therapy aims to restore tissue androgen exposure in androgen-deficient men due to pathological hypogonadism (disorders of the reproductive system) to levels comparable with those of eugonadal men. Using the natural androgen testosterone and a dose limited to one that maintains blood testosterone levels within the eugonadal range, androgen replacement therapy aims to restore the full spectrum of androgen effects while replicating the efficacy and safety experience of eugonadal men of similar age. Androgen replacement therapy is unlikely to prolong life because androgen deficiency, whether due to castration (274-278) or biological disorder (279) has minimal effect in shortening life expectancy (280). As an alternative, pharmacologic androgen therapy uses androgens without restriction on androgen type or dose but aims to produce androgen effects on muscle, bone, brain, or other tissues. In such pharmacological treatment, regardless of androgen status, an androgen is used therapeutically to exploit the anabolic or other effects of androgens on muscle, bone, and other tissues as hormonal drugs in various non-reproductive disorders. Such pharmacological androgen therapy is neither constrained to using the natural androgen, testosterone, nor it is limited to physiological replacement doses or their equivalent. Rather, it is judged on its efficacy, safety, and relative cost-effectiveness for that specific indication just as any other hormonal or xenobiotic non-hormonal therapeutic drug. Many older uses of pharmacologic androgen therapy are now considered second-line therapies as more specific treatments are developed (281). For example, erythropoietin has largely supplanted androgen therapy for anemia due to marrow or renal failure and improved first-line drug treatments for endometriosis, osteoporosis and advanced breast cancer have similarly relegated androgen therapy to a last resort while newer mechanism-based agents in development for hereditary angioedema may displace 17α-alkylated androgens (282-283). Nevertheless in many clinical situations, pharmacological androgen therapy remains a cost-effective option with a long-established efficacy and safety profile.

Androgen Replacement Therapy
The sole unequivocal clinical indication for testosterone treatment is in replacement therapy for androgen deficient men suffering from pathological disorders of their reproductive system (hypothalamus, pituitary, testis) that prevent the testes producing sufficient testosterone supply to meet the body's usual needs. Establishing a pathological basis for androgen replacement therapy requires identifying well-defined disorders of the hypothalamus, pituitary or testis which have a known and clearly defined pathological basis. These disorders can, and often do, lead to persistent testosterone deficiency either due to disorders of the testis, where damaged Leydig cells cannot produce sufficient testosterone, or disorders of the hypothalamus and/or pituitary, where impaired pituitary luteinizing hormone (LH) secretion reduces the sole driving force to testosterone production by Leydig cells.

The principal goal of androgen (testosterone) replacement therapy is to restore a physiologic pattern of net tissue androgen exposure in androgen deficient men whose damaged reproductive systems are unable to secrete adequate testosterone to levels comparable with those of eugonadal men. This treatment uses only the natural androgen, testosterone, aimed at restoring a physiologic pattern of androgen exposure using a dose limited to that which maintains blood testosterone levels within the eugonadal range. Such treatment aims to

restore the full spectrum of androgen effects when endogenous testosterone production fails due to pathological disorders of the reproductive system (testicular-hypthalamic-pituitary axis). This requires restricting replacement therapy to the major natural androgen, testosterone, which aims to not only replicate physiological circulating testosterone levels but also to provide testosterone's two bioactive metabolites, DHT and estradiol, so that all 3 bioactive sex steroids are available to androgen target tissues. Synthetic androgens are unsuitable because they are incapable of metabolism to the more potent 5α reduced metabolites or aromatized to estrogens. The overall goal of such replacement therapy is to replicate the efficacy and safety experience of eugonadal men of similar age by recreating the full spectrum of endogenous natural androgen effects on tissues so as to recapitulate the natural history of efficacy and safety of endogenous testosterone.

The prevalence of male hypogonadism requiring androgen repacement therapy in the general community can be estimated from the known prevalence of Klinefelter's syndrome (15.6 per 1000 male births in 33 prospective birth survey studies (284)) because Klinefelter syndrome accounts for 25-35% of men requiring androgen replacement therapy. The estimated prevalence of ~5 per 1000 men in the general community makes androgen deficiency the most common hormonal deficiency disorder among men. Although life expectancy is not reduced by castration as an adult (274-278) or only minimally (~2 years) shortened (279) by life-long androgen deficiency, the hormonal deficit causes preventable morbidity and a suboptimal quality of life (284). Due to its variable and often subtle clinical features, androgen deficiency remains significantly underdiagnosed, thus denying sufferers simple and effective medical treatment with often striking benefits. Only ~20% of men with Klinefelter syndrome characterized by the highly distinctive tiny (<4 mL) testes, are diagnosed during their lifetime (285) indicating that most men go through life without a single pelvic examination by any medical professional in stark contrast to the usual expectation of reproductive health care for women.

The testis has two physiological functions, spermatogenesis and steroidogenesis, either of which can be impaired independently, resulting in infertility or androgen deficiency, respectively, so the term hypogonadism is inherently ambiguous. However, hypogonadism of any cause may require androgen replacement therapy if the deficit in endogenous testosterone production is sufficient to cause clinical and biochemical manifestations of androgen deficiency. Androgen deficiency is a clinical diagnosis with a characteristic presentation and underlying pathological basis in hypothalamus, pituitary or testis disorder, and confirmed by blood hormone assays (see Chapter 4 for details). The clinical features of androgen deficiency vary according to the severity, chronicity, and epoch of life at presentation. These include ambiguous genitalia, microphallus, delayed puberty, sexual dysfunction, infertility, osteoporosis, anemia, flushing, muscular ache, lethargy, lack of stamina or endurance, easy fatigue, or incidental biochemical diagnosis. For each androgen deficient man, his leading clinical symptoms of androgen deficiency are distinctive, reproducible and corresponds to a specific blood testosterone threshold for any individual but both the symptom(s) and threshold vary between men (286). Because the underlying disorders are mostly irreversible, lifelong treatment is usually required. Androgen replacement therapy can rectify most clinical features of androgen deficiency apart from defective spermatogenesis (287). When fertility is required in gonadotropin-deficient men,

spermatogenesis can be initiated by treatment with pulsatile GnRH (288) (if pituitary gonadotroph function is intact (289)) or gonadotropins (290) to substitute for pituitary gonadotropin secretion (291) (see also Endotext, Endocrinology of Male Reproduction, Chapter 5, Hypogonadotropic Hypogonadism (HH) and Gonadtropin Therapy). The short half-life of LH would require multi-daily injections rendering it unsuitable for gonadotrophin therapy (292). Instead practical gonadotropin therapy uses hCG, a placental heterodimeric glycoprotein which has a much longer duration of action allowing it to be administered every two or three days. The chorionic gonadotropin hCG consists of an identical α subunit as LH (also the same as in FSH and TSH) combined with a distinct β subunit that is highly homologous to the LH β subunit except for a C terminal extension of 22 amino acids which includes four O-linked sialic acid-capped, carbohydrate side chains. This C terminal extension markedly prolong the circulating half-life of hCG relative to LH thereby making it a naturally occuring long-acting LH analog. Both endogenous LH and hCG act on the Leydig cell LH/hCG receptor to stimulate endogenous testosterone production. Pharmaceutical hCG, originally purified from pregnancy urine and more recently its recombinant form, can be administered 2-3 times weekly for several months. Where spermatogenesis remains persistently suboptimal, recombinant FSH may subsequently be added (290). Once fertility is no longer required and any pregnancy has passed the 1st trimester, androgen replacement therapy usually reverts to the simpler and cheaper use of testosterone while preserving the ability subsequently to reinitiate spermatogenesis by gonadotropin replacement (290). The potential value of hCG therapy in gonadotropin-deficient adolescents to produce timely testis growth replicating physiologic puberty (293), rather than reliance on exogenous testosterone which leaves a dormant testis but remains standard management, has yet to be fully evaluated (294-295).

The extension of testosterone replacement therapy to men with partial, subclinical or compensated androgen deficiency states remain of unproven value. Biochemical features of Leydig cell dysfunction, notably persistently elevated LH with low to normal levels of testosterone constituting a high LH/testosterone ratio are observed in aging men (296-298), in men with testicular dysfunction associated with male infertility (299), or after chemotherapy-induced testicular damage (300-303). Although such features may signify mild androgen deficiency, substantial clinical benefits from testosterone replacement therapy remain to be demonstrated (304-305). Furthermore, testosterone administration may have deleterious effects on spermatogenesis so that its potential adverse effect on men's fertility must be considered with regard to their marital and fertility status.

Hormonal male contraception can be considered a form of androgen replacement therapy because all currently envisaged regimens aiming to suppress spermatogenesis (and thereby endogenous testosterone production) by inhibiting gonadotropin secretion, use testosterone either alone or with a progestin or a GnRH antagonist (306) (see also Endotext, Endocrinology of Male Reproduction, Chapter 15, Male Contraception). As a consequence, exogenous testosterone is required to replace endogenous testosterone secretion.
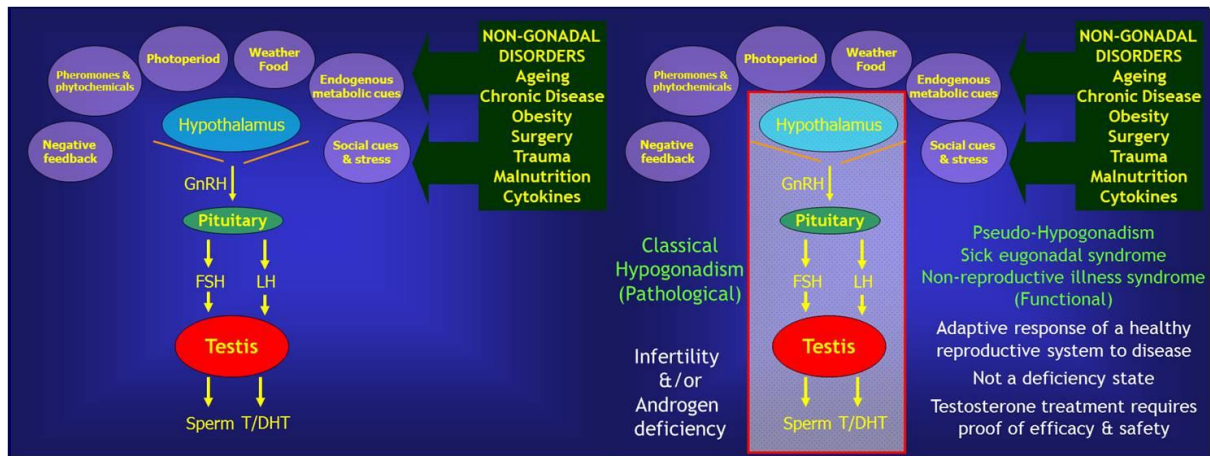
FIGURE 3. The Hypothalamo-Pituitary Testicular Axis in Health and Intrinsic and Extrinsic Diseases. *Schematic representation of the hypothalamo-pituitary-testicular (HPT) system in health (left panel) and in disease (right panel). Note in the right panel the distinction between organic disorders of the reproductive system causing pathologcal hypogonadism leading to male infertility and/or androgen deficiency and, alternatively, non-reproductive disorders which lead to an adaptive hypothalamic response to systemic non-reproductive disorders. While these non-reproductive disorders may lead to a reduced serum testosterone, that is neither a testosterone deficiency state nor warrants testosterone treatment without convincing evidence of safety and efficacy.*

Pharmacologic Androgen Therapy

Pharmacologic androgen therapy uses androgens to maximal therapeutic efficacy within adequate safety limits but without regard to androgen type, dose, duration of treatment or gender. In this, the goal is to improve mortality and/or morbidity of an underlying non-gonadal disease through eliciting androgen effects on muscle, bone, brain, or other target tissues. To obtain morbidity benefits requires that androgens must modify the natural history of an underlying disease, a goal not yet achieved in any nongonadal disorder. Morbidity benefits are more achievable in aiming to improve quality of life by enhancing muscle, bone, brain, or other androgen-sensitive function (including mood elevation) as an adjuvant therapy in non-reproductive diseases. Such treatment is judged by the efficacy, safety, and cost-effectiveness standards of other drugs but very few studies fulfill the requirements of adequate study design (prospective design, randomization, placebo control, objective and validated end points, adequate power, and appropriate duration) (281, 307). Accordingly, the role of pharmacological androgen therapy is mostly relegated to an affordable but second line, supportive or adjunctive therapy (307).

The range of pharmacologic uses of androgens include: treatment of anemia due to marrow or renal failure; osteoporosis especially where estrogen therapy is contraindicated; advanced ER-positive breast cancer; hereditary angioedema (C1 esterase inhibitor deficiency); and for immunologic, pulmonary, and muscular diseases (reviewed in detail (307)). In anemia due to renal or marrow failure, androgens have proven beneficial effects on morbidity by improving hemoglobin levels, reducing transfusion requirements and improving quality of life. However, characteristically androgens do not improve mortality as they do not change the natural history of the underlying disease. In renal anemia, androgens are equally effective with

erythropoeitin in maintaining hemoglobin levels and reducing transfusion requirement (308-310). However, their virilizing effects in women are limiting so that the affordability and augmentation of erythropoeitin effects by androgens provides an ongoing adjuvant role in older men or where erythropoeitin is unavailable (308-310). Similarly, in anemia due to marrow failure androgens reduce transfusion dependence but do not improve survival from the underlying marrow disorders. They remain secondary line, supportive therapy for men in whom marrow transplantation is not feasible or fails.

Although these traditional indications for androgen therapy are often superseded by more specific, effective but costly treatments, androgens usually persist as second-line, empirical therapies for which the lower cost and/or equivalent or synergistic efficacy may still favor androgen therapy in some settings. For historical reasons, pharmacologic androgen therapy has often involved synthetic, orally active 17α-alkylated androgens despite their hepatotoxicity including cholestasis, hepatitis, adenoma and peliosis (311-312). Other than in treating angioedema, in which direct hepatic effects of 17α-alkyl androgens (rather than androgen action per se) may be crucial to increasing circulating C1 esterase inhibitor levels to prevent attacks (313-315), safer (nonhepatotoxic) testosterone preparations should generally be favored for long-term clinical use, although the risk-benefit balance may vary according to prognosis. For hereditary angioedema, newer mechanism-based, more specific and costly therapies such as purified or recombinant C1 inhibitor and bradykinin or kallekrein antagonists may overtake the traditional role of 17α-alkylated androgens such as danazol for long-term prophylaxis of hereditary angioedema (282-283, 316) or endometriosis. In most clinical applications, pharmacological androgen therapy remains a cost-effective option relative to newer, more costly therapies.

An important watershed was the proof via a well-designed, placebo-controlled randomized clinical trial that pharmacologic testosterone doses increase muscular size and strength even in eugonadal men (317), overturning prior belief to the contrary (318). Testosterone has clear dose-dependent effects, extending from below to well above the physiological concentrations without evidence of a plateau, on muscle size and strength (but not performance function or fatigue) in young (319) and older (320) men with similar magnitude of ultimate effect (321). Nevertheless, ageing reduced the responsiveness of older muscle to testosterone as the same doses produced higher blood testosterone concentrations in older men. The higher blood testosterone concentrations are the result of decreased testosterone metabolic clearance rate due to age-related higher blood SHBG concentrations (322). Similarly, erythropoeitic effects of testosterone are greater in older men who developed a higher rate of polycythemia (323). Diverse androgen-sensitive effects including changes in metabolic function, cognition, mood and sexual function were minimal at physiological testosterone doses (324-325). The wide dose-response to testosterone through and beyond the physiologic range suggests that androgens may have beneficial effects in reversing the frailty observed in many medical settings. Whether such effects can be applied effectively and safely (326) to improve frailty and quality of life in chronic disease or in male ageing remains an important challenge to be determined.

Pharmacological androgen therapy for human immunodeficiency virus (HIV) infection in the absence of classical hypogonadism has been investigated for its effects on disease-

associated morbidity, notably AIDS wasting. However pharmacologic androgen therapy does not alter the natural history of underlying disease and the objective functional benefits remain modest being confined to reversing some aspects of AIDS wasting. The rationale for pharmacologic androgen therapy in AIDS wasting is that body weight loss is an important determinant of survival in AIDS and other terminal diseases with death estimated to occur when lean body mass reaches 66% of ideal (327). This leads to the hypothesis that androgens may delay death by increasing appetite and/or body weight. Meta-analysis of randomized, placebo-controlled studies of pharmacologic androgen therapy in HIV-positive men with AIDS wasting indicate modest increase in lean and decreased fat mass with additive effects from resistance training but inconsistent improvement in quality of life (328-329). Among HIV-positive men without wasting there is less improvement in body composition and none in quality of life although, in affluent countries, there is a popular subculture of androgen abuse (330). The oral progestin, megestrol acetate, used alone as an appetite stimulant induces profound gonadotropin and testosterone suppression to castrate levels and predominanty increases fat mass rather than reversing the loss of muscle (331-332).

It is now well recognized that chronic use of opiates has multiple effects on the human endocrine system (333), including prominent mu-opioid receptor mediated effects on the hypothalamus resulting in suppression of pituitary LH secretion and thereby testicular testosterone production (334-335). However, despite an open-label study suggesting quality of life benefits for testosterone replacement therapy (336), placebo-controlled studies show no clinically significant benefit (337-338) possibly due to failure to rectify non-androgenic effects of opiates.

A special application of pharmacologic androgen treatment is its use in women with estrogen-resistant menopausal symptoms such as loss of energy or libido. The similarity of blood testosterone in women, children, and orchidectomized men indicates that the term female androgen deficiency is not meaningful in women (339) with normal adrenal function (340). In women with adrenal failure due to hypothalamo-pituitary or adrenal disease, DHEA replacement therapy (14) has significant but modest clinical benefits in some (340-341) but not all (342-343) studies with relatively frequent, mild virilizing side-effects. Similar effects are observed using testosterone instead of DHEA (344). Well controlled studies of testosterone administration for menopausal symptoms or sexual hypofunction in women with normal adrenal function show strong placebo effects (345-346) but minimal or no consistent symptomatic benefits (347) despite supraphysiological blood testosterone levels (345). High-dose testosterone used at male androgen replacement therapy doses (348-349) produce markedly supraphysiologic blood testosterone levels and virilization including voice changes and androgenic alopecia (350-352). Lower but still supraphysiologic testosterone doses and blood levels increase bone density in menopausal women (353) but produce virilizing adverse effects (hirsutism, acne) in short-term studies. Overall the long-term efficacy and safety risks for cardiovascular disease and hormone dependent cancers (breast, uterus, ovary) for testosterone therapy in women remain unclear (354-356). Studies of testosterone administration as a form of adjuvant pharmacologic androgen therapy in women with chronic medical disorders such as anorexia nervosa (357), HIV (358) and systemic lupus

erythematosus (359) have little consistent effect on disease activity or quality of life including sexual function.

Many important questions and opportunities remain for pharmacologic androgen therapy in nongonadal disease, but careful clinical trials are essential for proper evaluation (307). Recent well designed placebo-controlled clinical studies of pharmacologic androgen therapy in chronic disease have been reported. In men with severe chronic obstructive pulmonary disease it produces modest increases in muscle mass and strength with improved quality of life but no effect on underlying lung function (360-362) whereas oral megestrol administration had similar effects despite marked suppression of blood testosterone levels (363). Similarly, although in an observational study chronic heart failure is associated with lower blood testosterone that is proportional to the decrease in cardiac function and which predicts survival (364), a placebo-controlled prospective study of testosterone administration showed improvement in effort-dependent exercise capacity but not in left ventricular function or survival (365). This discrepancy suggests that the lowered blood testosterone is the consequence of a non-specific adaptive reaction of the reproductive hormonal axis to chronic disease (ontogenic regression (366)) rather than a detrimental effect susceptible to being overcome by androgen supplementation. Both testosterone and its non-aromatizable derivative nandrolone, produce increased bone density in men with glucocorticoid-induced osteoporosis with minimal short-term side-effects (367-368). The best opportunities for future evaluation of adjuvant use of androgen therapy in men with nongonadal disease include steroid-induced osteoporosis; wasting due to AIDS or cancer cachexia; and chronic respiratory, rheumatologic, and some neuromuscular diseases. In addition, the role of pharmacologic androgen therapy in recovery and/or rehabilitation after severe catabolic illness such as burns, critical illness, or major surgery are promising (369) but requires thorough evaluation because detrimental effects may occur (370). Future studies of adjuvant androgen therapy require high-quality clinical data involving randomization and placebo controls as well as finding the optimal dose and authentic clinical, rather than surrogate, end points.
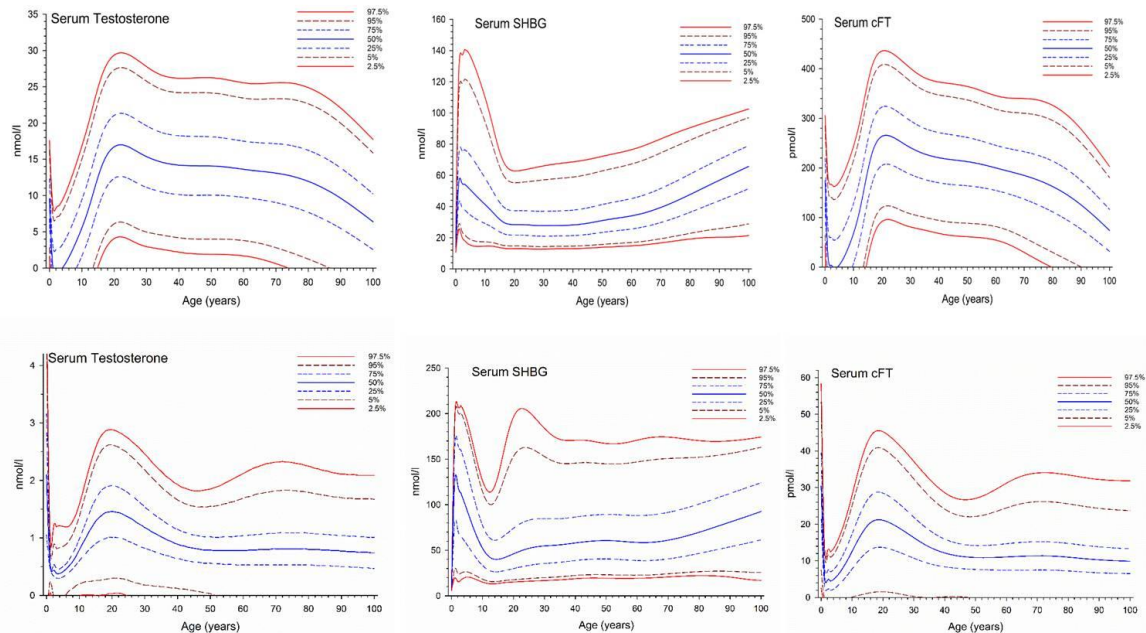
FIGURE 4. Serum Testosterone Across the Lifespan in Men and Women. *Serum testosterone, SHBG & calculated "free" testosterone in males and females over the lifetime as determined in >100,000 consecutive blood samples from a single laboratory. After Handelsman et al. Ann Clin Biochem 2016.*

TESTOSTERONE TREATMENT FOR MALE AGEING

The prospect of ameliorating male ageing by androgen therapy has long been of interest and recently has been the subject of many observational and short-term interventional controlled clinical trials. The consensus from population-based cross—sectional (296-297) and longitudinal studies (27, 371-372) is that circulating testosterone concentrations fall by up to ~1% per annum from mid-life onward, an age-related decline that is accelerated by the presence of concomitant chronic disease (372) and associated with decreases in tissue androgen levels (373-374) as well as numerous co-morbidities of male ageing (298, 375). Numerous cross-sectional and longitudinal observational studies show that low blood testosterone is associated with greater all-cause and/or cardiovascular mortality summarised in multiple meta-analyses (376-383). An observational study of older war veterans reported testosterone treatment was associated with better survival (384); however, bias in the non-randomised design allowing for preferential treatment of healthier men with testosterone may explain those findings (385). However, as observational studies cannot ascribe causality it remains likely that such reductions in blood testosterone may be a consequence rather than a cause of the increased mortality.

Interventional studies have remained too small and short-term to resolve this dilemma. Definitive evidence as to whether androgen treatment ameliorates age-related changes in bodily function and improves quality of life requires high quality, randomized placebo-controlled clinical trials using testosterone (386), DHT (387-388) or hCG (389) or synthetic androgens (390); however, so far the only consistent changes observed in well controlled studies of at least 3 months duration have been small increases in lean (muscle) and decreases in fat mass.

The best available summary evidence from meta-analyses indicates no or only inconsistent benefits in bone (391-392), muscle (393), sexual function (394-395) and detrimental effects on cardiovascular disease/risk factors (376-383) and polycythemia (396). As a result, the 2004 Institute of Medicine report (397) recommended a priority to acquire more convincing, target-defining feasibility evidence to justify a large-scale clinical trial to weight potential benefits against risks of accelerating cardiovascular and prostate disease.

Arising from that recommendation, the NIH-funded series of inter-related 'Testosterone Trials' reported their main result in which 790 older men (>65 years), mostly obese, hypertensive, ex-smokers (ie men with "andropause/lowT" but not pathological hypogonadism), treated daily with testosterone for one year showed a modest improvement in sexual function compared with placebo (398). The improvement in sexual function, about 1/3 increase over baseline sexual activity, waned during the year's treatment and there was no concomitant improvement in either vitality or physical activity compared with placebo. The benefit in sexual function was less robust than the effects of PDE5 inhibitors (398) and of uncertain clinical significance, insufficient to warrant initiating testosterone treatment of older men (399). These findings do not materially change the unfavourable balance of evidence for testosterone treatment for functional causes of a low serum testosterone in the absence of pathological hypogonadism.

The major hypothetical population risk from androgen therapy for male ageing remains increased cardiovascular disease (280) as was proven unexpectedly by the WHI study for the risks of estrogen replacement for menopause (400). Cardiovascular disease has earlier onset and greater severity in men resulting in a 2-3-fold higher age-specific risks of cardiovascular death compared with women (401). The male disadvantage in cardiovascular disease has a complex pathogenesis with androgens having apparently beneficial effects including in regulating cardiac ion channel fluxes that dictate QT interval length, cardiac ventricular repolarization and lesser risk of arrthymia (402-410) as well as angiogenesis (411-412) which must be integrated with other apparently deleterious effects (280, 413). Prospective observational data remains conflicting, with low blood testosterone predicting subsequent cardiovascular death in some (414-415) but not other (416-418) studies. Testosterone therapy for older, frail men may increase adverse cardiovascular events (326), side-effects that may be under reported (419) in previous studies not reporting such hazards (420). Observational data linking cardiovascular disease with low blood testosterone levels may however be the consequence of non-specific effects of chronic cardiovascular disease and/or confounding effects by major cardiovascular risk factors, like diabetes and obesity. The latter interpretations are supported by Mendelian randomization studies which report only non-causal relationships (421-422) albeit with important methodological caveats (423).

Similarly, for the more feared but quantitatively less significant late-life prostate diseases, although their androgen dependence is well established, it is also known that life-long androgen deficiency (Klinefelter's syndrome) reduces risk of fatal prostate cancer (424) and prevailing endogenous testosterone levels in healthy men do not predict risk of subsequent prostate cancer (425-426).

These epidemiological observations are consistent with either circulating testosterone levels being a biomarker, a non-specific barometer of ill-health, or else that restoring circulating testosterone to eugonadal levels could reduce age-related cardiovascular and prostate disease (the "andropause" hypothesis, also known as "LowT" or"late-onset hypogonadism"). Independent critical analyses have concluded that it is not valid to extrapolate the features of pathological hypogonadism in younger men to older men with possibly age-related hypogonadism (427). Nor did a comprehensive meta-analysis identify any valid basis for testosterone treatment of such older men (428). Decisive testing of these alternatives requires an adequately powered, placebo-controlled, prospective, randomized clinical trial (397). As the decisive safety and efficacy evidence on testosterone supplementation for male ageing remains distant, interim clinical guidelines have been developed by academic and professional societies (429-432) ostensibly aiming to restrain the unproven testosterone prescribing which nevertheless escalated over recent decades in Australia (433), Europe (434-435) and most dramatically in North America (436-439). Consequently, more recent clinical guidelines have curbed the tacit promotion of testosterone prescribing for men without pathological hypogonadism (440-441).

At present, testosterone treatment cannot be recommended as routine treatment for male ageing (see also Endotext, Endocrinology of Male Reproduction, Chapter 11, Age-Related Changes in the Male Reproductive Axis). Nevertheless, androgen replacement therapy may be used cautiously even in older men with pre-existing pathological pituitary-gonadal disorders causing androgen deficiency if contraindications such as prostate cancer are excluded.

ANDROGEN MISUSE AND ABUSE

Misuse of androgens involves medical prescription without a valid clinical indication and outside an approved clinical trial, and androgen abuse is the use of androgens for nonmedical purposes. Medical misuse includes prescribing androgens for male infertility (442) or sexual dysfunction in men without androgen deficiency (394) where there are no likely benefits or as a tonic for non-specific symptoms in older men ("male menopause", 'andropause", "late-onset hypogonadism") (397) or women (339) where safe and effective use is unproven. Although there is no exact boundary defining overuse, mass marketing and promotion to fend off ageing in the absence of reliable evidence are hallmarks of systemic misuse of androgens. Androgens have a mystique of youthful virility making them ideal for manipulative marketing to the wealthy, worried well as they grow older.

Androgen Misuse: Patterns of Testosterone Prescription

Despite the absence of any new approved indications beyond the treatment of pathological classical hypogonadism, testosterone prescribing has displayed major and progressive increases especially since 2000 (443). The market for testosterone prescribing has increased 100-fold from $18 million in the late 1980's (436) to $1.8 billion in this decade (439). In Australia, for example, where a national health scheme provides accurate prescription data, striking differences between states, increasing use of costlier newer products and partially effective regulatory curbs on unproven testosterone prescribing were reported (433, 444). Similar increases are also described in the USA (436, 438), Switzerland (435) and UK (434). A 2013 study of international trends in testosterone prescribing based

on per capita sales of testosterone usage and pooling all products into standardized testosterone usage estimates (per person per month), showed testosterone usage increased in every world region and for 37 of 41 countries surveyed over the 11 years (2000-11) (439). The increases were most striking in North America where they rose 40-fold in Canada and 10-fold in the USA over only a decade. Other estimates from the US confirm an increase in testosterone prescribing although with a lower increase when based on selective sources such as private insurance databases (438, 445-446) or the Veterans Administration (VA) system (447). These lower increases underestimate the national usage, indicating the efficacy of formulary or other restrictions constraining unjustified testosterone prescribing but implies much greater increases in testosterone usage outside those populations served by private medical insurance and the VA system.

The increased testosterone prescribing appears to be primarily for older men and driven by clinical guidelines that endorse testosterone prescribing for age-related low circulating testosterone concentration (430, 448-449), commonly referred to as "LowT" or "late-onset hypogonadism". The major factors driving these increases include direct-to-consumer advertising as part of a broad spectrum of pharmaceutical promotional activities as well as permissive clinical prescribing guidelines from professional and single-issue societies. The latter have, in concert, tacitly encouraged, facilitated, and promoted increased off-label testosterone prescribing, bypassing the requirement for high quality clinical evidence of safety and efficacy. Prescribing guidelines that systematically eliminated the fundamental distinction between pathological hypogonadism and functional causes of a low circulating testosterone have significantly contributed to legitimizing epidemic-like increases in testosterone prescription overuse based upon highly inflated incidence data for "hypogonadism" (439).

The known prevalence of pathological androgen deficiency (~0.5% of men (450)) equates to a figure of ~15 defined monthly doses per year per 1000 population. Population linkage registry data from the UK (424) and Denmark (285) prove severe under-diagnosis of Klinefelter's syndrome, the most frequent cause of pathological AD. Nevertheless, it is highly unlikely that recent steep increases in testosterone prescribing and use can be attributed to rectifying the under-diagnosis of KS, which is generally diagnosed in young male adults. Not only does total testosterone prescribing in some countries exceed the maximal amount that could be attributed to pathological AD, there is no evidence the diagnosis of KS has increased in recent years (444, 451).

By contrast, the estimated population prevalence of "andropause" among older men is up to 40% (452) or more usually claimed in the range 10-25% (297, 453-454) with even the lowest estimates of 2-3% (455) representing major (5-100 fold) increases in potential market size over pathological hypogonadism.

Androgen Abuse
(see also Endotext, Endocrinology of Male Reproduction Section, Chapter 20, Performance Enhancing Hormone Doping in Sport)
Androgen abuse originated in the 1950s as a product of the Cold War (456) whereby communist Eastern European countries could develop national programs to achieve short-

term propaganda victories over the West in Olympic and international sports (457). This form of cheating was readily taken up by individual athletes seeking personal rewards of fame and fortune in elite competitive sports. Over decades, androgen abuse has become endemic in developed countries with sufficient affluence to support drug abuse subcultures. Androgen abuse is cultivated by underground folklore among athletes and trainers, particularly in power sports and body building, promoting the use of so-called "anabolic steroids" to enhance personal image and sports performance. A lucrative illicit industry is fostered through wildly speculative underground publications promoting the use of prodigious androgen doses in combination ("stacking") and/or cycling regimens. The myotrophic benefits of supraphysiologic androgen doses in eugonadal men were long doubted (318) in the belief that alleged performance gains were attributable to placebo responses involving effects of motivation, training and diet, This belief was overturned by a randomized, placebo-controlled clinical study showing decisively that supraphysiologic testosterone doses (600 mg testosterone enanthate weekly) for 10 weeks increases muscular size and strength (317). In well-controlled studies of eugonadal young (458) and older (321) men, testosterone shows strong linear relationships of dose with muscular size and strength throughout and beyond the physiologic range. The additional dose-dependent increases in erythropoesis (323) and mood (459) may also enhance the direct myotrophic benefits of supraphysiologic androgen dose. While these studies prove the unequivocal efficacy of supraphysiological androgen dosage to increase muscle size and strength even in eugonadal men, the specific benefits for skilled athletic performance depend on the sport involved with greatest advantages evident in power sports. The overall safety of the sustained supraphysiological androgen exposure in these settings remains undefined, notably for cardiovascular and prostate disease as well as psychiatric sequelae (460).

Progressively, the epidemic of androgen abuse has spread from elite power athletes so that the majority of abusers are no longer athletes but recreational and cosmetic users wishing to augment body building or occupational users working in security-related professions (461). A remarkable meta-analysis of 271 papers reporting prevalence of androgen abuse within various populations comprising 2.8 million people (461), deduced a lifetime ("ever use") prevalence of 6.4% for males and 1.6% for females with higher rates for recreational sports (18.4%), athletes (13.4%), prisoners (12.4%), drug users (8.0%), high school students (2.3%) compared with non-athletes (1.0%). As an illicit activity, the extent of androgen abuse in the general community is difficult to estimate, although point estimates of prevalence are more feasible in captive populations such as high schools. The prevalence of self-reported lifetime ("ever") use is estimated to be 66 in the United States (462), 58 in Sweden (463), 32 in Australia (464), and 28 in South Africa (465) per 1000 boys in high school, with a much lower prevalence among girls. Predictors of androgen abuse in high schools are consistent across many cultures include truancy, availability of disposable income, minority ethnic or migrant status and there is significant overlap with typical features of adolescent abuse of other drugs. Voluntary self-report of androgen abuse understates prevalence of drug use among weight lifters (466) and prisoners (467-468).

Abusers consume androgens from many sources including veterinary, inert, or counterfeit preparations, obtained mostly through illicit sales by underground networks with a small proportion obtained from compliant doctors. Highly sensitive urinary drug screening methods

for detection of natural and synthetic androgens, standardized by the Word AntiDoping Agency (WADA) for international and national sporting bodies as a deterrent, has contributed to the progressive elimination of known androgens from elite sporting events. The persistent demand for androgens as the most potent known ergogenic drugs has led to the production in unlicensed laboratories of illicit designer androgens such as norbolethone (469), tetrahydrogestrinone (470-471) and dimethyltestosterone (472) custom-developed for elite professional athletes to evade doping detection. The rapid identification of these designer androgens has meant that they have been seldom, if ever, used (473). Corresponding legislation has also been introduced by some governments to regulate clinical use of androgens and to reduce illicit supply of marketed androgens. While overall, the community epidemic of androgen abuse driven by user demands shows little signs of abating (474-475), rigorous detection is reducing demand in elite sports and similar trends have been reported in the long running Monitoring the Future Project (http://www.monitoringthefuture.org/) whereby self-reported androgen abuse peaked in US high schools around 2000 and is now abating.

Androgen abuse is associated with reversible depression of spermatogenesis and fertility (476-480), gynecomastia (481), hepatotoxicity due to 17α-alkylated androgens (482), HIV and hepatitis from needle sharing (483-487) although the infectious risks are lower than among other iv drug users due to less needle and syringe sharing (488), local injury and sepsis from injections (489-490), overtraining injuries (491), rhabdomyolysis (492), popliteal artery entrapment (493), cerebral (494) or deep vein thrombosis and pulmonary embolism (495), cerebral hemorrhage (496), convulsions (497) as well as mood and/or behavioral disturbances (498-499). The medical consequences of androgen abuse for the cardiovascular system have been reviewed (500-504), but only few anecdotal reports are available relating to prostate diseases (505-507). However, for both, long-term consequences of androgen abuse based on anecdotal reporting are likely to be significantly underestimated due to underreporting of past androgen use and non-systematic follow-up. Few well controlled prospective clinical studies of the cardiovascular (508-509) or prostatic (321, 458, 510) effects of high dose androgens have been reported. Most available clinical studies consist of non-randomized, observational comparisons of androgen users compared with non or discontinued users (511-523). However, such retrospective observational studies suffer from ascertainment, participation and other bias so that important unrecognized determinants of outcomes may not be measured. Given the low community prevalence of androgen abuse, well designed, sufficiently powerful retrospective case-control studies are required to define the long-term risks of cardiovascular and prostate disease (524). The best available evidence suggests elite athletes have longer life expectancy due to reduced cardiovascular disease (525-526). This benefit, however, is least evident among power athletes, the group with highest likelihood of past androgen abuse, a finding confirmed by a small study finding a greater than 4-fold increase in premature deaths (from suicide, cardiovascular disease, liver failure and lymphoma) among 62 former power athletes compared with population norms (527). More definitive studies are required, but, at present, largely anecdotal information suggests that serious short-term medical danger is limited considering the extent of androgen abuse, that androgens are not physically addictive (528-529) and that most androgen abusers eventually discontinue drug use. After cessation of prolonged use of high-dose androgens, recovery of the hypothalamic-pituitary-testicular axis

may be delayed for months and up to 2 years (480), creating a transient gonadotropin deficiency state (530-532). This may lead to temporary androgen deficiency symptoms and/or oligozoospemia and infertility that eventually abate without requiring additional hormonal treatments. Although hCG can induce spermatogenesis (478, 533), like exogenous testosterone, it further delays recovery of the reproductive axis and perpetuates the drug abuse cycle (534). There remains anecdotal evidence from experienced observers that prolonged hypothalamic-pituitary suppression by high dose exogenous androgens may not always be fully reversible after even a year off exogenous androgens, resembling the incomplete reversibility of GnRH analog suppression of circulating testosterone in older men after cessation of prolonged medical castration for prostate cancer (535-536). An educational program intervention had modest success in deterring androgen abuse among secondary school footballers (537) and more effective interventions to prevent and/or halt androgen abuse capable of overcoming strong contrary social incentives of fame and fortune are yet to be defined.

PRACTICAL GOALS OF ANDROGEN REPLACEMENT THERAPY

The goal of androgen replacement therapy is to replicate the physiologic actions of endogenous testosterone, usually for the remainder of life as the pathological basis of hypogonadism is usually irreversible disorders of the hypothalamus, pituitary or testis. This requires rectifying the deficit and maintaining androgenic/anabolic effects on bone (141, 538), muscle (320), blood-forming marrow (323, 539), sexual function (70, 540), and other androgen-responsive tissues. The ideal product for long-term androgen replacement therapy should be a safe, effective, convenient, and inexpensive form of testosterone with long-acting depot properties providing steady-state blood testosterone levels due to reproducible, zero-order release kinetics. Androgen replacement therapy usually employs testosterone rather than synthetic androgens for reasons of safety and ease of monitoring. The aim is to maintain physiologic testosterone levels and resulting tissue androgen effects. Synthetic steroidal and non-steroidal androgens are likely to lack the full spectrum of testosterone tissue effects due to local amplification by 5α reductase to DHT and/or diversification to act on ERα by aromatization to estradiol. The practical goal of androgen replacement therapy is therefore to maintain stable, physiologic testosterone levels for prolonged periods using convenient depot testosterone formulations that facilitate compliance and avoid either supranormal or excessive fluctuation of androgen levels. The adequacy of testosterone replacement therapy is important for optimal outcomes (541) as suboptimal testosterone regimens, whether due to inadequate dosage or poor compliance, produce suboptimal bone density (542-544) compared with maintenance of age-specific norms achieved with adequate testosterone regimens (541, 545). Differences in testosterone-induced bone density according to type of hypogonadism (546) may be attributable to delay in onset and/or suboptimal testosterone dose in early onset androgen deficiency (547-548) leading to reduced peak bone mass achieved in early manhood. Similarly, the severity of the androgen deficiency also predicts the magnitude of the restorative effect of testosterone replacement with greatest effects early in treatment of severe androgen deficiency (538, 541) whereas only minimal effects are evident for testosterone treatment of mild androgen deficiency (304-305). The potential for individual tailoring of testosterone replacement dose according to an individual's pharmacogenetic background of androgen sensitivity has been proposed by a study showing that the magnitude of the prostate growth response to exogenous

testosterone in androgen deficient men is inversely related to the CAG triplet (polyglutamine) repeat length in exon 1 of the androgen receptor (206). However, this polyglutamine repeat is inversely related to ambient blood testosterone levels (549) consistent with the reciprocal relationship between repeat lengths and AR transactivational activity. Hence this polymorphism is only a weak modulator of tissue androgen sensitivity. Whether the magnitude of this pharmacogenetic effect is sufficiently large and significantly influences other androgen-sensitive end points will determine whether this approach is useful in practice.

Pharmacologic Features of Androgens

The major features of the clinical pharmacology of testosterone are its short circulating half-life and low oral bioavailability, both largely attributable to rapid hepatic conversion to biologically inactivate oxidized and glucuronidated excretory metabolites. The pharmaceutical development of practical testosterone products has been geared to overcoming these limitations. This has led to the development of parenteral depot formulations (injectable, implantable, transdermal), or products to bypass the hepatic portal system (sublingual, buccal, gut lymphatic absorption) as well as orally active synthetic androgens that resist hepatic degradation (108, 550).

Androgens are defined pharmacologically by their binding and activation of the androgen receptor (1). Testosterone is the model androgen featuring a 19-carbon, four-ring steroid structure with two oxygens (3-keto, 17β-hydroxy) including a Δ^4 nonaromatic A ring. Testosterone derivatives have been developed to enhance intrinsic androgenic potency, prolong duration of action, and/or improve oral bioavailability of synthetic androgens. Major ring structural modifications of testosterone include 17β-esterification, 19-nor-methyl, 17α-alkyl, 1-methyl, 7α-methyl, and D-homoandrogens. Most synthetic androgens are 17α-alkylated analogs of testosterone developed to exploit the fact that introducing a one (methyl) or two (ethyl, ethinyl) carbon group at the 17α position of the D ring allowed for oral bioactivity by reducing hepatic oxidative degradative metabolism. In 1998 the first nonsteroidal androgens, modified from nonsteroidal aryl propionamide antiandrogen structures, were reported (551) followed by quinoline, tetrahydroquinoline and hydantoin derivatives (552).

The identification of a single gene and protein for the androgen receptor in 1988 (553-555) explains the physiologic observation that, at equivalent doses, all androgens have essentially similar effects (556). The term "anabolic steroid" was invented during the post-WWII golden age of steroid pharmacology to define an idealized androgen lacking virilizing features but maintaining myotrophic properties so that it could be used safely in chidren and women. Although this quest proved illusory and was abandoned after all industry efforts failed to identify such a hypothetical synthetic androgen, the obsolete term "anabolic steroid" persists mainly as a lurid descriptor in popular media despite continuing to make a false distinction where there is no difference. Better understanding of the metabolic activation of androgens via 5α-reduction and aromatization in target tissues and the tissue-specific partial agonist/antagonist properties of some synthetic androgens may lead to more physiological concepts of tissue-specific androgen action ("specific androgen receptor modulator") governed by the physiological processes of pre-receptor androgen activation as well as post-

receptor interaction with co-regulator proteins analogous to the development of synthetic estrogen partial agonists with tissue specificity ("specific estrogen receptor modulator") (557). The potential for new clinical therapeutic indications of novel tissue-selective androgens in clinical development remain to be fully evaluated (558).

Formulation, Route, and Dose

Unmodified Testosterone
Testosterone Implants
Implants of fused crystalline testosterone provide stable, physiologic testosterone levels for as long as 6 months after a single implantation procedure (559). Typically, four 200-mg pellets are inserted under the skin of the lateral abdominal wall or hip using in-office minor surgery and a local anesthetic. No suture or antibiotic is required, and the pellets are fully biodegradable and thus do not require removal. This old testosterone formulation (560) has excellent depot properties, with testosterone being absorbed by simple dissolution from a solid reservoir into extracellular fluid at a rate governed by the solubility of testosterone in the extracellular fluid resulting in a standard 800 mg testosterone dose releasing ~5 mg per day (561) replicating the testosterone production rate in healthy eugonadal men (49-51, 562). The long duration of action makes it popular among younger androgen-deficient men as reflected by a high continuation rate (563). The major limitations of this form of testosterone administration are the cumbersome implantation procedure and extrusion of a single pellet after ~5% of procedures. Extrusions are more frequent among thin men undertaking vigorous physical activities (563) but surface washing (564), antibiotic impregnation (565) or varying the site of implantation or track geometry (566) do not reduce extrusion rate. Other side effects such as bleeding or infection are rare (<1%) (563). Recent studies using a smaller (75 mg) implant reproduce these features although requiring administration of a larger number of pellets (567-569). Despite its clinical advantages and popularity, this simple, non-patented technology has limited commercial marketing appeal and, consequently, is not widely available apart from compounding chemists and niche manufacturers (567).

Transdermal Testosterone
Delivery of testosterone across the skin has long been of interest (162). More recently products delivering testosterone via adhesive dermal patches and gels have been developed to maintain physiologic testosterone levels by daily application. The first transdermal patch was developed for scrotal application where the thin, highly vascular skin facilitates steroid absorption (570-571) and scrotal patches showed long-term efficacy (572) including minimal skin irritation (573-574). However, their large size, need for shaving and disproportionately high increase blood DHT levels due to 5α-reduction of testosterone during transdermal passage led to the development of a smaller non-scrotal patch (575) effective for long-term use (576). For non-scrotal patches, the smaller size and application to less permeable non-scrotal (trunk, proximal limb) skin limit testosterone absorption. Although this can be enhanced by heating (577), in practice this required inclusion of absorption enhancers that cause skin irritation (573-574) of varying severity (578). Although skin irritation may be reduced by topical corticosteroid cream (579), the majority of users experience some skin reaction with ~25% having to discontinue due to dermal intolerance (365).

Dermal testosterone (580) or DHT (581-582) gels developed in Europe are now more widely available as topical gels (540, 583-588), solution (589) or a cream (71). They must be applied daily on the trunk or axilla, and the volatile hydroalcoholic gel base evaporates rapidly with a short-lived stinging sensation but is relatively nonirritating to the skin so there are few discontinuations for adverse skin reactions (540, 590). Transdermal testosterone delivery depends on a small fraction (typically <5%) of testosterone applied to the skin in the dermal gel or solution transfering into the skin where it forms a secondary reservoir in the stratum corneum. From this depot, testosterone is gradually released into the circulation by diffusion down a concentration gradient into the blood stream. As a large amount of testosterone remains on the skin after topical application, transfer of testosterone by direct skin contact is a risk for an intimate partner (591-593) or children (594-599). Serum testosterone concentrations are increased in non-dosed female partners making direct skin contact with men using transdermal testosterone products (600). Creating a physical barrier such as using a testosterone transdermal patch (601) or covering the application site with clothing (600, 602) reduces this risk. Washing off excess gel from the application site after a short time (<30 min) may reduce the risk of transfer (600, 602-603) but also reduces effective testosterone absorption in some (604) but not all (603) studies. Unlike transdermal patches, topical gel or solutions have considerable misuse and abuse potential.

Testosterone Microspheres
Suspensions of biodegradable microspheres, consisting of polyglycolide-lactide matrix similar to absorbable suture material and laden with testosterone, can deliver stable, physiologic levels of testosterone for 2 to 3 months after intramuscular injection (605-606). Subsequent findings (607) suggest that the practical limitations of microsphere technology such as loading capacity, large injection volumes, and batch variability may be overcome.

Oral Testosterone
Finely milled testosterone (155, 608) or testosterone suspended in an oil vehicle (609-610) have low oral bioavailability requiring high daily doses (200–400 mg) to maintain physiologic testosterone levels. Such a heavy androgen load causes prominent hepatic enzyme induction (611) without hepatotoxicity (612). Although effective in small studies (613), oral testosterone is not commercially available and little used. Sufficiently high oral testosterone doses (400-900 mg daily) also reduce serum SHBG (614) which may explain the concomitant acceleration of testosterone metabolism (155, 613, 615).

Buccal or sublingual delivery of testosterone is an old technology (158) designed to bypass the avid first-pass hepatic metabolism of testosterone that is inevitable with the portal route of absorption. Once absorbed into the general circulation, however, testosterone is rapidly inactivated in accord with its short circulating half-time. Revivals of this technology include testosterone in a sublingual cyclodextrin formulation (616) and in a buccal lozenge (159, 617). The multiple daily dosing required to maintain physiologic testosterone levels are drawbacks for long-term androgen replacement using such products, and their effectiveness and acceptability remain to be established. Like all transepithelial (nonparenteral) testosterone delivery systems, disproportionate amounts of testosterone undergo 5α-reduction during local absorption, resulting in higher blood DHT levels than those in eugonadal men (618). Because intraprostatic DHT is produced locally within the prostate and unlikely to be affected by changes in circulating DHT levels as well as the fact that

prostate diseases remain rare among androgen deficient men receiving androgen replacement therapy, the higher blood DHT levels appear to pose no real risk of accelerating prostate disease (619).
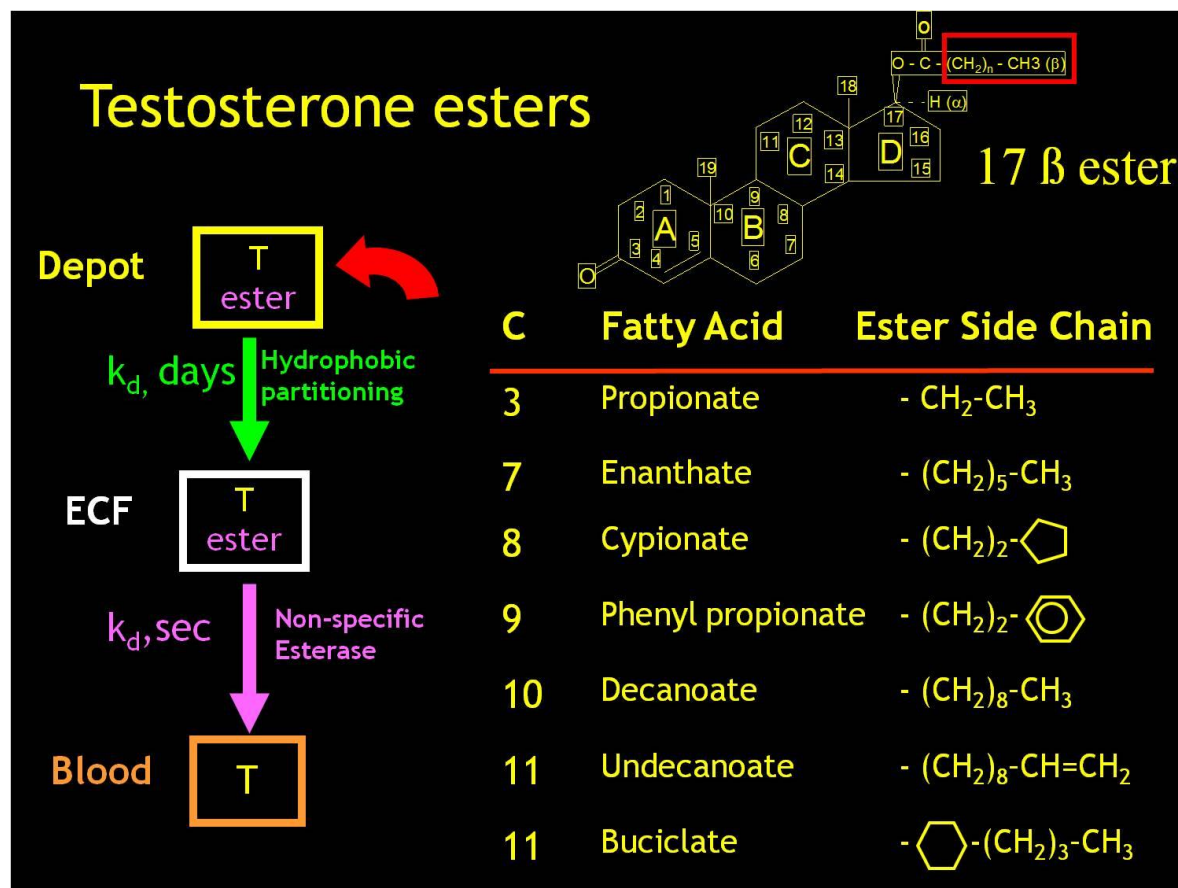
Testosterone Esters

FIGURE 5. Schematic overview of the pharmacology of testosterone esters.
Testosterone is esterified through the 17 β hydroxyl group with fatty acid esters of different aliphatic or other chain length which is a biologically inactive pro-drug. The esterified testosterone in an oil vehicle is injected deeply into a muscle forming a local drug depot from which the testosterone ester is released at a slow rate determined by its physico-chemical partitioning according to the testosterone ester's hydrophobicity. Once the testosterone ester exits the depot and enters the extracellular fluids, it is rapidly hydrolysed by ubiquitous non-specific esterases thereby releasing the testosterone into the general circulation.

Injectable
The most widely used testosterone formulation for many decades has been intramuscular injection of testosterone esters, formed by 17β-esterification of testosterone with fatty acids of various aliphatic and/or aromatic chain lengths, injected in a vegetable oil vehicle (620). This depot product relies on retarded release of the testosterone ester from the oil vehicle

injection depot because esters undergo rapid hydrolysis by ubiquitous esterases to liberate free testosterone into the circulation. The pharmacokinetics and pharmacodynamics of androgen esters is therefore primarily determined by ester side-chain length, volume of oil vehicle, and site of injection via hydrophobic physicochemical partitioning of the androgen ester between the hydrophobic oil vehicle and the aqueous extracellular fluid (621).

The short 3-carbon aliphatic ester side-chain of testosterone propionate gives the product a brief duration of action requiring injections of 25 to 50 mg at 1-2 day intervals for effective testosterone replacement therapy. In contrast, the 7-carbon side-chain of testosterone enanthate has a longer duration of action so that it is used at doses of 200 to 250 mg per 10 to 14 days for androgen replacement therapy in hypogonadal men (622-624) and has been for decades the most widely used form of testosterone used in replacement therapy. Other testosterone esters (cypionate, cyclohexane carboxylate) have simillar pharmacokinetics making them pharmacologically equivalent to testosterone enanthate (625). Similarly, mixtures of short- and longer acting testosterone esters also have essentially the same pharmacokinetics of the longest ester.

Longer acting testosterone esters, testosterone buciclate and undecanoate, intended to provide depot release over months rather than weeks, have been developed. Testosterone buciclate (trans-4-n-butyl cyclohexane carboxylate) is an insoluble testosterone ester in an aqueous suspension that produces prolonged testosterone release due to steric hindrance of ester side-chain hydrolysis slowing the liberation of unesterified testosterone. Although the buciclate ester produces blood testosterone levels in the low-normal physiologic range for up to 4 months after injection in nonhuman primates (626) as well as hypogonadal (627) and eugonadal (628) men, product development has not progressed. Injectable testosterone undecanoate, an ester of an 11 carbon aliphatic fatty acid, in an oil vehicle provides a longer (~12 weeks) duration of action (629-631) now widely marketed as a long-acting injectable depot testosterone product. Due to its limited solubility in the castor oil vehicle, testosterone undecanoate is administered as a 1000 mg dose in a large (4 mL) injection volume at 12 week intervals after the first and one 6 week loading dose or multiple loading doses (632) or in the USA, 750 mg in 3ml volume administered at the start of treatment and then 4 and subsequently at 10 weekly intervals. Once available, the rapid uptake of long-acting injectables show that they are very popular among younger hypogonadal men whereas transdermal products are more suited for older men in case of need to rapidly discontinue testosterone treatment such as after diagnosis of prostate cancer. The relatively long duration of action is also well suited to male hormonal contraception either alone in Chinese men (633) or as part of an androgen-progestin combination (634-636). For treatment of androgen deficiency, although its longer duration of action entails fewer injections with advantages for convenience and compliance, the efficacy and safety does not differ from that of the shorter acting testosterone enanthae (637).

Oral Testosterone Undecanoate
Oral testosterone undecanoate, a suspension of the ester in 40-mg oil-filled capsules, is administered as 160 to 240 mg in two or more doses per day (638). The hydrophobic, long aliphatic chain ester in a castor oil/propylene glycol laurate vehicle favors preferential absorption into chylomicrons entering the gastrointestinal lymphatics and largely bypassing hepatic first-pass metabolism (161). Oral testosterone undecanoate is not absorbed under

fasting conditions but is taken up when ingested with food (639) containing a moderate amount (at least 19 gm) of fat (640). Although oral testosterone undecanoate produces a disproportionate increase in serum DHT which is unaffected by concomitant administration of an oral 5 α reductase inhibitor (641); such modest increases in circulating DHT would have no impact on prostate size (642) or apparent risk of prostate cancer (425, 643) presumably because DHT of extra-prostatic origin fails to increase intra-prostatic DHT concentrations (644). Its low oral bioavailability (645) and short duration of action requiring high and multiple daily doses of testosterone lead to only modest clinical efficacy compared with injectable testosterone esters (624, 646). Widely marketed except in the United States, it may cause gastrointestinal intolerance but has otherwise well established safety (643). Two new formulations of oral testosterone undeanoate have been developed (647) but not yet marketed. Its limitations in efficacy, notably its capricious bioavailability, make it a second choice (624), unless parenteral therapy is best avoided (e.g., bleeding disorders, anticoagulation) or a low dose, as for induction of male puberty, must be provided (648-649) as a better option than the hepatotoxic alkylated androgen, oxandrolone (650).

Synthetic Androgens
Synthetic androgens include both steroidal and non-steroidal androgens. Synthetic steroidal androgens, most developed by 1970, comprise categories of 17α-alkylated androgens, 1-methyl androgens and nandrolone and its derivatives.

17β-OH ester ——→ R

17α-alkyl

GENERIC NAME	YEAR OF PATENT	R (17β)	X (17α)	OTHER MODIFICATIONS		
NATURAL ANDROGENS						
Testosterone		H	H			
5α-Dihydrotestosterone	1960	H	H	4,5-ane		
UNMODIFIED 17β ESTERS						
Testosterone propionate	1941	COCH₂CH₃	H			
Testosterone cypionate	1956	CO(CH₂)₂—⬡	H			
Testosterone enanthate	1958	CO(CH₂)₅CH₃	H			
Testosterone undecanoate	1975	CO(CH₂)₉CH₃	H			
Testosterone buciclate	1987	CO⬡(CH₂)₃CH₃	H			
MODIFIED ANDROGENS						
Methenolone	1958	H	H	4,5-ane	:1,2-ene	:1-CH₃
Nandrolone	1955	H	H	19-norCH₃		
Mesterolone	1962	H	H	4,5-ane	:1α-CH₃	
MENT (7α-methyl nandrolone)	1994	H	H	19-norCH₃	:7α-CH₃	
MODIFIED 17β ESTERS						
Methenolone acetate	1958	COCH₃	H	4,5-ane	:1,2-ene	:1-CH₃
Nandrolone phenylpropionate	1959	CO(CH₂)₂—⬡	H	19-norCH₃		
Nandrolone decanoate	1961	CO(CH₂)₈CH₃	H	19-norCH₃		
17α ALKYLATION						
Methyltestosterone	1945	H	CH₃			
Fluoxymesterone	1957	H	CH₃	9α-F	:11β-OH	
Methandrostenolone	1959	H	CH₃	1,2-ene		
Oxandrolone	1964	H	CH₃	4,5-ane	:C2-replaced by O	
Oxymetholone	1959	H	CH₃	4,5-ane	:2-methyleneOH	
Stanozolol	1962	H	CH₃	4,5-ane	:[2,3-c]pyrazole	:2,3-ene
Danazol	1962	H	C≡CH	2,3-ene	:[2,3-d]isoxazole}	
Norethandrolone	1955	H	CH₂CH₃	19-norCH₃		
Ethylestrenol	1959	H	CH₂CH₃	19-norCH₃	:3-H₂	

FIGURE 6. Testosterone and its pharmacological derivatives. *Listed are the most common synthetic androgens displaying their structural relationship with testosterone.*

Most oral androgens are hepatotoxic 17α-alkylated androgens (methyltestosterone, fluoxymesterone, oxymetholone, oxandrolone, ethylestrenol, stanozolol, danazol, methandrostenolone, norethandrolone) making them unacceptable for long-term androgen replacement therapy. The 1-methyl androgen mesterolone is an orally active DHT analog that undergoes neither amplification by 5α reduction nor aromatization but it is free of hepatotoxicity. Mesterolone is not used for long-term androgen replacement due to the need

for multiple daily dosing, its poorly defined pharmacology (651) and suboptimal efficacy at standard dose (539, 546). For historical reasons, the other marketed 1-methyl androgen methenolone is used almost exclusively in anemia due to marrow failure (652-653) although it has no specific pharmacological advantage over testosterone or other androgens.

Nandrolone (19-nor testosterone) is a widely used injectable androgen in the form of aliphatic fatty acid esters in an oil vehicle mainly for treatment of postmenopusal osteoporosis where it is effective at increasing bone density and reducing fracture rate (654-655). It is also the most popular androgen abused in sports doping and in body building. Nandrolone is a naturally occuring steroid but is not normally secreted in the human bloodstream although it occurs as an intermediate in the aromatization of testosterone to estradiol by the aromatase enzyme (656). This enzyme complex undertakes two successive hydroxylations on the angular C19 methyl group of testosterone followed by a cleavage of the C10-C19 bond to releases formic acid and aromatize the A ring (657). Nandrolone represents a penultimate step of the molecule undergoing aromatization bound to the enzyme complex with the C19 methyl group excised but a still non-aromatized A ring. Paradoxically, despite being an intermediate in the aromatization reaction, nandrolone is virtually not aromatized after parenteral administration in men (658-659), presumably because it is a very poor substrate for the human aromatase enzyme (660). It is susceptible to amplification by 5α reductase with its 5α reduced metabolites being moderately activated in androgenic potency (661). The minimal aromatizability of nandrolone makes it suitable for treatment of osteoporosis in women in whom estrogen therapy is contraindicated due to hormone sensitive cancers (breast, uterus) or for older women, although virilization limits its acceptability (662).

Synthetic nandrolone derivatives 7α-methyl 19-nortestosterone (MENT) (663) and 7α, 11β-dimethyl 19-nortestosterone (dimethandrolone) (664) are potent, non-hepatotoxic androgens. MENT is being developed as a depot androgen (665) for androgen replacement (666) and male contraception in an androgen-progestin combination regimen (667) while dimethandrolone has potential for male contraception as a single steroid with dual androgen and progestin activity (668). As nandrolone derivatives, these synthetic androgens are less susceptible to amplification by 5α-reduction (660, 669) whereby their 5α-reduced metabolites have reduced AR binding affinity (670). Disparities in reported susceptibility to aromatization vary from minimal using a recombinant human aromatase assay (660) whereas greater aromatization is reported using purified human or equine placental aromatase (669, 671-672). The inability of MENT to maintain bone density in androgen deficient men (544) may be due to underdosing rather than an intrinsic feature of this synthetic androgen but illustrates the need for thorough dose titration in different tissues for synthetic androgens that may not possess the full characteristic spectrum of testosterone effects.

Nonsteroidal Androgens
The first nonsteroidal androgen was reported in 1998 (551) and the first placebo-controlled randomized clinical trial in 2013 (673). None are yet approved for clinical use but registration studies are underway for enobosarm (674). Based on structural modifications of the nonsteroidal class of the aryl-propionamide antiandrogens (bicalutamide, flutamide), these compounds offer the possibility of orally active, potent androgens. Subsequently, additional

classes of non-steroidal androgen based on structures including quinolines, hydantoins, tetracyclic indoles and oxachrysensones have been reported. Lacking the classical steroid ring structure such androgens are likely to be not subject to androgen activation either by 5α reductase or aromatization but, if taken orally, subject to first-pass hepatic metabolism. Such hepatic metabolism can eliminate in vivo bioactivity of analogs with potent in vitro androgenic effects (675) whereas metabolically resistant analogs can produce potent and disproportionate androgenic effects on the liver in transit. Many of the novel non-steroidal androgens demonstrate potent androgenic effects experimentally on muscle, bone and sexual function while minimizing prostate effects in experimental animals but none have yet undergone full clinical evaluation. These selective effects may be attributable to the tissue-selective distribution of 5α-reductase as a local tissue, pre-receptor androgen amplification mechanism (676) or more complex mechanisms involving ligand-induced receptor conformation changes and/or post-receptor co-regulator interaction mechanisms such as define the tissue selectivity and agonist/antagonist specificity of non-steroidal estrogen partial agonists (677). These features suggest that non-steroidal androgens have potential for development into pharmacologic androgen therapy regimens as tissue-selective mixed or partial androgen agonists ("selective androgen receptor modulators", SARM) (390, 678). Conversely, they are not ideal for androgen replacement therapy where the full spectrum of testosterone effects including aromatization is ideally required, especially for tissues such as the brain (136, 147) and bone (141) where aromatization is a prominent feature of testosterone action. The clinical efficacy and safety of non-steroidal androgens have yet to be reported and none are yet marketed. Whether the hepatotoxicity of antiandrogens (679-680) will also be a feature of non-steroidal androgens remains to be determined.

Choice of Preparation

The choice of testosterone product for androgen replacement therapy depends on physician experience and patient preference, involving factors such as convenience, availability, familiarity, cost, and tolerance of frequent injections. Preparations of testosterone or its esters are favored over synthetic androgens for all androgen replacement therapy applications by virtue of their long record of safety and efficacy, ease of dose titration and of monitoring of blood levels as well as the possibility that synthetic androgens lack the full spectrum of testosterone effects through pre-receptor tissue activational mechanisms (5α reduction, aromatization). The hepatotoxicity of synthetic 17α-alkylated androgens (311-312) makes them unsuitable for long-term androgen replacement therapy.

Cross-over studies indicate that patients prefer testosterone formulations that maintain stable blood levels and smoother clinical effects. This is best achieved by testosterone products that form effective depots for sustained release such as long-acting testosterone implants (6 monthly) (624) and injectable testosterone undecanoate (3 monthly) (681-682) or shorter-acting daily transdermal gels (70). These are an improvement over the previous standard of intramuscular injections of older testosterone esters (enanthate, mixed esters) in an oil vehicle every 2-3 weeks (622, 624-625) which produce characteristically wide fluctuations in testosterone levels and corresponding roller-coaster symptomatic effects.

There are few well-established formulation or route-dependent differences between various testosterone products once adequate doses are administered. As with estrogen replacement

(683-684), testosterone effects on SHBG are effectively manifestations of hepatic overdose (685) so that oral ingestion of either 17α-alkylated androgens (686) or oral testosterone undecanoate (624) cause prominent lowering of SHBG levels due to prominent first-pass hepatic effects. By contrast long or short acting sustained-action testosterone depot products cause at most minor transient decreases, mirroring blood testosterone levels, or no effects on blood SHBG (559, 606, 624, 627, 681). The more convenient and well tolerated depot testosterone products which maintain steady-state delivery patterns (70, 559, 624, 681-682) are supplanting the older, short-term (2-3 week) injectable testosterone esters (enanthate, mixed esters) as the mainstays of androgen replacement therapy.

Side Effects of Androgen Therapy

Serious adverse effects from androgen replacement therapy using physiological testosterone doses for appropriate indications are rare. This corresponds to the observation that testosterone is the only hormone without a well defined, spontaneously occurring clinical syndrome of hormone excess in men. However, supraphysiological doses of synthetic androgens in pharmacological androgen therapy or the massive doses of androgen abusers as well as unphysiological use of androgens in chidren or women may produce unwanted androgenic side effects. Oral 17α alkylated androgens also risk a wide range of hepatic adverse effects. Virtually all androgenic side effects are rapidly reversible on cessation of treatment apart from inappropriate virilization in children or women in which voice deepening, terminal body hair, or stunting of final height may be irreversible.

Steroidal Effects

Androgen replacement therapy activates physical and mental activity to enhance mood, behavior, and libido, thereby reversing their impairment during androgen deficiency (687). In otherwise healthy men, however, additional testosterone at doses equivalent to testosterone replacement doses (eg for male contraception or andropause) mood or behaviour changes are not evident (325, 688-694) or minimal (636). Even among healthy young men having very high androgen doses there are few mood or behaviour changes (459, 695-698) except for a small minority (~5%) of paid clinical trial vounteers who display a hypomanic reaction, reversible on androgen discontinuation (459). However, such adverse behavioural reactions were not observed in larger studies of testosterone administration to unpaid healthy men (633, 636, 699-700). The higher prevalence of adverse behavioral effects reported among androgen abusers may be related not only to the massive androgen doses but also to high levels of background psychological disturbance (498), drug habituation (528), and anticipation (701) which predispose to behavioral disturbances reported during this form of drug abuse (687, 702).

Excessive or undesirable androgenic effects may be experienced during androgen therapy due to intrinsic androgenic effects in inappropriate settings (e.g., virilization in women or children). In a few untreated hypogonadal men, mainly in newly diagnosed older men, initiation of androgen treatment with standard doses occasionally produces an unfamiliar and even intolerable increase in libido and erection frequency. If this occurs, more gradual acclimatization to full testosterone dose with counseling of men and their partners may occasionally be helpful but usually adequate advice before starting treatment is sufficient.

Seborrhea and acne are commonly associated with high androgen levels in either the steep rise in endogenous testosterone during puberty or among androgen abusers. In contrast to the predominantly facial distribution of adolescent acne, androgen-induced acne with onset well after puberty is characteristically truncal in distribution and provides a useful clinical clue to androgen abuse (703). Acne is unusual during testosterone replacement therapy being mainly restricted to a few susceptible individuals during establishment of treatment with shorter-acting intramuscular testosterone esters, probably related to their generation of transient supraphysiologic testosterone concentrations in the days after injection (539, 622). Acne is rare with depot testosterone products that maintain steady-state physiologic blood testosterone levels. Androgen-induced acne is usually adequately managed with topical measures and/or broad-spectrum antibiotics, if required, with either dose reduction or a switch to steady-state delivery (gel, long-acting injectable) that avoids supraphysiologic peak blood testosterone concentrations. Increased body hair and temporal hair loss or balding may also be seen even with physiologic testosterone replacement in susceptible men.

Modest weight gain (up to 5kg) reflecting anabolic effects on muscle mass is also common. Gynecomastia is a feature of androgen deficiency but may appear during androgen replacement therapy, especially during use of aromatizable androgens such as testosterone that increase circulating estradiol levels at times when androgenic effects are inadequate (e.g., a too low or infrequent dose or unreliable compliance with treatment).

Obstructive sleep apnea causes a mild lowering of blood testosterone concentrations that is rectified by effective continuous positive airway pressure treatment (704). Although testosterone treatment has precipitated obstructive sleep apnea (705) and has potential adverse effects on sleep in older men (706), the prevalence of obstructive sleep apnea precipitated by testosterone treatment remains unclear. The risk is rare in younger androgen deficient men, but is higher among older men with the steeply rising background prevalence of obstructive sleep apnea with age. Hence, screening for obstructive sleep apnea by asking about daytime sleepiness and partner reports of loud and irregular snoring, especially among overweight men with large collar size, is wise for older men starting testosterone treatment but not routinely required for young men with classic androgen deficiency.

Hepatotoxicity
Hepatotoxicity is a well-recognized but uncommon side effect of 17α-alkylated (311) whereas the occurrence of liver disorders in patients using non-17α alkylated androgens such as testosterone, nandrolone and 1-methyl androgens (methenolone, mesterolone) are no more than by chance (312). This is consistent with the evidence of direct toxic effects on liver cells of alkylated but not non-alkylated androgens (707). The risk of 17α alkylated androgen-induced hepatotoxicity is unrelated to the indication for use, although association with certain underlying conditions may be related to intensity of diagnostic surveillance (312). It is possible, but unproven, that the risks are dose-dependent although relatively few cases are reported among women using low dose methyl-testosterone (708-709) while clinical management of children using the alkylated androgen oxandolone often omits liver function tests. However, even if the risks are dose-dependent, the therapeutic margin is narrow. By contrast, the rates of hepatotoxicity among androgen abusers who typically use supraphysiological, often massive, doses remain difficult to quantify due to underreporting of

the extent of illicit usage and dosage but abnormal liver function tests are common in androgen abusers when checked incidentally as part of other health evaluation.

Biochemical hepatotoxicity may involve either a cholestatic or hepatitic pattern and usually abates with cessation of steroid ingestion. Elevation of blood transaminases without gamma-glutamyl transferase may be attributable to rhabdomyolysis rather than to hepatotoxicity if confirmed by increased creatinine kinase (710). Major hepatic abnormalities are related to use of 17-alkylated androgens include peliosis hepatis (blood-filled cysts) (711) and hepatic rupture, adenoma, angiosarcoma (712-713) and carcinoma; however, these risks do not apply to testosterone or other nonalkylated androgens such as nandrolone or 1-methyl androgens. Prolonged use of 17α-alkylated androgens, if unavoidable, requires regular clinical examination together with biochemical monitoring of hepatic function, the latter not required for non-alkylated androgens. If biochemical abnormalities are detected, treatment with 17α-alkylated androgens should cease and safer androgens may be substituted without concern. Where structural lesions are suspected, radionuclide scan, ultrasonography, or abdominal computed tomography scan should precede hepatic biopsy during which severe bleeding may be provoked in peliosis hepatis. Because equally effective and safer alternatives exist, the hepatotoxic 17α-alkylated androgens should not be used for long-term androgen replacement therapy. By contrast, pharmacological androgen therapy often uses 17α alkylated androgens for historical reasons rather than the non-hepatotoxic alternatives. In these situations, the risk-benefit analysis needs to be judged according to the clinical circumstances.

Formulation-Related Effects

Complications related to testosterone products may be related to dosage, mode of administration or idiosyncratic reactions to constituents. Intramuscular injections of oil vehicle may cause local pain, bleeding, or bruising and, rarely, coughing fits or fainting due to pulmonary oil microembolization (POME) (714) as a minor variant of accidental self-injection oil embolism (715-716). In a study of over 3000 consecutive injections by experienced nurses, POME occurred at a rate of ~2% (717) but is often unrecognised or under-reported (718) due to the transient symptoms. There was also no bruising or bleeding reported even among men using anticoagulants and/or antiplatelet drugs (upper confidence limit of risk ~1%) (717). Inadvertent subcutaneous administration of the oil vehicle is highly irritating and may cause pain, inflammation, or even dermal necrosis. Allergy to the vegetable oil vehicle (sesame, castor, arachis) used in testosterone ester injections is very rare, and even patients allergic to peanuts may tolerate arachis (peanut) oil. Self-injection by body-builders of large volumes of sesame or other oils may cause exuberant local injection site reactions (719) or even oil embolism (715). Long-term fibrosis at intramuscular injection sites might be expected but has not been reported. Oral testosterone undecanoate may causes gastrointestinal intolerance due to the castor oil/propylene glycol laurate suspension vehicle. Testosterone implants may be associated with extrusion of implants or bleeding, infection, or scarring at implant sites (563). Parenteral injection of testosterone undecanoate (681) or biodegradable microspheres (607) involves a large injection volume that may cause discomfort. Transdermal patches applied to the trunk cause skin irritation in most men, some with quite severe burn-like lesions (578, 720) with a significant minority (~20%) are unable to continue use . Skin irritation may be reduced in prevalence or ameliorated by concurrent use of topical corticosteroid cream at the application site (579) while transdermal testosterone

gels (540) or solution (590) are rarely irritating. Topical testosterone gels can cause virilization via transfer of androgens through topical skin-to-skin contact with children (594-599) or sexual partners (591-592). These problems can be avoided by covering the application site with clothing or washing off excess gel after a short time (603).

Monitoring of Androgen Replacement Therapy

Monitoring of androgen replacement therapy involves, primarily, clinical observations to optimize androgen effects including ensuring the continuation of treatment and surveillance for side effects. Once testosterone dosage is well established, androgen replacement therapy requires only very limited, judicious use of biochemical testing or hormone assays to verify adequacy of dosage when in doubt or following changes of product or dosage. Testosterone and its esters at conventional doses for replacement therapy are sufficiently safe not to require routine biochemical monitoring of liver, kidney or electrolytes.

Clinical monitoring depends on serial observation of improvement in the key presenting features of androgen deficiency. Androgen-deficient men as a group may report subjective improvement in one or more of a variety of symptoms (some only recognized in retrospect) including energy, well-being, psychosocial drive, initiative, and assertiveness as well as sexual activity (especially libido and ejaculation frequency), increased truncal and facial hair growth and muscular strength and endurance. Individual men will become familiar with their own leading androgen deficiency symptom(s), and these appear in predictable sequence and at consistent blood testosterone thresholds towards the end of any treatment cyce (286, 721). Subjective symptoms of genuine androgen deficiency are alleviated quickly, typically within 3 weeks and reach plateau within 2-3 months (722) whereas persistent symptoms after 3 months may represent placebo responses reflecting the non-specificity of androgen deficiency symptoms and the unusually prominent expectations in the community for testosterone treatment. Objective and sensitive measures of androgen action are highly desirable but not available for most androgen-responsive tissues (723). The main biochemical measures available for monitoring of androgenic effects include hemoglobin and trough reproductive hormone (testosterone, LH, FSH) levels. In androgen deficient men, hemoglobin typically increases by ~10% (or up to 20 g/L) with standard testosterone doses (323, 539, 724). Excessive hemoglobin responses (hematocrit ≥0.54, or ≥0.50 with higher risk of cardio- or cerebrovascular ischemia) occur as a rare (~1%) idiosyncratic reaction which is more frequent at older age (323) explaining the higher prevalence of polycythemia in older testosterone-treated men (725). Testosterone-induced polycythemia is dose-dependent (323, 726) being related to the supraphysiological peak blood testosterone levels observed with shorter-acting testosterone ester injections (539) or trough blood testosterone during treatment with injectable testosterone (726) although it can occur at high enough androgen doses in older men even with transdermal products (727). Such androgen-induced secondary polycythemia is characteristically negative for *JAK2* mutations distinguishing it from primary polycythemia rubra vera (728) and usually resolves with reducing testosterone dose and/or switching to more steady-state testosterone delivery systems (implants, injectable testosterone undecanoate or transdermal gel) (729) and only very rarely is venesection and/or anticoagulation required. Circulating testosterone and gonadotropin levels must be considered in relation to time since last testosterone dose. Trough levels (immediately before next scheduled dose) may be helpful in establishing adequacy of depot

testosterone regimens. In the presence of normal testosterone, negative feedback on hypothalamic GnRH and pituitary LH secretion (in men with hypergonadotropic hypogonadism), plasma LH levels are elevated in rough proportion to the degree of androgen deficiency. In severe androgen deficiency, virtually castrate LH levels may be present, and, conversely, circulating LH levels provide a sensitive and specific index of tissue testosterone effects (559, 622) especially with more steady-state testosterone delivery by depot-type products. Suppression of LH into the eugonadal range indicates adequate androgen replacement therapy, whereas persistent nonsuppression after the first few months of treatment is an indication of inadequate dose or pattern of testosterone levels. In hypogonadotropic hypogonadism, however, impaired hypothalamic-pituitary function diminishes circulating LH levels regardless of androgen effects, so blood LH levels do not reflect tissue androgenic effects.

Blood testosterone measurements are valuable before treatment for diagnosis and after start of treatment to check adequacy of dosage if in doubt and, during long-term treatment, only to evaluate changes in treatment dosage or product. During depot testosterone treatment which achieves quasi steady-state blood testosterone levels, trough blood testosterone levels taken prior to the next dose may detect patients whose treatment is suboptimal and whose dose and/or treatment interval need modification. Blood testosterone levels are not helpful for monitoring of oral testosterone undecanoate while pharmacological androgen therapy using any synthetic androgens would lower endogenous blood testosterone levels. Serial evaluation of bone density (especially vertebral trabecular bone) by dual photon absorptiometry at 1- to 2-year intervals may be helpful as a time-integrated measure to verify the adequacy of tissue androgen effects (391, 541).

Although chronic androgen deficiency protects against prostate disease (118, 730-731), prostate size of androgen-deficient men receiving androgen replacement therapy is restored to, but does not exceed, age-appropriate norms (732-733). Even prolonged (2 years) high doses of exogenous DHT did not significantly increase age-related prostate growth in middle-aged men without known prostate disease (642). Between-subject variability in response to testosterone replacement is partly explained by genetic sensitivity to testosterone, which is inversely related to length of the CAG triplet (polyglutamine) repeat polymorphism in exon 1 of the androgen receptor (206). Furthermore, because neither endogenous blood testosterone nor circulating levels of other androgen predicts subsequent development of prostate cancer (425), maintaining physiologic testosterone concentrations should ensure no higher rates of prostate disease than eugonadal men of similar age (734).

The potential long-term risks for cardiovascular disease of androgen replacement and pharmacologic androgen therapy remain uncertain. Although men have two to three times the prevalence (401) as well as earlier onset and more severe atherosclerotic cardiovascular disease than women, the precise role of blood testosterone and of androgen treatment in this marked gender disparity is still poorly understood (280). Although low blood testosterone concentration is a risk factor for cardiovascular disease and testosterone effects include vasodilation and amelioration of coronary ischemia as well as potentially deleterious effects, it is not possible to predict the net clinical risk-benefit of androgen replacement therapy on cardiovascular disease. Hence, during androgen replacement therapy, it is prudent to aim at

maintaining physiologic testosterone concentrations and surveillance of cardiovascular and prostate disease should be comparable with, and no more intensive than, that for eugonadal men of equivalent age (734). The effects of pharmacologic androgen therapy, in which the androgen dose is not necessarily restricted to eugonadal limits, on cardiovascular and prostate disease are still more difficult to predict, and surveillance then depends on the nature, severity, and life expectancy of the underlying disease.

Contraindications and Precautions for Androgen Replacement Therapy

Contraindications to androgen replacement therapy are prostate or breast cancer, because these tumors may be androgen responsive, and pregnancy, in which transplacental passage of androgens may disturb fetal sexual differentiation, notably risking virilization of a female fetus.

The Nobel Prize-winning recognition in the 1940's that prostate cancer was androgen dependent led to castration being ever since the main treatment for advanced prostate cancer for which it prolongs life but is not curative. This approach led to long-held concern about testosterone treatment of men with advanced prostate cancer (257) for fear of relapse, based however, largely on anecdotal observations (735-736). Recent studies have challenged this belief as intermittent rather than sustained androgen blockade (737), rapid androgen cycling (738), androgen priming (739-740) or even testosterone administration (741-742) have all shown promising, albeit counter-intuitive, results. Meta-analyses suggest that neither ambient circulating testosterone concentrations nor testosterone treatment predict future prostate cancer (425-426, 743). Furthermore, the increasing diagnosis of organ-confined prostate cancer detected by PSA screening among younger men requires different considerations including the continuation of testosterone replacement therapy following curative treatment of the prostate cancer with careful monitoring (744-746). This is consistent with the fact that endogenous circulating androgens (testosterone, dihydrotestosterone) do not predict subsequent prostate cancer (425) and even prolonged (2 year) administration of high doses of exogenous DHT does not accelerate mid-life prostate growth rate in middle-aged men without prostate disease (642) presumably because exogenous DHT does not increase intra-prostatic androgen concentration (644). Hence local, organ-confined prostate cancer following treatment with curative intent may be an exception to the otherwise absolute contraindication to testosterone for men with a diagnosis of prostate cancer.

Precautions and/or careful monitoring of androgen use are required in (1) initiating treatment in older men with newly diagnosed androgen deficiency who may experience unfamiliar and intolerable initial changes in libido; (2) men subject to occupational monitoring by drug testing (including elite athletes) who may be sanctioned or disqualified for drug use; (3) androgen deficient men with residual spermatogenesis who are planning fertility in the near future who may wish to delay or bank sperm prior to starting treatment; (4) women of reproductive age, especially those who use their voice professionally, who may become irreversibly virilized; (5) prepubertal children in whom inappropriate androgen treatment risks precocious sexual development, virilization and premature epiphyseal closure with compromised final adult height; (6) patients with bleeding disorders or those undergoing anticoagulation or antiplatelet treatment when parenteral administration may cause severe

bruising or bleeding; (7) sex steroid–sensitive epilepsy or migraine; and (8) older and especially obese men with subclinical obstructive sleep apnea.

Some traditional warnings about risks of androgen treatment which appear on older product information appear to be rarely or never observed in modern clinical practice. An example of this is hypercalcemia, originally described during pharmacological androgen therapy for advanced breast cancer with metastases (747) although direct causation was not well established (748), but this not been reported with androgen use for other indications. Similarly, fluid overload from sodium and fluid retention due to cardiac or renal failure or severe hypertension is rare and probably confined to high dose pharmacological androgen therapy (747) whereas controlled clinical trials suggest androgens may improve cardiac function and quality of life (365), rather than having detrimental effects, in men with chronic heart failure.

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