

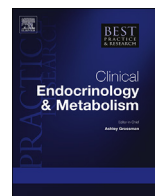


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History of androgens and androgen action



Those who don't know their history are condemned to repeat it (Santayana), first as tragedy and then as farce (Marx) and, even when it does not exactly repeat itself, it often rhymes (Twain). But what we learn most from history is that we do not learn from history (Hegel)

History is bunk (Sir Alan Parkes, after Henry Ford)

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Introduction

Progress in science consists of discoveries and inventions enlivened not just by the epiphany of insight and the bright radiance of enlightenment, but also by dark blind alleys and false leads. Far from the simplistic linear progress from the darkness to the light, at any one moment in real time, it is not always clear, and easy to mistake, a new high road for a doomed *cul de sac*. This review will try to highlight both the pathways to ultimate success over time as well as some blind alleys, not all of which are yet abandoned as dead ends in the history of androgens and of androgen action.

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Pre-history

The concept that the testis was the seat of masculinity, virility and fertility has ancient roots. Over millennia, it has been common knowledge from domesticating and farming animals that castration produced more docile, sterile male animals. Perceptive, timeless observations of the effects of castrating male animals were recorded by Aristotle with astonishing accuracy in the 4th century BC (cited at length by Wilson [1]) with comparable knowledge is likely to have been discovered in non-European cultures [1,2]. That received wisdom was applied throughout human history into the early 20th century in Europe for preserving operatic singing voice [3] as well as in other cultures to create asexual prisoners of war, loyal harem guardians and civil servants and to punish sexual violators among other ritual practices in many cultures [4,5].

Using proto-scientific logic, these traditional observations were interpreted as that substance(s) originating from the testis were responsible for the somatic physical and psychological features that create the distinctive sexual dichotomy of males and females. Contemporaneous with these intuitive beliefs was superimposed an equally ancient wishful thinking about finding ways to revive youthful



Fig. 1. Painting of the **Fountain of Youth** by Lucas Cranach the elder. Held in the Gemäldegalerie, Staatliche Museen zu Berlin, Berlin. Reproduced with permission. For details see text. **Cranach, Lucas (der Ältere), Der Jungbrunnen, 1546, Cat. no. 593** Staatliche Museen zu Berlin, Gemäldegalerie/Jörg P. Anders.

vigor and virility in ageing men, reversing the lamentable declines of older age. The ancient fantasies of rejuvenation represent the product of humanity's cognitive dissonance between an unrequited desire for immortality confronting the inevitability of death. The demise of the ageing body is tangible evidence of a person's decline towards death, a foretaste of dying's waiting room, from which one's imagination cannot be averted. It is therefore no surprise that the age-old wishful thinking about revival of youthful vigor flourishes most avidly when there is sufficient leisure and affluence to facilitate pursuit of health hobbies such as life extension. Within that framework, the rejuvenation mystique forms an overarching canopy under which wishful thinking and the imagination reigns supreme over real, if mundane, evidence. As an unending quest, eruptions of the rejuvenationist mystique have appeared repeatedly in the past in such episodes as the Fountain of Youth and the Organotherapy fads (see Fig. 1).

..Locomotor Ataxia, Neurasthenia..
AND OTHER NERVOUS DISEASES.

EXTRACTS OF ANIMAL ORGANS.
GRAY MATTER, TESTICLE EXTRACT,

Prepared at the **New York Biological and Vaccinal Institute**, according to the method of **PROFESSOR BROWN-SEQUARD.**

If the treatment of Locomotor Ataxia, Neurasthenia, and other nervous diseases with "**Extracts of Animal Organs**," has not obtained in America the great favor that it enjoys in Europe, it is chiefly owing to the numerous unreliable preparations of so-called "**Extracts**" which have been placed on the market.

Physicians desirous to try the injections of fresh and reliable extracts, may obtain them from the **New York Biological and Vaccinal Institute**, at the following prices:

TESTICLE EXTRACT, 1 vial, 25 c. c.,	\$2.50.
GRAY MATTER,	2.50.
SPECIAL SYRINGE, 3 c. c.,	2.50.

Literature sent on application.

NEW YORK BIOLOGICAL AND VACCINAL INSTITUTE,
Pasteur Institute Building, 1, 3, 5 and 7 West 97th Street, New York, N. Y.

Advert for organ extracts, Bulletin of the Pasteur Institute, New York, 1897

Fig. 2. Advertisement for public sale of extracts of animal organs by the method of Professor Brown-Sequard and sold by the Pasteur Institute. New York in 1897.

Among the best-known examples was the 16th century expeditions of Ponce de Leon in the Caribbean which landed in Florida in search of the fabled Fountain of Youth, depicted famously by Lucas Cranach 1546 painting as legendary spring waters which magically restore youthfulness [6]. Cranach's painting depicted old crones entering the magical Fountain to emerge on the other side as nubile bodies. In the assumed male gaze of the time, it may have seemed sufficient for revitalization of men that the female form alone be restored to youthfulness. This voyage, possibly as a lucrative sideline to piracy, was sponsored by the King of Spain who had recently married a woman 35 years his junior.



Fig. 3. Contemporary cartoons satirising Brown-Sequard's elixir (Left) From Puck magazine 1889, labelled "It beats Brown-Sequard – Tanner's elixir of life for pension grabbers only". (Right) From American magazine Judge, 31 August 1889. Bert Hansen Collection, Harvey Cushing/John Hay Whitney Medical Library of Yale University.

Even if that tale is apocryphal [6], its elaborate embroidery over time since reveals the underlying widespread popularity of fantasies that repel ageing.

The most elaborate eruption of medical revivalism was the era of organotherapy, a rejuvenation quackery fad over the turn of the 20th century. Ushered in by Charles-Edouard Brown-Sequard's 1889 announcement that self-injection of his aqueous extract of animal testes produced miraculous, prolonged revival of his mental and physical health [7]. Although now debatably considered a flawed pioneer of internal medicine by some [8], Brown-Sequard had retired from a distinguished career as Claude Bernard's successor at the Collège De France which included discoveries of hormones in tissue extracts. At a post-retirement meeting, he announced that self-injection of his extract of animal testes produced months of resurgent energy and restoration of youthful health and vigor [7]. Derided as fantasy by contemporary transatlantic professional colleagues [9–12] who reported testing it on unaware patients without benefits and with even fatal consequences [13]. Subsequently, this was proven to be a placebo response by modern replication of Brown-Sequard's carefully described methods showing his aqueous extracts contained negligible amounts of hydrophobic testosterone [14].

Nevertheless, based on his report, *methode sequardienne* became a phenomenally popular commercial success for male rejuvenation [15,16] boosted by a veneer of scientific respectability reflecting Brown-Sequard's medical prestige. Soon after, competing methods of rejuvenation emerged in Europe based on Steinach's unilateral vas ligation [15] and Voronoff's grafting of non-human testis slices onto the testis capsule [17,18]. Using his ineffective surgical technique, Steinach was reputed to have operated on 100 University professors including Freud as well as the great Nobel Prize-winning Irish poet WB Yeats [16]. In America, organotherapy and rejuvenation surgery practitioners undertaking testis transplantation from executed convicts, suicides, or accidental deaths as well as non-human animals also appeared in the early 20th century [19,20]. Influential advocates included Frank Lydston who apparently emulated Brown-Sequard by self-experimentation [21] using testes from a young man dying accidentally [22] with reporting of subjective benefits in a small series of uncontrolled experiments [23]. A prominent advocate was the San Quentin prison doctor Leo Stanley [24], whose work drawing on reports by Brown-Sequard, Voronoff and Lydston, experimented with testis transplantation from executed prisoners and, when human supplies were too scarce, animal testes [25]. These were avascular tissue implants or an injected slurry of homogenized testis parenchyma with some proportion observed to be gradually absorbed or to slough off and suppurate leaving only necrotic tissue [26]. Stanley believed that transplanting testis tissue was preferable to testis extracts as they might last longer in the body [21] unaware of the immunological consequences of allografting and his uncritical reporting of results [21] would now be considered implausible or non-therapeutic. Such popular innovations were taken up with flourish by men lesser integrity including the notorious John R Brinkley, an audacious charlatan who developed entrepreneurial scheme in the early 20th century to market surgical rejuvenation by testis transplantation [27].

Non-Western beliefs in the origins of the testicular hormone(s) are often overlooked. In ancient Chinese medicine, Daoist alchemy is reported to have included using extracts of placenta or urine for treatment of probable hypogonadism [28]. In several Chinese dynasties, Emperors had been pursuing legendary “immortality herbs” which ensured eternal life [29]. Most impressively, the astonishing 15th century seafaring expeditions of the famous eunuch Admiral Zheng He involved 7 ocean voyages over 28 years (1405–1433). Commissioned by the Ming Emperor, Zhu Di, Zheng He's armada of over 37,000 men (including 180 doctors) and 62 large “treasure” ships [30], far larger than European ships of that time, had a mission to collect worldly tribute to their Emperor. The voyages also renewed the search for an elixir of youth to ensure the ruler's longevity [28,29]. After that Emperor's demise, however, all seafaring expeditions were cancelled (see Figs. 2, 3).

As quickly as organotherapy emerged over the turn of the 20th century [19,31,32], all these rejuvenation fads disappeared abruptly in the 1930's. At that time, the Depression removed discretionary spending on frivolous hobbies coinciding with the 1935 discovery of testosterone, fatally undermining organotherapy's façade of scientific credibility. However, rather than vanishing forever, the impulse for rejuvenation merely went into decades-long hibernation to re-emerge over the turn of the 21st century under a variety of synonymous neologisms including “andropause”, “LowT”, “male menopause”, “climacteric”, “viropause”, **“partial androgen deficiency in the aging male”**, “late-onset hypogonadism”, and “functional hypogonadism” all seeking medical gravitas for this invented “disease”. The

pre-scientific faith, prevailing remarkably in the face of 21st century medical science, that male ageing can be considered a treatable testosterone deficiency state has led to the 100-fold increase in largely evidence-free, off-label testosterone prescribing over the last 3 decades [33]. This quasi-epidemic of testosterone prescribing, mainly as an anti-ageing and sexual tonic for middle-aged and older men, persisted despite the absence of convincing evidence or medical indications [34]. Virtually all aspects of the body deteriorate with ageing, including all endocrine systems as well as non-reproductive organs and tissues. Beyond simple wear-and-tear deterioration, speculative theories about possible more fundamental drivers of ageing abound, including many elaborate and specific mechanistic theories [35]. Projecting beyond this, the rejuvenationist fantasy connects the almost universal decline of virility and energy during male ageing with a possible decrease in testicular function. In that framework, this makes testosterone treatment seem a logical remedy, a speculation reinforced by superficial analogies with menopause or authentic hypogonadism in young men, neither of which is valid on any careful analysis.

Discovery of testosterone: birth of modern androgen physiology

The earliest scientific findings that gave objective credibility to the speculated role of the testis as the source of a virilizing principle originated in the 18th century with the unpublished testis transplantation experiments of Hunter in the course of exploring the feasibility of tissue transplantation [36]. Subsequently, Berthold's experiments published in 1849 proved that transplanting testis into castrated roosters or hens caused appearance of the masculine characteristic of comb and wattle. However, the nature of the testicular substance remained entirely unknown till the early 20th century.

The modern history of androgen physiology dates from the discovery of testosterone as the principal mammalian androgen in 1935 [37]. This was the culmination of a race in the early decades of the 20th century to identify the cardinal sex steroids, including estradiol and progesterone, both identified before testosterone. Using whole animal androgen bioassays (see below) to guide the purification of the principal androgen from biological fluids, in 1931 the German scientist Adolf Butenandt, a University academic funded by the Schering pharmaceutical company, isolated androsterone from human urine believing it to be the sought-after testicular substance. However, androsterone is an oxidized metabolite of testosterone although its residual androgenic bioactivity (~10% of testosterone) allowed its purification despite the misidentification, due to the limitation of the non-specific whole animal androgen bioassay which cannot distinguish between different androgens. It was soon apparent from androsterone's low androgenic potency and minimal levels in testis that it was not the native testicular substance being sought. The correct isolation, identification and naming of testosterone was first reported in 1935 by Dutch scientist Ernst Laqueur's group working for the Organon pharmaceutical company. Based in Organon's Oss facility, located for its proximity to abattoirs that provided ample source material for purification of animal insulins, Lacquer reasoned correctly from the higher androgenic potency of testicular extracts compared with urine-purified materials, that the testicular hormone was rapidly metabolized after secretion and excreted in urine as largely inactive metabolites. Hence, he made the successful choice to purify testosterone from extracts of testis for which his location was convenient, instead of the 25,000 L of human urine that Butenandt had used as starting material for his purification of androsterone. Later in the same year (1935), Butenandt [38] and, independently, Leopold Ruzicka's Swiss group from Ciba-Geigy [39], confirmed the structure and reported the synthesis of testosterone finally confirming its identity as the principal human androgen. The 1939 Nobel Prize in Chemistry was awarded to Butenandt and Ruzicka although Laqueur was not even nominated for that year's Prize. Previously, Butenandt had been nominated 17 times (1934–39) and Ruzicka 22 times (1931–9) for Nobel Prizes, whereas Laqueur was nominated only 4 times before, and once after, the 1939 Prize. Following the award of the 1935 Nobel Peace Prize to a German pacifist, Hitler decreed in 1937 that no German could accept a Nobel Prize which prevented Butenandt from receiving his share of the Prize until 1949. The award of a Nobel prize for characterizing testosterone to two German-speaking scientists but omitting the Dutch Jew, who first isolated and named it, is striking. How much the tense, gathering storm of the imminent pre-war atmosphere influenced that decision remains a speculation among other anomalies of Nobel Prize awards.

The discovery of the other major mammalian androgen, dihydrotestosterone (DHT), was only achieved more than 30 years later in Jean Wilson's lab [40,41] as the last major canonical steroid hormone to be characterized. DHT is the most potent, pure natural androgen and is produced mainly locally within androgen target tissues as a form of paracrine amplification of androgen action such as the prostate, liver, skin and hair follicles [42] with subsequent spillover into the circulation. DHT is formed by the irreversible conversion from testosterone via the action of 5α reductase enzymes 1 and 2 [43,44], a dual isozyme system first fully identified in Daryl Russell's lab [45]. This rectified the original misidentification of the type 1 isozyme in a report which prematurely assumed it was THE 5α -reductase expressed in the prostate [46], a mistake that became evident when patients with genetic 5α -reductase deficiency proved to have normal levels of the type 1 enzyme [47]. These discoveries of local tissue-based amplification of androgen action laid the foundations for a new class of steroid enzyme inhibitors, the 5α reductase inhibitors, that have found a therapeutic role for treatment of prostate disease and alopecia (see below). More recent discoveries have identified multiple alternative pathways for DHT production bypassing testosterone [48–50], including finding a role for 11 keto testosterone in mammals long after it was known as a major fish androgen [51]. The ultimate significance of these recent findings, which might equally identify novel targets for modulating androgen action, are yet to be fully unraveled.

Androgen physiology and genetics

Prior to discovery of testosterone in 1935, most experiments investigating androgen action were confined to analysis of castration effects for inferring androgen action. The discovery of testosterone created new opportunities for experimental analysis of androgen action adding an important new dimension of testosterone replacement to castration experiments. As a result, descriptive experiments using the new androgen accrued rapidly before, and increasingly after, the hiatus of World War II. An early pioneer of androgen physiology, Charles D Kochakian, an American of Armenian background, edited the landmark 1976 *Anabolic-Androgenic Steroids* [52] which comprehensively summarized knowledge of experimental and clinical androgen physiology till the mid-1970s. Contributing seven of 12 chapters on experimental androgen physiology from his wide-ranging research since the 1930s, he was the first to prove the anabolic effects of testosterone on nitrogen retention and linking androgen action with muscle physiology. He was also a major proponent of the hypothesis that the anabolic effects of androgens could be separated from androgenic (virilizing) effects, a proposal he later admitted failed [53]. Many others contributed to Kochakian's compendium on the descriptive clinical and experimental features of androgen action in growth and development of males as well as, more speculatively, on various androgen effects in non-reproductive organ functions and in women.

Astute clinical observations had long led to understanding the genetic origins of classical phenotypes of impaired androgen action long before the genes involved were identified. Key informative paradigms were reported by Fuller Albright with his fellow Harry Klinefelter describing Klinefelter's syndrome in 1942 [54], a German eugenicist describing Kallmann's syndrome in 1944 (recapitulating the original 1856 report of a Spanish physician-anatomist Maestre de San Juan [55]), androgen insensitivity first described by John Morris in 1953 [56], 5α reductase type 2 deficiency identified by Julianne Imperato-McGinley in 1979 [57] and the first probable androgen receptor (AR) coregulator deficiency in 2000 by Nawata and Yanase [58]. Strikingly, thanks to modern genetic technologies, such as massive parallel sequencing and genome-wide association studies, the gap in time between clinical description and gene discovery has dramatically narrowed as the size of groups responsible for discoveries and inventions has equally dramatically expanded.

The pathophysiology of androgen effects on mood, behavior and sexual function are the subject of a vast but largely inconclusive literature making it hard to discern unequivocal and durable landmarks in human androgen physiology. While many clever psychological paradigms are employed, the inaccessibility of the brain tissue and reliance on observational studies of healthy eugonadal individuals using convenient but unreliable salivary testosterone measurement - rather than interventional studies using full block-replace paradigm [59–61] and LCMS measurements - have thwarted objective progress leaving opaque even headline issues of androgen action on psychological state and trait.

Androgen receptor

The single most important advance in understanding androgen action has been the molecular characterization of the androgen receptor which developed over decades as the genetic technologies improved. The first key discovery was by Mary Lyon of the X-linked gene for androgen (in)sensitivity in *tfm* mice [62,63], subsequently proven to be the AR gene. The most salient advances in understanding androgen action in the 20th century arrived through the identification and characterization by cloning of the AR gene by four independent groups in 1988–9 [64–67]. This facilitated discoveries of genetic mutations in AR creating a wide spectrum of androgen insensitivity (once known as *testicular feminization*) ranging from mild to complete with a broad category of partial in between [68]. There are over 1000 mutations reported in the Androgen Receptor Database maintained by Bruce Gottlieb (<http://www.androgendb.mcgill.ca/>) the high number reflecting that the AR is essential for reproduction but is not required for life so that an inactive AR is compatible with a normal life expectancy. The most severe form, complete androgen insensitivity syndrome, and some of the partial androgen insensitivity syndromes form important components of the range of 46XY Disorders of Sexual Development (DSD) characterized by impaired androgen action in a genetic male body [69].

In addition, the cloning of the AR gene also facilitated experimental studies by multiple groups rapidly defining the functional topography of the receptor with its distinct DNA and ligand binding domains, hinge region and a large N terminal domain regulating gene expression and activation in experimental studies [70,71] and from natural mutations [72,73]. By 1995 functional studies of AR conformation identified the chaperoned, inactive AR residing in the cytoplasm prior to androgen ligand binding leading to shedding the chaperone and translocation to the nucleus. There in the homodimerized antiparallel state, the receptor binds to AR-specific nucleotide motifs in androgen-target genes [74]. Further research identified a large retinue of coregulators that amplify, restrain, or redirect androgen action mediated by the androgen-loaded, activated receptor [75].

Additional natural genetic defects in key reproductive genes provided complementary insight into androgen action beyond AR mutation in *tfm* mice. For example, defects in the GnRH gene in the *hpg* mouse [76,77] created a null reproductive hormone platform to allow investigation of the selective effects of testosterone and gonadotropins on spermatogenesis [78], making it feasible to separate the otherwise intertwined effects of LH and/or testosterone from that of FSH. However, the limited number of ideal naturally occurring genetic mutations available was overcome with technical advances in molecular methods for gene targeting, resulting in the award of the 2007 Nobel Prize in Physiology and Medicine. In creating customized transgenic, knockout and knock-in of specific genes in the mouse, and combining them with traditional pharmacological tools, these novel pharmacogenetic models greatly expanded the scope for analytical research into androgen and related hormone action providing new depth of insight into the molecular mechanisms of androgen action. For example, knockouts of LH β subunit [79] or its LH/CG receptor [80,81], recreating the *hpg* phenotype, allowed further depth of investigation of LH in driving testosterone production and actions. More broadly these include androgen-responsive tissues and organs such as classical androgen targets including the testis, ovary, breast, prostate, uterus as well as non-classical targets such as muscle, skin, liver, kidney, adipocytes, bladder, and immune cells. For example, the original insight into AR-mediated androgen action in the ovary reported in the 1970's through clever embryonic recombination experiments of Susumu Ohno [82] and Mary Lyon [83,84] was greatly expanded by genetic experiments using targeted AR inactivation to advance insight into androgen action in the ovary [85–87].

Male puberty

A major area of genetic discovery of impaired androgen action has been in the identification of a growing cascade of molecular causes of delayed or failed male puberty. While the initial trigger for puberty remains one of the deepest, most significant mysteries in reproductive endocrinology [88,89], numerous genetic as well as acquired environmental factors influence the timing and tempo of male puberty. The earliest reports of genetic causes of failed male puberty were considered “eunuchoidism” [90,91]. Despite meticulous, astute clinical descriptions with early laboratory testing (urinary

gonadotropins), these reports lacked critical knowledge of modern reproductive endocrine physiology, so the interpretation and extrapolations were often inaccurate by current standards.

After excluding pubertal delay due to the most frequent cause, self-limited constitutional delayed puberty which starts late but ultimately progresses to completion without treatment, and functional disorders, such as chronic diseases (including undernutrition and malabsorption) or testicular failure, the remainder of delayed or failed puberty are due to congenital onset of genetic gonadotrophin deficiency (isolated hypogonadotropic hypogonadism). This is only evident clinically after full testicular development, expected in adolescence with male puberty, fails to start or complete. The known genetic causes involve a cascade of at least 70 genes activated by puberty with defects in any one causing delayed or failed male puberty [92]. The increasing impact of massive parallel (“nextgen”) sequencing is facilitating identification of pathogenic gene mutations while gradually reducing the cost and increasing feasibility of identifying genetic mutations underlying delayed puberty.

An important corollary of male puberty is the natural history of testosterone secretion and action in women. From the earliest measurements using valid testosterone immunoassays in 1970 [93], it has been known that circulating testosterone is 15–20 times higher in post-pubertal men than in children or women at any age, a change originating from the testosterone surge during male puberty. The tolerance of this remarkable natural hormonal torrent probably explains why there is no naturally occurring testosterone excess syndrome in men, unlike virtually every other hormone.

Androgens in women

Testosterone's mystique as the conduit of masculine energy and virility has long fostered the belief that testosterone treatment, using male doses, may benefit some women with “frigidity” or other disorders, long before testosterone could be measured [94,95]. Ultimately this led to disease-mongering invention of a new disease “hypoactive sexual desire disorder” for which placebo-controlled trials of testosterone were conducted [96,97]. Using testosterone doses aiming to replicate female testosterone production rates, these studies demonstrated modest efficacy beyond placebo effects and only when testosterone concentrations exceeded physiological concentrations for women. These higher circulating testosterone concentrations developed because in women, unlike men, exogenous testosterone is additive with endogenous testosterone as it arises from three source (adrenal, ovary, extra-glandular conversion) none subject to strong negative testosterone feedback. By contrast in men exogenous testosterone effects suppress endogenous testosterone as men's circulating testosterone originates from a single source governed by tight negative feedback.

An important insight into androgen effects in women was enunciated by Ferdinand Labrie whose concept of intracrinology [98] referred to the importance of local tissue generation of bioactive sex steroids in women and in men with advanced prostate cancer; circumstances where despite circulating testosterone at castrate male or female/childhood levels, substantial evidence of androgen action could be demonstrated indirectly by pathophysiological androgen effects and/or urinary steroid metabolites of androgens, none of which were unambiguous in isolation. This concept evolved from the concept of total androgen blockade in prostate cancer (see below) which was only partly vindicated (see below). On the other hand, interesting vindication of the intracrinology concept comes from experimental studies in AR knockout female mice. Technically challenging AR inactivation in females (as androgen insensitive males as obligate fathers are sterile), has significant effects on female reproductive function despite female circulating testosterone levels first shown in the 1970s by Susumu Ohno [82] and Mary Lyon [84,99] and updated by modern genetic pathophysiological investigations [85–87].

Invention

The Golden Age of Steroid Pharmacology, the decades after World War II till 1970, produced hormonal contraception and synthetic glucocorticoids which remain key elements of the modern pharmaceutical armamentarium. This Golden Age also encompassed a third major but failed quest, that to invent a pure anabolic steroid, a synthetic androgen having purely anabolic effects but so devoid of any virilizing effects it could be used safely in women and children. Despite massive, invested effort, this failed quest left behind a legacy of thousands of synthetic androgens recorded in the expired patent

literature, almost none ever tested in humans. Decades later, this public archive proved a windfall for enterprising organic chemists who used it as a free resource to manufacture illicitly never-marketed designer androgens for the unregulated illicit body-building internet market as well as for illicit doping purposes, aiming to evade detection by conventional anti-doping tests [100,101]. The comprehensive failure of this quest was ultimately due to its limited understanding of androgen physiology. These limitations included the subsequent discovery of a singular androgen receptor (AR), a final common pathway of androgen action that differs from other steroid receptors (estrogen/progestin, glucocorticoid/mineralocorticoid) that feature complex dual receptor isoform control mechanisms. More grievously, screening of steroid analogs was based on a misguided simplistic modification of the whole animal androgen bioassay. This purported to separate anabolic (represented by the levator ani muscle) from androgenic (represented by the ventral prostate) effects, an *ad hoc* refinement which made a false distinction where there was no difference. This flawed approach is ultimately inconsistent with modern androgen physiology, findings rediscovered in more recent pharmaceutical research showing that the apparent differences between androgenic and anabolic endpoints in whole animal androgen bioassays reflected (a) whether the bioassay used maintenance or recovery of androgen effects and (b) differences in the speed of recovery from castration of androgen effects making the results depend on the timing of the endpoint [102]. Nevertheless, the term “anabolic” (or “androgenic-anabolic”) steroid persists in the common lexicon, serving largely as a journalistic pinata, long after it was known to be making a tautological distinction where there was no real difference – in reality, all androgens are anabolic, and all anabolic steroids are androgens [103].

The discoveries of 5 α reductase enzymes prompted by the identification of genetic mutations causing selectively impaired androgen action paved the way for the development of 5 α reductase inhibitors finasteride [104] and dutasteride [105] for highly tissue-selective abrogation of androgen action on the prostate [106] and hair follicles [107]. These drugs, together with a wide array of newer 5 α reductase inhibitors in development [108], have established important therapeutic roles for 5 α reductase inhibition in treatment and prevention of prostate disease and androgenic alopecia, respectively. Similarly, the clinical characterization of mutations leading to 46XY DSDs which feature selective impairment of testosterone action in genetic males have provided clues leading to development of steroidogenic enzyme inhibitors for treatment of androgen-dependent diseases, notably prostate cancer. These include abiraterone, a CYP17 (17 hydroxylase/lyase) an inhibitor of C19 and C18 steroid synthesis and other novel androgen metabolizing enzymes in development [109,110] (including ones that might target the testis-specific enzyme 17 β hydroxysteroid dehydrogenase type 3) which may be effective for selective inhibition of testosterone synthesis and treatment of androgen-dependent disease such as prostate cancer.

The next invention was that of the first non-steroidal androgen by Dalton et al. [111] in 1998, six decades after the first non-steroidal estrogen [112]. This creates a new class of non-steroidal synthetic androgen, often termed Specific Androgen Receptor Modulators (SARM), a misleading marketing term rather than an accurate pharmacological description [113,114], usurping a speculative but unsound analogy with Specific Estrogen Receptor Modulators (SERM). Non-steroidal androgens were developed by a rational drug design using computer-based structural modelling, an iterative approach exploiting a library of chemical substituents to optimize physicochemical interactions with the therapeutic target, in this case the androgen receptor using non-steroidal anti-androgens as lead compounds. Non-steroidal androgens are pharmacologically distinct from testosterone in being obligatorily pure androgens as they inherently lack testosterone's wider spectrum of activity. Pure (including non-steroidal) androgens lack testosterone's capacity for amplification of androgenic potency by tissue-based 5 α reduction to DHT as well as diversification by aromatization to estradiol to act upon estrogen receptors. Hence, while non-steroidal androgens may have potential roles in pharmacological androgen therapy, subject to rigorous efficacy, safety, and cost-effectiveness criteria, they could not replace testosterone for replacement therapy in men with pathologic hypogonadism due to their lack of testosterone's full spectrum of effects. Nevertheless, although intended for pharmacological androgen therapy in indications such as prevention and treatment of muscle wasting in cancer [115], none of the non-steroidal androgens under development [116,117] are marketed by 2021. Yet hope springs eternal for this new attempt to separate anabolic from androgenic properties of androgens to facilitate marketing for muscle wasting and other selective effects of testosterone.

Androgen measurement

The first measurements of androgens were based on whole animal androgen bioassays invented early in the 20th Century, mostly using castrated rats administered crude testis extracts or other biological fluids. These bioassays have been in continuous use ever since with harmonization and refinement into the contemporary Hershberger androgen bioassay [118] which remains used in toxicology and pre-clinical characterization of new drugs [119,120]. Although instrumental in the original purification of testosterone, these whole animal bioassays are laborious, costly with slow throughput, insensitive and non-specific in their inability to distinguish one androgen (or anti-androgen) from another (see Fig. 4).

Refinement of whole animal androgen bioassays have included development of androgen receptor (AR) binding assays and in vitro cell-based androgens bioassays, both enhancing the throughput, reducing the costs and use of animals. The first quantitative AR binding assays were developed in the mid-1970s using AR-rich purified rat prostate cytosol in a quantitative androgen binding assay using a synthetic androgen tracer (R1881) being competitively displaced by androgens being tested [121,122]. AR binding assays were better standardized by using cultured cell lines stably transfected with human AR. However, binding assays remain unable to distinguish either agonists from antagonists or one AR binding substance from another [123].

In vitro androgen bioassays based on yeast or mammalian host cell expressing human AR are more efficient in sparing animal use, more sensitive than whole animal androgen bioassays and can distinguish between agonists and antagonists but not between specific androgens. The earliest in vitro androgen bioassays were formed using primary cell isolations of androgen-dependent cells, typically derived from the prostate, which avoided the need to transfect the AR. However, although these were androgen responsive, they were laborious and hard to standardize assays lacking in the versatility and throughput needed for wide application. The availability of the cloned AR facilitated the next step in the custom development of AR-expressing cell lines with simple chemical read-out to achieve better standardization and reproducibility for in vitro androgen bioassays. The first was using AR-transformed yeast as the host cell for in vitro androgen bioassays during the 1990s [124,125]. Subsequently, analogous in vitro androgen bioassays were developed in stably AR-transfected mammalian host cells

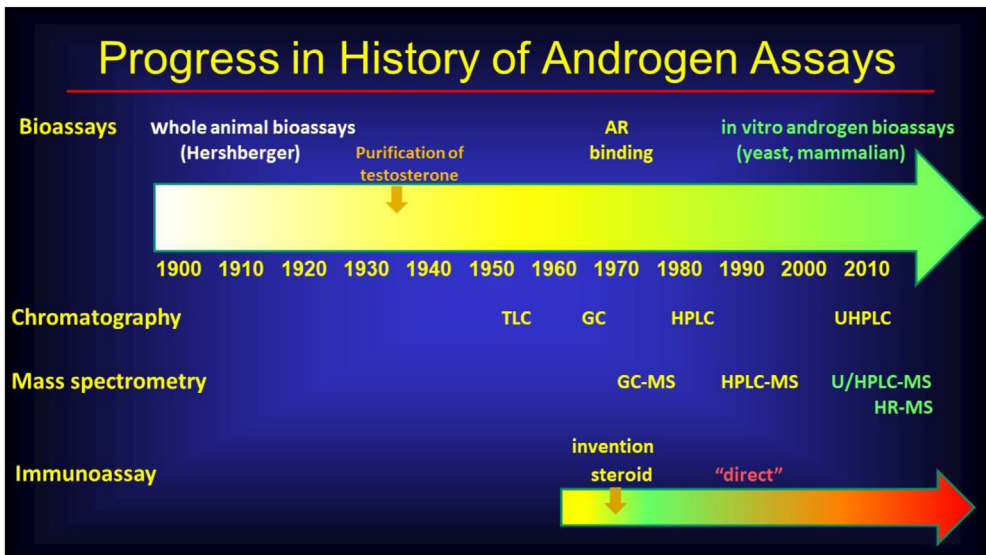


Fig. 4. Depiction of progress since 1900 in androgen measurements using bioassays, chromatography, mass spectrometry and immunoassays. Progress is depicted as colouration going from white to yellow to green and retrogression from yellow to red. For further details see text.

[126,127]. The mammalian host cells are more sensitive, but also express steroidogenic enzymes and/or non-AR steroid receptors unlike yeast cells which express neither. For example, steroidogenic metabolism in mammalian host cell may enhance androgenic potency of pro-androgens or nullify potency of other androgens [128]. Hence although mammalian host cells sacrifice specificity for sensitivity, both provide complementary information.

The first methods to measure specifically testosterone or other synthetic androgens were based on radioimmunoassay. Following the Nobel Prize-winning invention of the immunoassay method by Berson and Yalow in 1959–60 [129,130], another decade passed before immunoassay was adapted to non-immunogenic small molecules like steroids as first reported for estradiol in 1969 [131] and then testosterone in 1970 [93]. These classical steroid immunoassays featured organic solvent extraction, chromatography, and authentic tracers, a triplet of validity criteria for steroid immunoassay, essential to avoid cross-reactivity from structurally related steroids and matrix artefacts. Based on these criteria, the steroid immunoassay findings from the pioneering era of the 1970–80s, remain valid. Subsequently the clinical demand for testosterone as well as other steroid assays in clinical practice has led to the adaptation of steroid immunoassays to fit into high throughput, semi-automated multiplex immunoassay platforms which were originally developed for peptide immunoassays which required no pre-assay preparation but save the costs of skilled scientist operators. Unfortunately, these platforms are incompatible with the solvent extraction and chromatography required for validity of steroid immunoassay. Discarding the triplet validity criteria to create “direct” steroid immunoassays has led to predictable inaccuracy of all steroid immunoassays, including for testosterone. Early in the 21st century the inaccuracies of “direct” testosterone immunoassays relative to the reference specificity of mass spectrometry-based measurement became well-known [132–134] so that the major inaccuracy at low circulating testosterone concentrations in women and children was described as virtually random number generation [135]. Yet currently serum testosterone measurements in clinical practice are still dominated by “direct” (i.e., non-extraction) steroid immunoassays implemented in chemical pathology laboratories. While the advent of bench-top mass spectrometers promises to make steroid liquid chromatography-mass spectrometry (LCMS) more widely available in clinical pathology and practice, limitations on availability, which include the higher costs of skilled staff and equipment, have still to be overcome to make this superior technology widely available in clinical practice.

Alternative methods for measuring circulating steroids such as testosterone have also been available since the 1970s including various forms of thin-layer, gas and liquid chromatography, with the latter two even more effectively linked to mass spectrometry, the so-called hyphenated technologies [136]. The development and wider availability of bench-top LCMS equipment in recent decades has led to it becoming the method of choice for steroid measurement in clinical research and practice [137,138]. LCMS combines reference level specificity with high sensitivity, matching or exceeding the best steroid immunoassays, and the flexibility to readily incorporate new steroids into multi-analyte profiles (“steroidomics”) thereby providing greater insight into complex steroid pathway fluxes. The multi-analyte profiling capability requires less sample than the inevitably single-analyte immunoassays requiring more sample and a different immunoassay for each steroid, if such assays exist. As the steroid immunoassay era closes, the ascendant LCMS methods are limited by their need for costly equipment and skilled operators while commercial steroid immunoassays remain locked into reagent rental contracts between pathologists and manufacturers, an arrangement that bypasses the needs of patients and doctors for more accurate steroid measurements.

Another conceptual invention, the so-called “free” testosterone hypothesis, has a less admirable and indeed regressive role in androgen history. This concept asserts that the small unbound fraction of circulating testosterone not bound to SHBG or albumin, (around 2%), had the greatest availability to tissue and was therefore the most bioactive fraction of testosterone while the protein-bound testosterone formed an inert circulating reservoir [139]. Logically, however, that concept equally explain that the unbound fraction is the least bioactive as it more rapidly metabolized [140]. Beyond its seriously flawed concept, the “free” testosterone hypothesis was further elaborated from a now defunct pharmacological theory of drug interactions [141]. That theory based on mutual displacement of drugs bound to circulating proteins invoked a hypothetical unbound (“free”) drug fraction [142–145]. However, that theory is now discarded in modern pharmacology [146] in favor of physiological mechanisms of drug interaction due to molecular receptor binding, cytochrome P450 induction/

inhibition, P-glycoprotein and ion channel blockade [147]. This “free” concept was grafted onto and reinforced by another contemporaneous concept, that of “free” vs “bound” moieties in the calculational basis of immunoassays [148].

Laboratory measurement of “free” testosterone was developed using dialysis-based methods masquerading as an analytical measure, despite lacking all the fundamentals of a certified reference standard, quality control program or verified reference ranges. Furthermore, as the dialysis-based methods are manual and laborious, calculational formulae have been devised as a substitute [149–151]; however, these deviate systematically from the laboratory measurements they purport to represent [152–155]. These formulae which embody equilibrium binding equation and incorporate several biases arising from untested assumptions. These include that the tissue unloading of testosterone is at equilibrium when capillary passage occurs in seconds, the mistaken stoichiometry for testosterone binding to SHBG [156] and use of arbitrary plug-in binding affinity constants adapted from empirical estimates that vary over a 5-fold range [157]. An alternative approach purporting to support the “free” testosterone concept has been the measurement of salivary testosterone. Saliva is the product of three sets of salivary glands, each with distinct fluids and spontaneous and stimulated secretory characteristics but containing minimal protein. Although that provides glancing resemblance to unbound testosterone in serum, salivary testosterone is two orders of magnitude lower than in serum [158] and correlates only weakly ($r = 0.68–0.71$) with serum “free” testosterone [159]. However, in studies of salivary testosterone 10–15% of samples are discarded as outliers due to microscopic blood contamination arising from minor oral trauma or infection, poor dental hygiene or eating hard foods despite precautions to avoid such contamination and when saliva is hard to produce, flow stimulants can distort measurements. Hence salivary testosterone is yet another distinct biological matrix with distinctive characteristics and limitations and has little bearing on the validity of the “free” testosterone concept so far but new critical studies using it are yet to emerge.

The remarkable uncritical acceptance of this concept is a clear example of Kahneman's Fast Thinking which distinguished intuitive, emotive, and irrational thinking from the rational Slow Thinking which is characteristic of sound scientific thought. The rapid, reflexive and intuitive “free” testosterone concept cannot rely on sound critical evidence which is lacking as, other than elaborating the theory [139,142,143,145,160], the “free” testosterone concept has virtually never been subjected to empirical testing in humans [161] before this exploratory heuristic became reified into an unquestioned quasi-axiom. Together with the direct testosterone immunoassays, this “free” testosterone concept is part of the regressive mismeasure of testosterone.

Androgen pharmacology

Prior to the discovery of testosterone, experimental approaches from the 18th century onward had provided indirect evidence that the testes secreted a masculinizing substance, the nature of which was unknown until the 20th century. During the purification of testosterone, it was learned that the urine-purified substance (androsterone) had a brief duration of action, was ineffective orally and had unexpectedly low efficacy compared with testicular extracts. These represent the challenging pharmaceutical features of testosterone for effective therapeutic delivery which requires a non-oral, depot route of delivery. Among the first depot androgen formulations was the development of subdermal implants by the pioneering researcher couple, Ruth Deanesly and Sir Alan Parkes [162]. Further, in parallel by 1936 Miescher et al. from Ciba laboratories reported, through astute observation during the purification of testosterone that the biological action of injected testosterone was substantially prolonged and enhanced by combination with various organic substances [163]. This was most effective and practical using esterification to fatty acids of various aliphatic chain lengths [164]. Ever since, intramuscular injection of testosterone esters dissolved in a vegetable oil vehicle has been the most cost-effective and widely used formulation for therapeutic delivery of testosterone [165]. In this injectable depot, longer fatty acid sidechains create greater hydrophobicity and prolong the duration of testosterone release and action by the delayed release of the testosterone ester by physicochemical partitioning between the hydrophobic oil vehicle depot and the hydrophilic bloodstream. Once liberated from the depot, the testosterone ester is rapidly hydrolyzed by ubiquitous circulating non-specific esterase that removes the fatty acid sidechain releasing unesterified testosterone to act on

tissues. In addition to physicochemical partitioning, steric hindrance of esterase action also tends to delay release of testosterone from longer aliphatic chain esters.

Only in recent decades has this dominance of injectable testosterone esters been challenged by another non-oral depot delivery, transdermal products in the form of adhesive patches [166,167], hydroalcoholic gels [168–170] or cream in women [171] and men [172,173]. A single oral formulation of testosterone, testosterone undecanoate in an oil-filled capsule, was marketed in the 1970s globally [174] except for the USA until recent modifications were marketed [175,176]. This formulation counters the very low oral bioavailability of unesterified testosterone by directing absorption via gut lymphatics [177]; however, the oral absorption of testosterone undecanoate is highly dependent on capsule ingestion with a high fat meal, without which it has no bioavailability [178]. Otherwise, testosterone formulations have been developed for application to every orifice or cavity including oral, buccal, nasal, rectal, trans-scrotal, scrotal, axillary, and umbilical routes.

The classical and sole unequivocal indication for testosterone is in replacement therapy for organic disorders of the male reproductive system due to pathological disorders of the hypothalamus, pituitary, or testes. More recently this indication extends the category of primary hypogonadism to female-to-male transgender seeking somatic masculinization to match their psychic gender orientation. In replacement therapy, testosterone displays its full spectrum of effects including not just direct action via the androgen receptor, but also indirect effects via amplification by 5 α reductase in some target tissues (prostate, skin, liver) as well as diversification of its effects via aromatization to estradiol to act on estrogen receptors in other target tissues (brain, bone). These effects are dose limited to replicate physiological androgen exposure intended to replicate the lifetime efficacy and safety of eugonadal men.

By contrast, synthetic androgens are exclusively pure androgens acting directly (or via metabolites) solely on the androgen receptor and are not suitable substitutes for testosterone in replacement therapy. However pharmacological androgen therapy uses synthetic androgens for their potent effects on muscle, liver and other androgen target tissues aiming to modify the natural history of non-gonadal diseases [179]. In these applications the dose and type of synthetic androgen are limited only by efficacy, safety, and cost-effectiveness of the androgen in placebo-controlled clinical trials. Many such applications have been developed for synthetic androgens which remain cost-effective second line alternatives even when more specific, purpose-designed therapeutics become available. For example, an oral alkylated androgens (stanozolol, danazol) can increase C1-inhibitor levels and reduce frequency of angioedema attacks; however for prophylaxis recombinant C1-inhibitor is more effective but costly [180]. Similarly, synthetic androgens are cost-effective at increasing hemoglobin in anemias due to renal or marrow failure [181] although recombinant erythropoietin or erythropoietin stimulating analogs are equally effective but higher cost.

Another important application of androgen action has been the abortive development of hormonal male contraception. Strong interest in male contraception early in the 20th century [182] even prior to the availability of testosterone and it gained further interest in the 1970s following the development of effective female hormonal contraception in the previous decade when prototype hormonal male contraceptive approaches were reported using an oral antiandrogen [183] and testosterone injections [184]. The major advances were from the 1980s when the World Health Organisation's Human Reproduction Programme through its Male Task Force (managed by Geoffrey Waites, chaired by Eberhard Nieschlag) produced two landmark proof-of-principle studies of hormonal male contraception [185,186]. Although these provided proof of contraceptive efficacy - counting unwanted pregnancies, not just sperm - the testosterone alone regimen required mildly supraphysiological testosterone doses to both suppress spermatogenesis as well as maintaining endogenous androgen exposure but was not universally effective in Western populations. Consequently, an alternative was developed using progestins to suppress spermatogenesis with testosterone used only at lower doses for replacement therapy for the ensuring acquired primary hypogonadism. Following the proof-of-principle study for contraceptive efficacy of a progestin/testosterone combination [187], progestin/androgen combination regimens remain the optimal approach for hormonal male contraception with variations investigated in larger contraceptive efficacy [188] and placebo-controlled safety [189] studies. Nevertheless, product development has stalled reflecting pharmaceutical industry disinterest for developing hormonal male contraception. Despite its status as low hanging fruit with virtually all

necessary efficacy and safety established in the public sector, the major motives for pharmaceutical disengagement are the lack of patentability, low profitability relative to other contraceptives and unacceptable risk of side-effects in healthy individuals as well as predatory product liability suits that follow every new marketed contraceptive for healthy individuals. As product development requires a commercial partner, its failure despite clear public and medical demand, largely attributable to the perverse incentives of the patent system, represents a market failure that awaits an angel investor such as the wealthy heiress Katherine McCormick served for Gregory Pincus, the conceptual developer of female oral contraception [190] if no government regards better gender sharing of contraceptive burden as a priority.

Anti-androgens

An important application of knowledge on androgen action is the development of anti-androgens for treatment of androgen-dependent disorders especially prostate diseases, but also other niche treatments for skin disorders (acne, seborrhea, hirsutism), polycystic ovary syndrome, precocious male puberty, male-to-female transgender, and forensic management of sex offenders. The original finding that blocking androgen action was effective in treatment of prostate disease was Charles Huggins's pioneering report in 1941 that surgical castration or high dose estrogen treatment was effective at palliating advanced, incurable prostate cancer [191] and was awarded the 1966 Nobel Prize for Medicine. In the 1960s, medical treatment aiming to avoid orchidectomy was developed using estrogens to suppress endogenous testosterone production but unacceptable rates of serious adverse effects of high dose diethylstilbestrol, a potent synthetic estrogen [192,193], prompted alternative development of steroidal anti-androgens. The first were spironolactone and cyproterone acetate, developed originally for steroidal anti-mineralocorticoid and anti-progestin properties, respectively, but both fortuitously found to also possess modest antiandrogenic properties leading to their repurposing for treatment of prostate diseases. By the mid-1970s, the first generation of custom-developed pure anti-androgens appeared with flutamide proven effective for treatment of advanced prostate cancer [194,195]. In the 1980s, additional first-generation anti-androgens, bicalutamide [196] and nilutamide [197], were reported to be effective treatment of prostate cancer. A second-generation of pure non-steroidal anti-androgens effective for treatment of advanced prostate cancer now include a growing list including enzalutamide in 2012 [198,199], apalutamide in 2018 [200,201] and darolutamide in 2019 [202,203].

In the 1980's a major alternative route of indirect anti-androgen therapy was developed using GnRH analogs. The Nobel Prize-winning identification of GnRH [204,205] led to hopes that superactive GnRH analogs might prove effective for stimulating reproductive function; however, the pivotal findings of Knobil in 1978 showed that only physiological pattern of pulsatile GnRH administration could maintain reproductive function, whereas continuous administration leads to paradoxical suppression of reproductive function [206]. The unexpected paradox of gonadal suppression by non-physiological GnRH administration led to the development of depot superactive GnRH analogs for effective long-term treatment of prostate cancer [207–210]. However, an unfavorable feature of superactive GnRH analogs was their initial “flare” reaction reflecting their residual agonist properties producing a transient increase in serum testosterone within early weeks of starting treatment, before the inhibitory effects became established [211,212]. This “flare” risk led Labrie to propose combined (or total) androgen blockade in combining a depot GnRH agonist with an oral anti-androgen [213,214]. Despite the minimal overall survival benefits of the combined androgen blockade [215], the early prevention of flare reactions from superactive agonists has led to persistence of the combined androgen blockade regimens. Alternatively, the agonist-induced “flare” reactions are eliminated by the further pharmaceutical development of pure GnRH antagonists [216], including orally active drugs [217], for inducing medical castration for treatment of advanced prostate cancer. A variant on combined androgen blockade is the development of intermittent androgen blockade which aims to maintain the benefits of androgen withdrawal on prostate cancer while reducing the adverse symptomatic effects of castration [218–220].

Another alternative anti-androgen treatment for prostate cancer has been the development of steroidogenic enzyme inhibitors that target enzymes selective for testicular testosterone synthesis. The

first marketed, abiraterone, an inhibitor of CYP17 (17-hydroxylase/lyase), is effective treatment for prostate cancer [221]. However, as CYP17 is an enzyme early in the cascade of steroidogenesis and inhibits adrenal as well as sex steroid synthesis so that abiraterone treatment requires concomitant administration of prednisone for glucocorticoid/mineralocorticoid replacement. A still more selective enzyme target for inhibition of testicular testosterone production is 17 β hydroxysteroid dehydrogenase type 3 which was suggested by naturally occurring enzyme mutations that produce an under-virilized male phenotype variant of 46XY DSD [222]. This enzyme is expressed selectively in the testis and inhibition has shown promise for treatment of prostate cancer [223].

The hunt for lucrative markets is the standard operational dynamic of the pharmaceutical industry to offset the costs of many drugs that fail along the long path from concept to marketing. This involves not just development of new drugs with patent-derived marketing exclusivity, but also much lower cost repurposing of older drugs with extended indications and wider markets. In this, synthetic androgens are no exception. Prominent in this arena is the wishful thinking about “andropause” (see above) and the refreshed goal to develop a pure non-virilizing androgen, under the marketing rubric of SARM.

Androgen misuse and abuse

Soon after testosterone and synthetic androgens became commercially available immediately after World War II [224], androgens were used by elite athletes in power sports for performance enhancement (“doping”) through their effects in gaining muscle mass and strength [225]. By the mid-1970s, this led the International Olympic Committee (IOC) to prohibit elite athlete's using androgens at any time, in and out of competition. That prohibition is now enforced by the World Anti-Doping Agency which issues an annually updated Prohibited List and accredits anti-doping laboratories to run sensitive urine detection tests for all prohibited drugs including androgens that must be robust to medico-legal challenge by professional athletes in the Court for Arbitration in Sports (CAS), operating as the superior court for sports. WADA-compliant international sporting federations and their athletes competing in elite sports are bound by a strict liability for drugs detected in an athletes' body making them at fault for sanctions regardless of intent or negligence. The net effect of these measures has been to maintain a very low level of positive antidoping tests (<1%) of samples, with about half due to androgens [226]. Although androgens, together with erythropoietin and its analogs, remain the most potent ergogenic doping drugs, androgens remain the most frequent source of positive antidoping offences. The net effects of this program of highly sensitive urine anti-doping tests with ever-widening windows of detection for exogenous natural and synthetic androgens has maintained an effective, if imperfect, suppression of androgen doping.

Wide public knowledge of androgen doping, epitomized by the 1988 Olympics disqualification of Ben Johnson for use of a synthetic androgen (stanozolol) as well as the unscrupulous national doping programs of East Germany, and more recently Russia, led from the 1980s to the crossing-over of androgen abuse into the general non-athletic community for image rather than performance enhancement. In the four decades since, androgen abuse for image enhancement and bodybuilding has become an endemic variant of drug abuse in communities with sufficient affluence and leisure creating the consumer demand to support an illicit drug industry. Contrasting with the tight prohibition and severe sanctions which strongly deter androgen doping in sports, over recent decades the black market for androgen abuse in the community for non-athletic image enhancement and bodybuilding has proliferated in a completely unregulated marketing environment. Androgens are widely available from illicit sales without prescription from manufacturing plants, mainly in China [227], as well as illicit local domestic manufacturing using imported raw materials by drug dealers who boost their sales by unrestrained flamboyance in internet advertising with unscrupulous, unverifiable, and usually baseless claims of efficacy and safety for their unlicensed products. This marketing environment is virtually the same as that of the 19th century when snake oil remedies were sold from covered wagons at Medicine shows [228], long before mandatory public safety standards for marketing of pure, properly labelled drugs were introduced early in the 20th century [229]. The magnitude of the illicit androgen abuse market is secretive making it difficult to gauge so no credible estimates are available; however, it is likely to greatly exceed the legitimate market for prescription androgens (mainly testosterone) which by the 3rd decade of 21st century is likely between \$2–5 billion per year [33].

Androgen misuse is mainly the prescription use of testosterone for non-approved, futile, or harmful off-label uses. This has led to a 100-fold increase in testosterone prescription sales over the last 3 decades without a single new approved indication [33]. Mostly this has been prescription of testosterone as an anti-ageing or sexual tonic without sound evidence but driven by figments of misunderstanding androgen action, a pre-scientific fantasy relationship of testosterone to sexual function and youthful vigor, the “The Fountain of Youth” reimagined. To buttress this rejuvenationist mystique, a new invented disorder known variously as “andropause”, “late-onset hypogonadism” and other even less credible neologisms. These fictions in search of a definition were designed to legitimize testosterone prescription for age-related “hypogonadism” by a disease-mongering widened redefinition of “hypogonadism” not recognized by the FDA as a disease or condition warranting testosterone treatment [230,231]. This aimed to replace the long-standing classical definition of hypogonadism as a pathological disorder of the hypothalamo-pituitary testicular axis with a widened definition of “disease”. This was to include functional states whereby circulating testosterone was reduced as part of a normal, non-specific hypothalamic response to systemic non-reproductive disorders with or without non-specific symptoms. This hijacking of the term “hypogonadism” represented a classical disease-mongering widened redefinition to boost drug marketing [232,233]. This infernal mechanism was augmented by employing so-called “free” testosterone to validate wider prescription of testosterone on a road to a dead end with imaginary form of testosterone the means to that dead end.

The adverse effects of androgen misuse and abuse are necessarily acquired gradually through observational studies as prospective safety studies are not feasible without well-established efficacy indications. After the earliest reports of androgen abuse [234], the first systematic reviews summarizing androgen misuse and abuse were by the great pioneer of androgen pathophysiology Jean Wilson [235,236] with more recent reports expanding on these safety issues [237–240].

Androgen dependence

Androgen dependence, recognized in ICD-10 and DSM-5, is an important but poorly appreciated consequence of androgen abuse. All closed loop endocrine systems feature suppression of the endogenous system during administration of exogenous agonists. First reported and studied by Kurt Brower in the early 1990s [241] to developed into a two-stage model [242] starting with voluntary recreational use transforming into compulsive drug-seeking habits as a gateway to addiction [243]. There is growing recognition that androgen dependence can complicate the long-range effects of androgen abuse, notably when individuals attempt to cease androgen abuse. The potent psychoactive effects of high dose androgens elevate mood and risk hypomania with aggression, impulsive reckless and addictive-type drug-driven, drug-seeking behaviors. Transient withdrawal symptoms during recovery are a crucial reinforcing feature [244]. Most abusers commence androgen intake in their early 20s [245], continue for several years but very few remain active androgen abusers over the age of 50 years [246–248]. No systematic studies of the reasons for discontinuing androgen abuse have been reported.

Although there is less evidence, prolonged use of unjustified testosterone treatment may also create iatrogenic androgen dependence resulting from androgen withdrawal/deficiency symptoms after stopping treatment. That creates a potential vicious circle of resuming testosterone treatment to alleviate withdrawal symptoms despite the loss of the original objective for treatment [249]. Most misuse of testosterone eventually ceases recognizing the futility of treatment and wishing to discontinue the habit.

Future research agenda

Many opportunities await further development of androgens in physiology and clinical use if based on careful pathophysiology and pharmacology. A brief, personal listing might include (a) the physical form of storage of the high testosterone concentrations in the testis, (b) mechanism of androgen action in target tissues especially muscle, erythropoiesis, skin/hair follicles, and intracrine mechanisms in females and in advanced prostate cancer, (c) identifying the primary trigger of male puberty and creating a comprehensive picture of the cascade it unfolds and their defects, (d) wider uses of higher

doses of synthetic pure androgens (including DHT) in non-reproductive disorders where fertility is not critical, and (e) controlled analytical clinical research into androgen dependence and recovery from androgen abuse.

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