Testosterone

From Basic to Clinical Aspects

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Editor

Second Edition

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Preface

When I received the invitation from Springer in 2014, I did not imagine exactly what this book project would become.

We launched the first edition in 2017 and I was surprised by the success of the book in different parts of the world. In 2019, the book was released in Portuguese in Brazil, my home country.

After a few years, we observed the need to expand on some important key points regarding the hormone "testosterone." Topics such as gynecomastia, male infertility, anabolic steroids, and transgenderism were incorporated in this edition.

In recent years, we have seen a worsening of testosterone use and abuse, not only among men, but also among women. The phenomenon is global and worries health authorities and medical societies in different countries.

Revisiting the hormonal physiology of sex steroids in men and women, the actions of androgens and their binding to the respective receptor, the development of male sexual characteristics that fundamentally depend on testosterone and its derivatives, form the basis of the book.

As well as the different causes of male hypogonadism, central and peripheral, organic and functional, the respective differential diagnoses are described in detail in different chapters.

Testosterone, the focus hormone of this book, is used in different forms and routes of administration. Several authors unravel its peculiarities and assist in choosing the most suitable form in each case, as well as the possible risks of its misuse or even abuse in men and women, seeking alternatives to help patients in this situation.

I hope every reader will take advantage of this book as a source of knowledge to improve medical practice in different parts of the world.

Good reading!

Florianopolis, Brazil — Alexandre Hohl

Acknowledgments

I would like to thank my family who are always supporting my professional projects.

I thank each of the authors who dedicated their time and knowledge to the construction of this work.

I would like to thank my doctor friends who work in the daily care of each patient.

Thanks to my patients who make it possible to further understand the hormonal changes described in this book.

Contents

1 The Cultural and Medical History of the Testes and Testosterone: From Antiquity to Modern Times

Eberhard Nieschlag and Susan Nieschlag

The Oldest Key to the Endocrine Treasure Trove: The Testicles

With this phrase Medvei [1], the master historian of endocrinology highlights the role played by the testes in unravelling mankind's knowledge of endocrine functions. The testes, in their exposed position, are vulnerable and easily accessible to manipulation including both accidental trauma and forceful removal. Loss of virility and fertility are easily recognizable, not only by physicians but also by laymen, so that the results of lost testicular function were known since antiquity and long before the discovery of sperm and their function in the seventeenth and eighteenth century, and long before testosterone, as the active agent, was isolated and synthesized in the twentieth century.

Far from being complete, this chapter describes how knowledge about androgenic functions of the testes evolved, which detours and blind alleys were taken towards the actual discovery of testosterone and finally, how testosterone preparations have been developed for clinical use. An earlier historical report may supplement this chapter [2].

Effects of Testis Removal

In Greek mythology Chronos (Saturn) castrated his father Uranos (Zeus) because he did not allow him to procreate children with his mother Gea, where- upon Chronos' testes fell into the sea, causing a gigantic foaming, from which Aphrodite (Venus) was born, already indicating the magic powers attributed to the testes in ancient times (Depicted masterly by Giorgio Varsari (1511–1574) in a fresco in the Palazzo Vecchio, Florence). In hellenistic times (4th to first century B.C.) the power attributed to the testes is also reflected in the cult devoted to Diana Ephesina. Seeking

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favors, good luck, health, and fertility, worshipers affixed testes of sacrificed bulls to wooden statutes of the goddess. In some places effigies of the so-decorated goddess were made of marble and colored. Some of these statutes have survived to our times as, e.g., exhibited in the Archeological Museum of Naples (Italy) (Fig. 1.1). Until clarified quite recently, the bull testes were mistaken as supernumerary breasts as signs of the goddess of fertility.

At the Chinese imperial court, documented since the Ming dynasty (1368–1644), eunuchs were not only custodians of the harem, but could obtain high-ranking political positions as exemplified by Admiral Zhèng Hé (1371–1435), leader of seven large expeditions into countries around the Indian and Pacific oceans, or Lin Yin (1451–1510), who is still counted among the richest persons in history. The last imperial eunuch, Sun Yaoting, died in 1996 at the age of 94. Castration has been used to produce obedient slaves, loyal to their masters and rulers. The Chinese castratos underwent the orchidectomy as adults and many lost their lives due to blood loss or infections. Only the expectation of wealth and influence enticed men to undergo this cruel operation.

Fig. 1.1 Diana Ephesina (=Artemis of Ephesus) decorated with sacrificed bulls' testes (Roman creation, second century BC—Archeological Museum Naples)

Not only in China was castration a means to obtain docile slaves. In the Byzantine Empire castration was also practised. Eutropius (350–399) is among the famous examples; he was born in slavery and castrated in Persia, serving as eunuch in harems and a callboy to wealthy men. Eventually he became chamberlain to the emperors Theodosisus I and Arcadius. He amassed vast wealth as consul, became involved in intrigues and was ultimately murdered. Another example is Narses (490–574) who was a castrated slave imported from Armenia and became chamberlain to emperor Justinian (527–565). He became famous as a general fighting against the Visigoths in Northern Italy.

Over the centuries in Islamic societies castrated slaves, predominantly imported from Sub-Saharan Africa, but also from Europe and Asia, were the work force and constituted elite troops deployed in wars of conquest. It has been estimated that between 650 and 1920 about 17 million slaves were deported from Sub-Saharan Africa, while between 1450 and 1870 transatlantic deportation consumed "only" 11.5 million Africans [3]. This constant depletion of young and healthy men can be considered as a factor contributing to why Sub-Saharan Africa remained underdeveloped for such a long period.

Castration was also used as lawful punishment. In medieval Scandinavia high treason was not subject to capital punishment, but to castration combined with blinding. This was adopted by the Normans who introduced this custom wherever they ruled [4]. For example, King William III of Sicily was castrated and blinded by Emperor Heinrich VI following a revolution against the emperor in 1194 [5]. After the invasion of England in 1066, William the Conqueror largely abolished the Anglo-Saxon death penalty and replaced it by castration and blinding: "I also forbid that anyone shall be slain or hanged for any fault, but let his eyes be put out and let him be castrated."

However, castration as punishment has survived over the centuries. A recent example occurred in the Chechenian War in 1996: "…. From crosses mounted in the square hang crucified Russian soldiers … each with multiple gunshot wounds. All of them were castrated. …. The (Russian) commander orders cleansing of the village. All the men are dragged to the square. One soldier presses the Chechenian to the ground, another strips off his trousers and removes the scrotum with 2 or 3 quick cuts. In half a day the whole village is castrated" [6].

For other, more benign purposes, prepubertal castration was carried out because it maintains the high voice of boys so that soprano and alto voices with the acoustic volume of an adult male result [7]. These were featured in operas of the seventeenth and eighteenth centuries, such as composed by Georg Friedrich Händel (1685–1779) or Nicola Porporra (1686–1768). In the Vatican choirs these voices could be heard until the early twentieth century. Some of these castrates became famous soloists, such as Carlo Farinelli (1705–1782) or Domenico Annibaldi (1705–1779). The last castrato, Alessandro Moreschi, born in 1858, died in 1922 and left behind the only recordings of the castrato voice as a collection of arias he sang in the Vatican.

Surgeons in the middle Italian cities of Norcia and neighboring Preci, secluded in the Sibelline Mountains in Umbria, were specialized on delicate operations including castration of young boys. Going back to the thirteenth century, 30 family

dynasties formed the Scuola Chirurgica di Preci [8] and monopolized the trade there, guaranteeing utmost secrecy concerning this operation which was forbidden by church law under Pope Benedict IV (1675–1758) and reinforced by Pope Clement XIV (1705–1774), although the Vatican itself was one of the foremost employers of castrated singers. The operation, carried out without anesthesia and under miserable hygienic conditions, must have cost the lives of hundreds of boys, but—if successful—chances for a lucrative career compensated for the risks. Because of their high-pitched voices, but with a significantly larger lung volume than in women, castrati were in high demand for opera performances.

Today the skills of the surgeons in Preci and Norcia have been adopted by local butchers working on animal "models," advertising for "agnello castrato" as their speciality. The Scuola Chirurgica di Preci was also famous for other surgical procedures, e.g., cataract extraction and these surgeons were in demand throughout Europe. For example, Cesare Scacchi (born 1555) was called to the English Court to operate on Queen Elizabeth I in 1588, and successfully removed her opaque lenses. Modest museums in the town hall of Preci and the San Eutizio Monastery nearby give a flavor of this fascinating piece of medical history and exhibit some of the tools used by the surgeons [9].

Those early castrates served in a kind of posthumous clinical trial to test the hypothesis that testosterone shortens male life expectancy: a comparison of the lifespan of 50 sixteenth to nineteenth century castrates with 50 contemporary intact singers demonstrated not only the stressful biographies both these groups of artists had to endure, but also revealed no difference in their life expectancy [10]. In contrast, biographies of eunuchs at the imperial court of the Korean Chosun Dynasty (1392–1910) showed a longer lifespan for the eunuchs than for normal men at the time [11]. However, the Korean eunuchs were castrated as adults and spent their life in a well-protected environment shielded from the hostile outside world. In addition, the time point of the loss of testes in the castrati singers and the Korean eunuchs may also account for the difference.

Lessons from Experimental Testis Transplantation

While the removal of endocrine glands is one basic tool of experimental endocrinology, replacing glands is the other. As surgeon in the Seven Years' War (1756–1763), John Hunter (1728–1793) saw the need for transplantation of organs and limbs. This stimulated his experiments of transplanting testes from cocks to hens, thereby demonstrating the "vital principle" of living organs. Far from any endocrine thought, his goal was to demonstrate the survival of the transplant due to nerve growth, with the intention of replacing limbs and organs in wounded soldiers. Thus Hunter, among many other achievements, can be considered as one of the fathers of modern transplant surgery.

Fig. 1.2 Arnold Adolph Berthold (1803–1861), "father of endocrinology," as depicted on the Berthold Medal, the highest decoration for achievements in endocrinology awarded by the German Society of Endocrinology

At the university of Göttingen Arnold Adolph Berthold (1803–1861) (Fig. 1.2) used chickens as an experimental model. In 1849 he observed and published that testes transplanted from roosters to capons restored androgenic functions: "[The transplanted capons] crowed quite considerably, often fought among themselves and with other young roosters and showed a normal inclination to hens." He concluded that these effects "must be affected through the productive relationship of the testes, that is to say, through their action on the blood, and then through the suitable ensuing action of the blood on the organism as a whole" [12]. He was thus the first to postulate a humoral effect of the testes (and of an endocrine gland in general) on distant organs. At the same time, Franz Leydig (1821–1908), at the University of Würzburg, described the interstitial Leydig cells in the testes in many species, without, however, knowing their real function and importance [13].

It would take another 50 years until an endocrine function was clearly attributed to the Leydig cells, when Ancel and Bouin [14] summarized their conclusions from extensive experimentation as follows: "In numerous previous studies we have assembled a group of morphological, physiological and chemical facts that, taken together, allow us to formulate the following hypothesis: that the general action of the testes on the organism, ascribed in the past to the testes as a whole, is actually due to the interstitial gland" (translated by [15]). They did not (yet) use the terms "*hormone*" or *hormone action* which would have been appropriate for their findings, as this term was coined and first published only a year later by Starling [16] in London.

Berthold's experiments were initially not accepted by the scientific community—including his own department director Rudolf Wagner (1805–1864)—for a long time, until Nussbaum [17] at the University of Bonn and Pézard [18] in Paris repeated and confirmed Berthold's results in frogs and chickens respectively.

Erroneous Conclusions from Berthold's Experiments

Probably prompted by these findings, surgeons turned to testes transplantation as a means to treat hypogonadism and bring about rejuvenation and therapy for all sorts of disorders. George Frank Lydston (1858–1923) at Cook County Hospital in Chicago was one of the first to perform human testicular transplantation from accident victims to recipients [19]. Also in Chicago Victor D. Lespinase (1878–1946) published his experience with transplanting human testes from donors to patients for rejuvenation [20] and Leo Stanley (1886–1976), at the California State Prison San Quentin, reported 20 cases of transplantation of testes from executed prisoners to other inmates who reported signs of revitalization. Later on he turned to rams as sources for his testicular grafts and reported satisfaction on the part of the patients, including 13 physicians [21, 22].

John Romulus Brinkly (1885–1942) a half-educated medic, turned goat testis transplantation in his clinic in Milford, Kansas, into a booming business between 1918 and 1930. However, in 1939 he was found guilty by a Texas judge of acting as a charlatan and quack, thus unleaching a series of lawsuits demanding millions of dollars as compensation. Brinkly declared bankruptcy and died of heart attacks soon thereafter.

In Vienna, Eugen Steinach (1861–1944) performed vasoligation for rejuvenation [23]. One of his followers, Serge Voronoff (1866–1951) turned to xenotransplantation and used monkey testes to be transplanted for rejuvenation [24]. He first offered his surgery in Paris, but after some scandals continued his questionable operations in Algiers, where he was visited by patients from all over the world. In many countries Voronoff's followers xenotransplanted animal testes and pieces thereof to patients demanding rejuvenation. When unrest among the medical profession grew concerning this quackery, in 1927 the Royal Society of Medicine (London) sent an international committee to Voronoff in Algiers. The committee concluded their investigations by declaring Voronoff's claims as "poppycock."

These scandals and the hope that steroid biochemistry would ultimately lead to the discovery and synthesis of the male sex hormone, following that of female sex hormones, finally terminated the questionable business of testes transplantation. However, before the chapter of modern testosterone biochemistry and pharmacology can be opened, another century-long medical misapprehension needs to be discussed.

Testes for Organotherapy

Since antiquity the knowledge of the powerful function of the testes in the normal male organism induced patients and healers to turn to the ingestion of these organs in various modalities. Early on Gaius Plinius Secundus (23–79) in Rome prescribed the consumption of animal testes for the treatment of symptoms of hypogonadism

and impotence. For the same purpose the Arabic physician Mesue the Elder (777–837) in Baghdad recommended testis extracts. Also in Chinese medicine—at least since 1132—Hsue Shu-Wei prescribed raw and desiccated animal testes. The "Universal Doctor" and founder of the University of Cologne, Albertus Magnus (1192–1280), concerned with the taste of his prescription, recommended powdered hog testes in wine as a vehicle [1].

The well-known scientist and member of several high-ranking learned societies, Charles-Edouard Brown-Séquard (1817–1894), gave organotherapy a new dimension when, at the age of 72, he published the results of his dubious self-experimentation in the Lancet [25]. He self-injected a mixture of testicular vein blood, semen, and juice extracted from dog or guinea-pig testes and observed signs of rejuvenation, which, at best, must have been placebo effects, since the testes synthesize testosterone, but do not store it (as, e.g., the thyroid does with its hormones), and the amount administered was minute [26]. However, in no time "extracts of animal organs by the Brown-Séquard method" were sold worldwide and factories sprang forth in Europe as well as in America. This elixir soon became not only the source of high revenues, but also the object of ridicule in comics and songs as demonstrated by "The greatest comic song of the day" (words and music by Winchell Forbes, 1889).

"Undertakers, wigmakers, and gravediggers swear,

- Till the air with their curses is blue,
- At the man who invented Elixir Séquard,
- And left them with nothing to do,
- And even the doctors are rattled at last,
- For when their best patients are sick, sir,
- They just step around to the corner drug store,
- And "shake" for a dose of "Elixir."

These elixirs were consumed by everyday patients as well as celebrities and were even used for doping in sports, as exemplified by Pud Galvin (1856–1902). This American National Association baseball pitcher was the first Major League 300-game-winner (elected to the Baseball Hall of Fame in 1965) who attributed his final successes to the Brown-Séquard elixir.

The craze for these products caused concern about the image of the young field of endocrinology. The famous neurosurgeon, Harvey W. Cushing (1869–1939), went so far as to talk about "endocr*imi*nology" in the context of this organotherapy. Nevertheless, many companies worldwide continued to manufacture extracts and pills, well into times when genuine testosterone was already long on the market. For example, Ciba (Switzerland) withdrew their Androstin® (="biologically titrated full extract from male gonads" for oral and parenteral use) only in 1961, after three decades of successful sales for the treatment of "male gonadal insufficiency, impotence, infantilism, premature aging and endocrine obesity" [27].

Isolation and Synthesis of Testosterone

Finally reacting to the hype generated by testicular transplantation and organotherapy, the young pharmaceutical industry and academic research cooperated in order to rehabilitate endocrinology and replace organotherapy by proper hormone substitution. In the dawn of testosterone emerging as a biochemical and marketable entity, in 1935 Ciba (Switzerland) and Schering (Germany), pharmaceutical companies active in the field, started a cooperative effort to inform each other about progress and forced their academic protagonists Leopold Ruzicka (1887–1976) at the Technical University of Zürich and Adolf Butenandt (1903–1995) at the University of Göttingen, to exchange their respective advances, to which the former rivals reluctantly agreed. In 1937 the Ciba-Schering cooperation was extended to include Boehringer (Germany), Chimio Roussel (France) and Organon (The Netherlands) to form a real cartel/syndicate to share knowledge, to stake their market claims worldwide and to agree on prices of the products [27].

Important steps in the isolation of testosterone were the development of biological tests for androgen activity. Loewe and Voss [28] described androgenic activity in urine and developed the "Loewe-Voss-Test" for measuring androgenic activity. Moore et al. [29] refined and standardized the capon comb test as the unit of androgenicity. This biological test helped to resolve the question whether *only one* or *several* androgenic steroids existed and, if more than one—which might be the more potent one.

In 1931 Adolf Butenandt isolated the androgenic steroid androsterone (androstan-3α-ol-17-one) from 15,000 l of urine provided by young policemen from Berlin and which were then processed by Schering to obtain 15 mg of this first androgen [30]. In 1935 Ernst Laqueur (1866–1947) and his group at Organon and the University of Amsterdam extracted and isolated 10 mg testosterone (17β-hydroxy-4-androstene-3one) from 100 kg of bull testes which they found to be more active than androsterone in biological tests [31]. They baptized this hormone "testosterone." In the same year Butenandt and Hanisch [32] in Göttingen, (Fig. 1.3) as well as Ruzicka and Wettstein [33] in Zurich/Basel (Fig. 1.4), published the chemical synthesis of testosterone, marking the beginning of modern clinical pharmacology of testosterone and male reproductive physiology.

The close cooperation behind the researchers reinforced by the two pharmaceutical companies may explain why these discoveries were published more or less at the same time. Marius Tausk, one of the former heads of research at Organon who knew the competing protagonists personally, describes the race to testosterone isolation and synthesis very vividly and how the key respective papers were submitted for publication in short sequence in 1935 [35]. In 1939 Butenandt and Ruzicka received the Nobel Prize for chemistry jointly, Butenandt "for his work on sex hormones" (estrogen, progesterone, and androsterone) and Ruzicka "for his work on polymethylenes and higher terpens," guiding him to the androgens. Why Laqueur's contribution was not recognized remains unclear. The Nazi regime prevented Butenandt from accepting the Nobel Prize and he received it only after World War II.

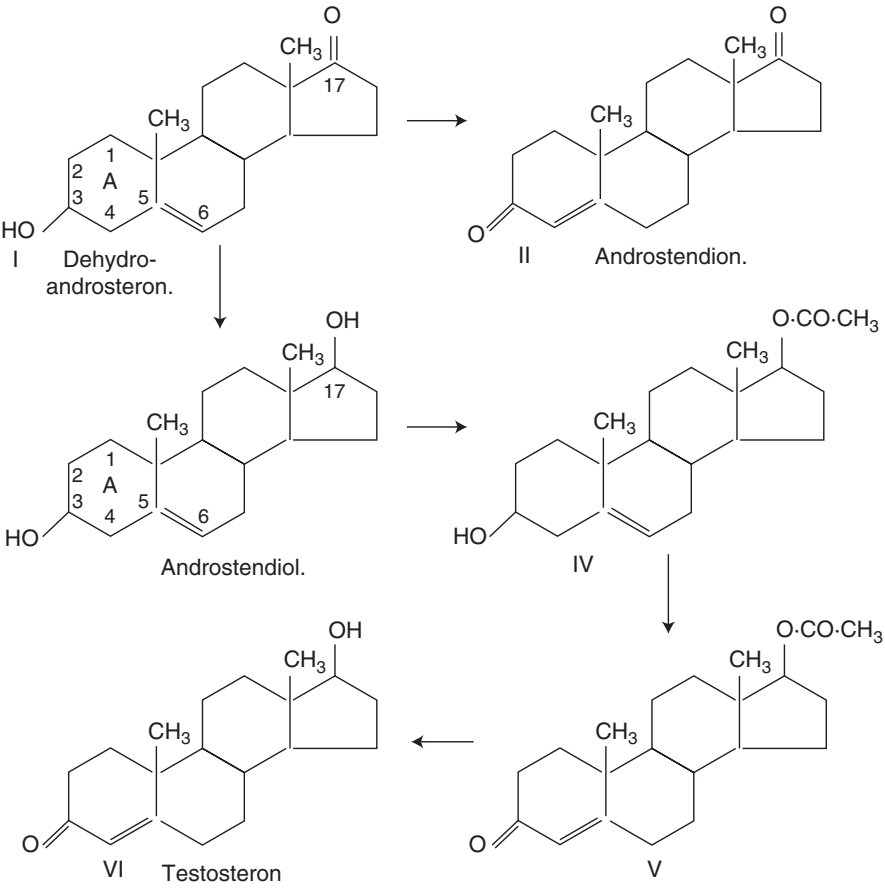

Fig. 1.3 The synthesis of testosterone from dehydroandrosterone as described and shown by Butenandt and Hanisch in their original paper in 1935

Beyond scientific recognition this research work was also financially rewarding, as Ruzicka wrote in autobiographical notes: "The patents for the degradative synthesis of testosterone and methyltestosterone earned me during subsequent years an enormous (compared with my professorial standard) amount of money as royalties from Ciba in Basel and Ciba in the USA" [34]. In the year 1939 alone Ciba transferred 56,744 Swiss Francs to Ruzicka as royalties [27]. Part of this money was invested in a collection of seventeenth century Flemish and Dutch paintings which Ruzicka donated to the Kunsthalle Zürich in 1947 [34].

However, while this pioneering work was well recognized in science and by the pharmaceutical companies, clinicians were very skeptical whether the early preparations of testosterone would contain enough of the hormone to produce any biological and clinical effects, as documented in a textbook of endocrinology of the time: "The number of testis hormone preparations is still very low. Comprehensive

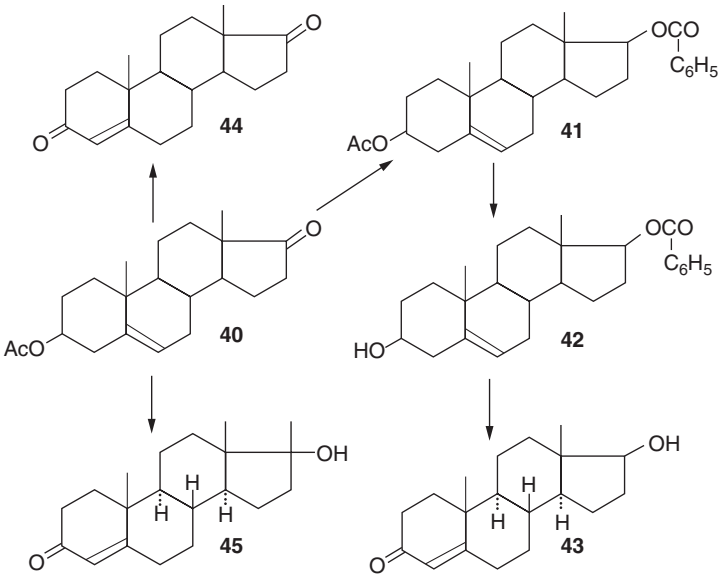

Fig. 1.4 Degradative synthesis of testosterone (43) and 17α-methyltestosterone (45) as described by Ruzicka and Wettstein in 1935. Interestingly, in his autobiography Ruzicka [34] considered the synthesis of 17α-methyltestosterone as "a greater intellectual achievement" than that of testosterone

clinical investigations are not yet available, determining the doses for human therapy. It is therefore unknown whether the available preparations can be administered in sufficient concentrations" [36]. Their skepticism was soon to be defeated by the chemists' continued efforts and skills.

Evolution of Testosterone Preparations for Clinical Use

Soon after its synthesis it became clear that, in reasonable doses, testosterone was not effective orally or—as we know today—would require extremely high doses which were simply not available and/or too expensive. Today we know that the lack of oral effectiveness is due to the inactivation of testosterone by the first-pass effects in the liver (Fig. 1.5) [37]. Three approaches were used to overcome this problem:

1. Chemical modification of the steroid molecule,
2. Parenteral application, and
3. Esterification in position 17β of the testosterone molecule.

For a complete description of the many testosterone preparations and routes of administration the reader is referred to reviews by Nieschlag and Behre [38] and Behre and Nieschlag [39] and to respective chapters in the current book.

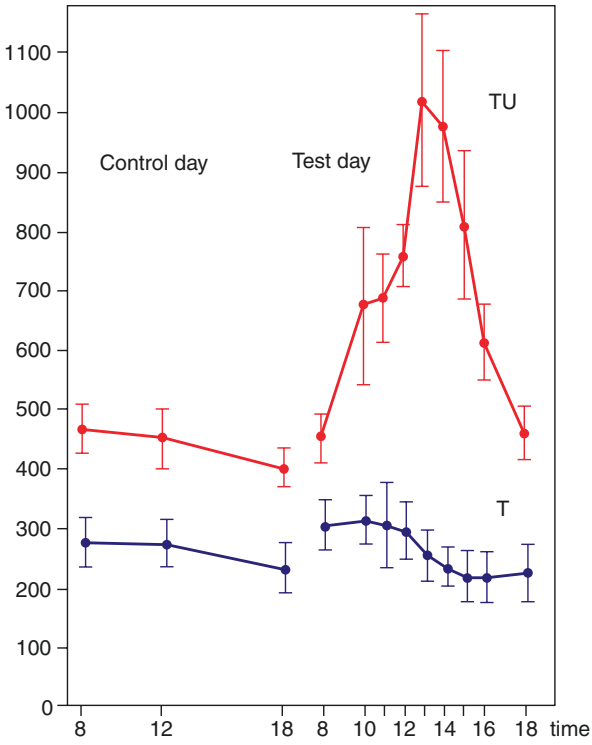

Fig. 1.5 Serum testosterone levels in normal volunteers after ingestion of either 65 mg crystalline testosterone (lower lines) or 100 mg testosterone undecanoate capsules (equivalent testosterone doses) (adapted from [37])

As early as 1935, the year when testosterone was first isolated and synthesized, Ruzicka et al. [40] also synthesized **17α-methyl-testosterone** (Fig. 1.4). Its oral effectiveness was demonstrated so that it soon was licensed for clinical use and well accepted because of the ease of oral application [41]. However, before long, liver toxicity due to the 17α-structure of this molecule became evident, especially under long-term use and at higher doses [42]. Later it became clear that this toxic effect would be shared by all 17α-substituted androgens [43], thus giving testosterone in general a bad name among physicians. Eventually, however, in the 1980s, when another orally effective preparation free of toxic side effects became available (see below), 17α-methyl-testosterone became obsolete for clinical use—at least in Europe.

As testosterone proved to be ineffective orally, parenteral routes were explored. Subdermal **testosterone pellet** implants were the first to be investigated [44] and pellets are still in use today [39]. They were manufactured by Organon (The Netherlands) until 2007, when the company was taken over by Schering Plough (USA) and then until 2009 when Schering Plough was bought by Merck, Sharp & Dohme/MSD (USA). Their application requires a small operation and harbors the risk of infection and extrusion. However, if enough pellets are implanted they may provide substitution for up to half a year. Today these pellets are predominantly used in Australia.

Other parenteral routes were tested in the course of the steroid's history during which **testosterone suppositories** were marketed by Ferring [45], but yielded rather unpredictable serum levels [46] and are no longer commercially available. A more recent development in this area is bioadhesive **buccal testosterone tablets**, placed on the gingiva and resulting in effective serum levels if applied twice daily [47]. However, due to low patient compliance they have never penetrated the market.

When injected, testosterone has an extremely short half-life of only 10 min and as such is not suited for substitution purposes. Therefore, the third possibility to make testosterone clinically effective is esterification at the 17β-hydroxy-group of the molecule, making for suitable intramuscular injection. **Testosterone propionate** was the first of these esters marketed by Ciba as Perandren® and by Schering as Proviron® in 1936. However, this ester has a short half-life so that effective serum levels are reached for only one to two days. When **mesterolone** (1α-methyl-5α-androstan-17βol-one) became available Schering used the then well-established name Proviron® for this new oral preparation [48, 49] and continued to market testosterone proprionate as Testoviron®. As mesterolone is not aromatizable it has little effect on gonadotropins, bones and other estrogen-dependent functions and could not be used for full substitution of hypogonadism, but was mainly employed for male infertility treatment—without evidence-based proof of its effectiveness for this indication [50].

Following the propionate ester, **testosterone enanthate** was synthesized by Junkmann [51, 52] at Schering and marketed as intramuscular Testoviron® Depot injection in 250 mg doses, provides substitution for 2–3 weeks [46]. However, the pharmacokinetics are characterized by transient supraphysiological peaks for a few days, followed by slow decline to levels below the lower limit of normal. Although patients do not appreciate these up and down swings in mood, activity, and libido between injections, this remained the major testosterone preparation for substitution of hypogonadism for half a century. In 2022 FDA licenced the subcutaneous application of testosterone enanthate as Tlando® thereby considerably extending the "half-life" of this early preparation for testosterone substitution [53].

In the late 1950s and 1960s, instead of improving modes of application, the pharmaceutical industry became more interested in the chemical modification of the testosterone molecule in order to disentangle its various effects and produced predominantly erythropoietic or anabolic androgenic steroids (AAS). Although hundreds of androgens were synthesized, it proved impossible to produce androgens with only *one* desired effect out of the wide spectrum of testosterone activities. Nevertheless, while some AAS were applied clinically, they disappeared again in the wake of evidence-based medicine. However, they retain a shadow existence for doping in sports and bodybuilding, potentially causing considerable undesired effects [43, 54]. Regrettably, at that time the pharmaceutical industry ignored the needs of hypogonadal patients, as pharmacokinetic studies had revealed that the existing testosterone preparations resulted in unphysiologically high or low serum levels, undesirable for substitution purposes so that the World Health Organization made an appeal for more physiologic modalities of substitution [55].

Unfortunately, the pharma industry also turned a blind eye on another potentially huge application of testosterone: hormonal male contraception. In the 1970s the WHO Human Reproduction Program as well as the Population Council of the Rockefeller Foundation had identified male contraception as an unmet need for family planning and as a means against global overpopulation. Hormonal male contraception based on the combination of testosterone and a progestin was at that time—and remains so to date—the most likely candidate for general use. However, the existing testosterone preparations required too frequent applications (for review of clinical trials see [56]). To overcome this deficiency both organizations started programs in search of long-acting testosterone preparations. Under the auspices of WHO **testosterone buciclate** was synthesized [57] and identified as a long-acting preparation, well suited for male contraception—and by the same token, also for substitution [58]. However, no pharmaceutical company could be inspired to further develop this promising preparation [59], so that in its ensuing clinical trials for male contraception WHO switched to intramuscular testosterone undecanoate as described in the following [60].

Meanwhile, the Population Council had turned to **7α-methyl-19-nortestosterone (MENT)** as its preferred androgen for male contraception. This androgen might have the advantage of lacking conversion to DHT and thereby have little effect on the prostate. As MENT has a rather short half-life it was administered in subdermal silastic implants, delivering the active substance for a year—or perhaps even longer—thus being well suited for contraception as well as for substitution [61–63]. However, the company, although interested in further clinical research with this androgen, dropped its plans in the wake of being taken over by another company not interested in the male.

In the late 1970s the **orally effective testosterone undecanoate**, absorbed from the gut via the lymph to avoid the first-pass effect in the liver (Fig. 1.5) [64, 65] had been added to the spectrum of testosterone preparations available for replacement therapy. Peter Kicovic, in charge of clinical development at Organon, approached the senior author for first clinical trials with this new substance as he had developed a radioimmunoassay for testosterone and was able to measure testosterone levels in small serum volumes lending themselves to pharmacokinetic studies [66]. As this assay and the testosterone-antiserum produced for the assay [67] were widely used and quoted both papers became Citation Classics in 1982. While initial clinical testing revealed that oral testosterone undecanoate was best absorbed with a meal, the testosterone peaks were short-lived so that 3–4 capsules had to be taken during the day [37, 68]. Oral testosterone undecanoate was introduced to the market worldwide in the late 1970s under the brand name Andriol®—except in the USA. Recently a new oral self-emulsifying delivery system for testosterone undecanoate has shown better pharmacokinetics than testosterone undecanoate in arachis oil [69, 70] and was licensed as Jatenzo® by FDA in 2019.

In the mid-1990s, **transdermal testosterone films** applied to the **scrotal skin** became the first transdermal testosterone preparation in clinical use. Invented by Virgil Place (1924–2012) at ALZA in Palo Alto (CA) and first tested in clinical trials in Münster [71–73], they showed excellent pharmacokinetic and clinical results

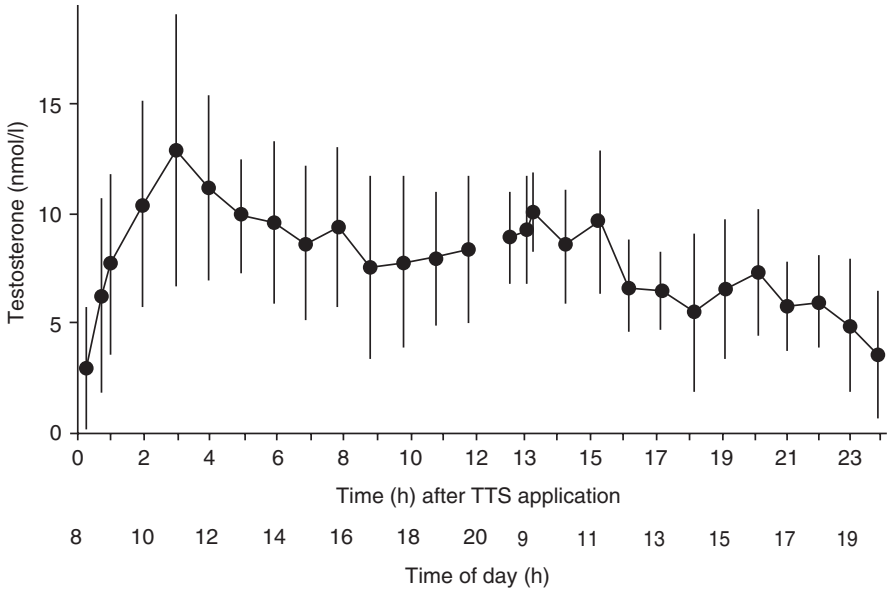

Fig. 1.6 First clinical trial of transdermal testosterone: serum testosterone values in 7 hypogonadal men following the scrotal application of a transdermal testosterone film (adapted from [71])

and, for the first time, physiological testosterone serum levels could be achieved (Fig. 1.6). Patients were very satisfied with this physiological pharmacokinetic profile, as long-term substitution revealed [74, 75]. However, physicians were reluctant to prescribe a medication to be applied to the scrotum, preferring a subsequently developed **non-scrotal testosterone system**, Androderm® [76]. This, however, caused unpleasant skin reactions as it required an enhancer to drive testosterone through the skin. For this reason the advent of the first transdermal testosterone gel in 2000 was welcomed for substitution [77]. Of the various gels available today, the one with the highest testosterone concentration (2.5% Testotop®) can be washed off the skin shortly after application, thereby reducing the danger of contaminating children or women. It has also been tested for scrotal application. Because of the high absorptive capacity of scrotal skin, only 20% of the gel needed for non-scrotal application is required, making this form of application economically and ecologically desirable [78].

Finally, in 2004, the **intramuscular testosterone undecanoate** preparation entered the market and soon achieved great popularity as a real depot preparation. Testosterone undecanoate, originally used in oral capsules (see above), had been turned into an injectable preparation by Chinese investigators using tea seed oil as a vehicle [79]. When the authors came across it at a meeting in Beijing in 1993, samples were brought to Germany, injected into monkeys and showed a surprisingly long half-life [80]. In a further study in monkeys the kinetics and biological effects of intramuscular testosterone undecanoate were compared with those of testosterone enanthate and with testosterone buciclate, a preparation duly synthesized under

WHO auspices primarily for male contraceptive purposes (see above), further demonstrating the superior properties of intramuscular testosterone undecanoate [81]. The long half-life and serum levels, consistently in the physiological range, could be confirmed in volunteering hypogonadal men who all showed serum levels in the normal range for several weeks [82]. Several pharmaceutical companies were contacted for further development of this preparation, however, although all were convinced of the excellent pharmacokinetic properties, none was interested in taking it on board, probably disregarding its potential because male hypogonadism was too small an indication for a financial commitment, and the aging male had not yet been "discovered." When finally Jenapharm could be interested in this fascinating preparation, tea seed oil was replaced by castor oil as vehicle and the injection intervals could be extended to 12 weeks of physiological serum testosterone levels [75, 82–84].

Meanwhile the new testosterone preparation had also been tested in trials for hormonal male contraception and had proven to be very effective in combination with norethisterone enanthate [85, 86]. Our 10-year experience with intramuscular testosterone undecanoate has been summarized [87] and demonstrated that the CAG repeat polymorphism in exon 1 of the androgen receptor influences the pharmacokinetic and biological effects of testosterone and may thus provide a clue to a personalized administration of testosterone.

In 2004 intramuscular testosterone undecanoate was first licensed in Germany under the trade name of Nebido® and licensing in over 100 countries followed either under this brand name or as Reandron®. The latest approval came from the FDA in 2014, licensing ampoules of 750 mg testosterone undecanoate under the brand name Aveed® for the treatment of hypogonadism in the USA.

Conclusion

The testes, with their exposed position, are vulnerable and easily accessible to external manipulation, including trauma and forceful removal. Thus, the effects of testosterone and its absence as a consequence of loss of the testes became known quite early in the history of mankind. Castration, leading to a loss of virility and fertility, has been used over centuries for punishment of criminal acts and high treason, for the creation of obedient slaves and servants and for preserving the prepubertal soprano voices for musical entertainment. Although the testes were correctly identified as the source of androgenicity, attempts to use them in organotherapy can only have led to placebo effects.

Testosterone itself was isolated only in 1935 and, since then, has been available as a powerful medication for treatment of hypogonadism. For many decades, substitution with testosterone enanthate or cypionate was the leading form of testosterone substitution. In the 1970s, orally active testosterone undecanoate was added and in the 1990s the first transdermal preparations in the form of membranes and patches were introduced. Later, testosterone gels became available allowing physiological serum testosterone levels to be reached. With injectable testosterone undecanoate, a true depot preparation came into clinical use. History demonstrates the early

knowledge about the testes and how this knowledge was misused by castration as punishment or to preserve or suppress certain male psychological features and fertility. History also shows the long-winding detours medicine took including organotherapy and transplantation until the active agent, i.e., testosterone was isolated and synthesized as clinical medication [88].

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Androgen Receptor in Health and Disease

2

Alexandre Hohl and Marco Marcelli

Androgenic Hormones

Biosynthesis of Testosterone (T) and 5α-dihydrotestosterone DHT

Fetal Leydig cells differentiate from mesenchymal-like stem cells within the interstitial spaces situated between the developing seminiferous tubules at gestational week (GW) 8 [1]. Transcripts encoding the enzymes required for the synthesis of testosterone (T) are detectable shortly after fetal Leydig cell differentiation, at which point T will be measurable in the fetal circulation. T level continue to raise until it peaks in the early second trimester [2]. During this phase of male development, from GW 8 to 12, T drives the events associated with normal fetal masculinization together with two other testicular hormones: the anti-Mullerian hormone (AMH), which causes regression of the Mullerian ducts, and insulin-like 3 (INSL3), which contributes to testicular descent into the scrotum. T, interacting with the androgen receptor (AR), is responsible for the maturation of the epididymis, vasa deferentia, and seminal vesicles from the Wolffian ducts. T is converted to dihydrotestosterone (DHT) by the enzyme 5α reductase, and DHT is the ligand interacting with AR in the urogenital sinus to give raise to the prostate and prostatic urethra, and in the urogenital tubercle, swelling and folds to give raise to the glans, scrotum and shaft of the penis, respectively [3].

At least two generations of Leydig cells [4] have been described in eutherian mammals; a fetal Leydig cell population producing T during gestational life and an adult Leydig cell population responsible for the surge of T occurring at the time of

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puberty. A third neonatal population of Leydig cells responsible for the postnatal surge of T has been hypothesized [5], however, it is not established whether this neonatal Leydig cell represents an independent entity or simply a reactivation of the fetal population. T production from fetal Leydig cells is hCG-dependent and LH-independent, as shown by the occurrence of normal fetal masculinization in carriers of inactivating mutations of the LHβ gene [6], and by the observations that placental hCG peaks before the onset of LH secretion at GW 8–12, when it stimulates steroidogenesis by interacting with the LH receptor on the surface of Leydig cells [7]. In contrast, differentiation of adult Leydig cells and their ability to synthesize T are exclusively LH-dependent processes, as shown by the occurrence of Leydig cells aplasia and severe T depletion in carriers of LHβ gene inactivation [6].

Cells that synthesize polypeptide hormones accumulate large quantities of preformed hormones in secretory vesicles, from where these hormones are readily available for release when proper physiologic circumstances occur. In contrast, steroidogenic cells store very little quantities of steroid hormones. This implies that when the physiologic pathways leading to the synthesis of steroid hormones are activated, there must be a mechanism generating their rapid de novo synthesis. The main mediator of this rapid steroidogenic response is the 37-kDa StAR (steroidogenic acute regulatory) protein [8]. In response to appropriate stimulation (for instance, the interaction of ACTH, angiotensin II or LH with their receptors), StAR mRNA transcription increases and StAR protein is rapidly translated, phosphorylated, directed to the mitochondria by its mitochondrial leader sequence, and cleaved upon mitochondrial entry to yield a 30-kDa intramitochondrial protein [9]. StAR stimulates the flow of cholesterol from the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM) where it is converted by the first steroidogenic enzyme, CYP11A1, into pregnenolone. Inactivating mutations of StAR are associated with the most common form of lipoid congenital adrenal hyperplasia, characterized by complete inability to synthesize steroid hormones [10]. Pregnenolone serves as a common substrate for the synthesis of T through the Δ^5 or Δ^4 pathways in the endoplasmic reticulum of Leydig cells (Fig. 2.1). Despite the two pathways run in parallel and entail the same number of enzymatic reactions, the majority of testosterone biosynthesis in the human testis takes place through the conversion of pregnenolone to dehydroepiandrosterone via the Δ^5 pathway, due to a higher affinity of the steroidogenic enzymes involved for the metabolites of the Δ^5 pathway [11]. T is released in the general circulation via the spermatic vein in a pulsatile way. In young males this occurs in a circadian manner, with a T peak observed in the early morning followed by a nadir between 4 and 8 PM [12]. Aging is associated with progressive loss of circadian T secretion [13].

Serum Testosterone

Testosterone is the main sex steroid produced by Leydig cells, with an average secretion rate of 7 mg/day [14] (Fig. 2.1). Based on calculations from spermatic vein/peripheral vein gradients, Leydig cells can also release intermediate

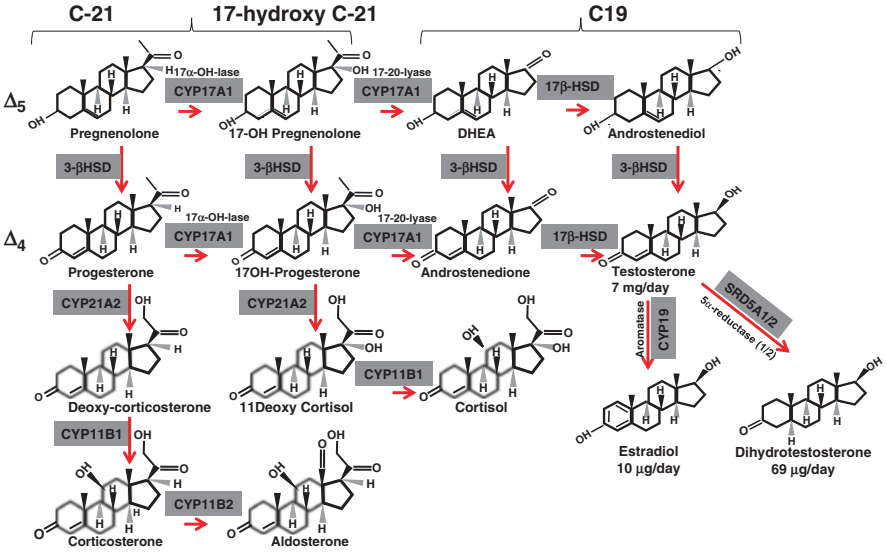

Fig. 2.1 Main pathways of adrenal, ovarian, and testicular steroidogenesis, showing structures of the most important intermediate metabolites and end products, and enzymes involved. D(delta)5: Metabolites of the D(delta)5 pathway characterized by the presence of a double bound between carbons 5 and 6. D4: metabolites of the D4 pathway, characterized by a double bound between carbons 4 and 5. C-21: all intermediates going from pregnenolone to aldosterone have 21 carbon atoms (C-21 steroids). 17-hydroxy C-21: all intermediates going from 17-OH-pregnenolone to cortisol have 21 C carbon atoms and a OH group in position 17. The enzyme 17-20-lyase removes carbon atoms 20 and 21 to yield C-19 steroids

metabolites such as androsterone, androstenedione, 17-OH progesterone, progesterone, and pregnenolone [14]. The testes also release 69 μg/day of DHT and about 10 μg/day of estradiol [14], however, the major sites of formation of these two sex steroids are extra-glandular. Approximately 5% of the T pool is of adrenal derivation. Studies in patients with prostate cancer demonstrated that the human adrenal produces approximately 200 μg of T regardless of whether the patient had intact testes or was castrated [15], and an additional 200 μg of T is formed in the periphery from the conversion of adrenal-derived androstenedione [15]. Plasma T is bound to sex-hormone binding protein (~44%) and albumin (~54%), and only 2% circulates free. These three fractions of T are measured together as "Total T," which represents a reliable first line tool to screen patients for hypogonadism. SHBG has a higher affinity for T than albumin (1.6×10^{-9} M vs. 4×10^{-4} M), however, the overall T-binding capacities of SHBG and albumin are similar because the concentration of albumin is higher. SHBG-bound T is not bioavailable due to the tight interaction existing between the two, which prevents SHBG-bound T to reach AR in the target cell. As a consequence, free and albumin-bound T represents the bioactive fraction

of T (i.e., the fraction of T that enters the target cell and interacts with the AR, also known as bioavailable T). It is important to remember that several conditions alter the absolute level of plasma SHBG and will be associated with an increased (or decreased) serum level of Total T. SHBG (and total T) decrease in patients affected by obesity, T2DM, hypothyroidism, and nephrotic syndrome, and increase with aging, pregnancy, hyperthyroidism HIV, and cirrhosis. Drugs such as estrogens, phenytoin, and tamoxifen increase SHBG, while androgens inhibit its synthesis [16]. Based on the high prevalence of some of these conditions [for instance, more than 30% of adult individuals are affected by obesity (http://www.cdc.gov/obesity/data/adult.html) and 9.3% by T2DM (http://www.cdc.gov/diabetes/pdfs/data/2014-report-estimates-of-diabetes-and-its-burden-in-the-united-states.pdf) in the USA], bioavailable T is a better indicator of the actual level of biologically active T in patients affected by conditions associated with abnormal SHBG concentrations. Free T by equilibrium dialysis represents the most reliable measurement of bioactive T, but this technique is cumbersome and not widely available. Total T by gas chromatography mass spectroscopy (GCMS) is rapidly becoming the most widely accepted technique to measure total T, but also this test is not widely available.

An important question in endocrine physiology is whether the circulating concentration of a hormone is a good harbinger of its concentration in the target organ. This question is important, because hormonal action takes place in the target organ, not in the blood stream. With regard to T and DHT, a conclusive answer to this question is not available. The Prostate Cancer Prevention Trial (PCPT) and Reduction by Dutasteride of Prostate Cancer Events (REDUCE) trials demonstrated that serum and tissue T levels increase and DHT decrease (i.e., they change in the same direction) in men who have been treated with the 5α-reductase inhibitors Finasteride for seven years [17] or Dutasteride for 4 years [18]. In contrast, other clinical trials reported that changes in serum T or DHT concentration observed after testosterone replacement therapy (TRT), castration or DHT replacement were not associated with parallel changes in the target tissue [19–21]. These data have important implications and suggest that maybe serum T level is not a good surrogate of tissue T level.

T and DHT Interact with AR in the Target Cell

From the general circulation, lipophilic T enters the target cell through a mechanism of passive diffusion across the plasma membrane. Inside the target cell T can be converted into the more active metabolite DHT by the 5α reductase isoenzymes (SRD5A1 or 2) or in alternative into estradiol (E_2) by the enzyme aromatase (CYP19). Both T and DHT bind with high affinity a unique cytoplasmic AR protein. This interaction is highly specific, and is ensured by the fact that normal concentrations of circulating T usually exceed by tenfold the equilibrium binding affinity for AR. When sufficient concentrations of T are not present, activation of AR can still take place in certain target tissues due to the conversion of T into DHT, an androgen with 4–10 times higher affinity for AR [22, 23]. AR is a class I member of the

nuclear receptor (NR) family of transcription factors together with other classic steroid receptors such as glucocorticoid, progesterone, mineralocorticoid, and estrogen receptors α and β (GR, PR, MR, ER, α and ERβ) [24]. Among class I nuclear receptors the model of two ligands interacting with a single receptor is unique to AR. In the case of MR, the model consists in a single receptor interacting with a single ligand. In the case of GR, a single ligand interacts with at least four GR isoforms arising from alternative mRNA splice variants or alternative translation initiations [25]. In the case of PR and ER a single ligand interacts with two isoforms, arising from alternate sites of transcription initiation [26] or two different genes [27, 28], respectively. In the case of AR, a second amino-terminally truncated isoform has been described, but there is no evidence that it regulates distinct biological functions [29].

Two Ligands and One Receptor

The presence of two ligands and one receptor is an enigma that has fascinated generations of endocrinologists. Owing to a faster dissociation rate of T from AR [30] and to differences in the way T interacts with the ligand binding pocket (LBP) of AR compared to DHT (discussed later), T is a weaker androgen compared to DHT by a factor of 10 [31]. During embryogenesis T is responsible for the virilization of the Wolffian structures, while DHT is required for the virilization of the anlagen that will generate the external genitals and the prostate. In agreement with this, the lack of DHT described in patients affected by the syndrome of SRD5A2 deficiency is associated at birth with a characteristic phenotype of undervirilized external genitalia and prostate [3]. Explaining the need for both T and DHT in adulthood is less simple. Pharmacologic inhibition of DHT synthesis in men aged 18–50 years for 20 weeks demonstrated that all androgen-dependent functions of post-pubertal males, including maintenance of muscle mass and strength, sexual function, erythropoiesis, prostate volume, PSA levels, and sebum production were interchangeably subserved by T and DHT [32]. Based on this, it could be argued that DHT is needed only during embryogenesis, when SRD5A provides local amplification of an androgenic signal, which leads to virilization (for instance, of the urogenital sinus) without inducing systemic hyperandrogenemia during critical periods of sexual differentiation. Further supporting the concept that in adult individuals T and DHT are interchangeable is the fact that the external genitalia of patients with SRD5A2 deficiency virilize at puberty, when maximal T production has been achieved [33].

Importance of Estrogens in the Male

It has been known for a long time that men produce minute amounts of estradiol in their bodies, and that several male organs express ERα and ERβ, but the physiological meaning of the estrogen → ER axis in males remained unknown until a unique case of ERα inactivation and several cases of CYP19 deficiency were described [34,

35], that lead to conditions of target organ insensitivity or of inability to synthetize estrogens. These phenotypes taught us that estrogens are very important in male physiology by contributing to functions such as skeletal development, epiphyseal fusion, subcutaneous fat deposition, glucose and lipid metabolism, and sperm maturation [36]. A recent study described the consequence of inhibiting estrogen production in men treated with a GnRH agonist followed by testosterone replacement and an aromatase inhibitor. This study demonstrated that in the adult man estrogens also regulate body fat deposition and sexual function [37].

The Androgen Receptor (AR)

Background

The AR gene (N3C4; Nuclear Receptor subfamily 3, group C, gene 4) is located in chromosome Xq11-12, spans more than 90 kB and contains 8 exons (Fig. 2.2a, b). A functioning AR is essential for normal virilization during embryologic

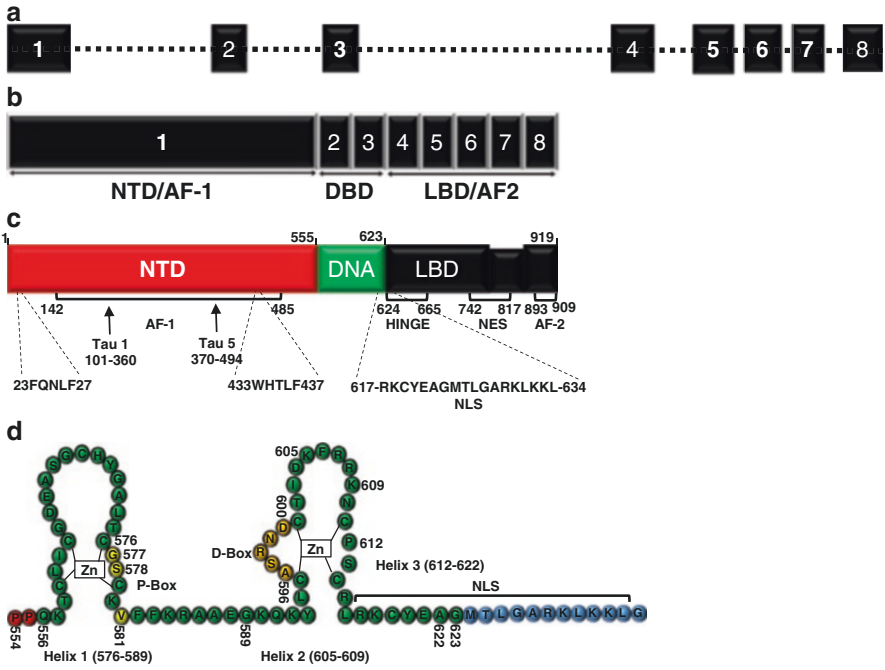

Fig. 2.2 (**a**) AR gene. (**b**) AR mRNA. (**c**) AR functional domains. Color code: NTD red. DNABD green. LBD black. (**d**) Cartoon representation of the AR DBD. Color code: P-Box yellow. D-Box orange. Exon 1: red. Exon 4: blue

development and puberty, and to maintain the adult male phenotype later in life. In agreement with this important function, the Xq11-12 region is highly conserved among mammals, marsupials, and monotremes, and dates back to a common ancestor of about 150 million years ago [38]. The AR gene encodes a protein of 98.8 kDa. The precise number of amino acids is 919, however, the number varies from individual-to-individual owing to the size of a polymorphic glutamine repeat located in the amino-terminal domain (NTD), that stretches in the normal population from 9 to 39 residues [39]. With the exception of the spleen, most tissues, and in particular reproductive organs, express AR at the immunohistochemical level [40].

In the absence of ligand, AR is inactive and localized in the cytoplasm (Fig. 2.3a). After T or DHT binding, AR undergoes a drastic conformational change and dissociates from anchoring proteins (Fig. 2.3b). The signal responsible for AR nuclear import is positioned in the AR hinge region and is structurally and functionally similar to the bipartite nucleoplasmin nuclear localization signal (NLS) [41]. Following the conformational change that occurs after ligand binding, NLS is exposed and controls AR translocation through the nuclear core complex into the nucleus (Fig. 2.3d). Inside the nucleus AR dimerizes with a second AR molecule (Fig. 2.3f, g), binds specific DNA cis-acting sequences known as androgen or hormone response elements (ARE or HRE) located within the regulatory regions of AR-responsive genes (Fig. 2.3h), or at considerable distances either upstream or downstream from the transcription initiation site. Following DNA binding, AR recruits coregulatory proteins and components of the basal transcriptional machinery, to induce (or inhibit) transcription of specific networks of genes (Fig. 2.3i). Post-translational modifications at 23 AR sites have been described to occur and consist in phosphorylation, acetylation, SUMOylation, methylation, and ubiquitination. These modifications are thought to regulate AR cellular localization, structure, activity, and stability, and can occur in the cytoplasm or nucleus of the cell. Phosphorylation, occurring at multiple Serine residues, is the most studied post-translational modification of AR, regulating AR transcriptional activity in a negative or positive fashion, and providing a platform for cross talking between growth factors and AR signaling mechanisms. Excellent reviews focused on the effects of AR post-translational modifications are published, and the reader is referred to them for additional information on this topic [42–44].

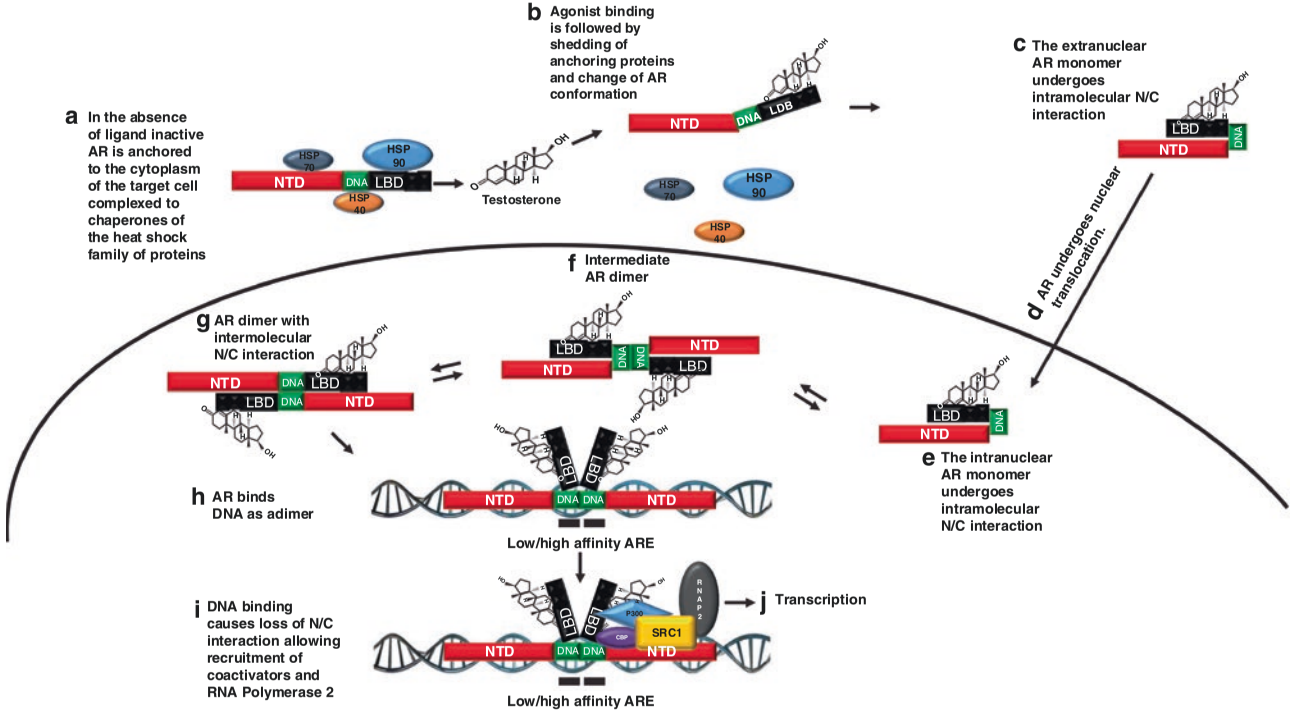

Fig. 2.3 Signaling pathway of the AR schematized in nine steps. Each step is described in the cartoon. (**a**) In the absence of ligand inactive AR is anchored to the cytoplasm of the target cell complexed to chaperones of the heat shock family of proteins. (**b**) Agonist binding is followed by shedding of anchoring proteins and change of AR conformation. (**c**) The extranuclear AR monomer undergoes intramolecular N/C interaction. (**d**) AR undergoes nuclear translocation. (**e**) The intranuclear AR monomer undergoes intramolecular N/C interaction. (**f**) Intermediate AR dimer. (**g**) AR dimer with intermolecular N/C interaction. (**h**) AR binds DNA as a dimer. (**i**) DNA binding causes loss of N/C interaction allowing recruitment of coactivators and RNA polymerase 2

Functional Domains of AR

Similar to the other NR, AR contains four modular domains (Fig. 2.2c), which include: (1) An N-terminal domain (NTD) of 555 amino acids corresponding to exon 1. This region contains a segment regulating hormone-independent AR transcription named activation function 1 (AF-1). NTD also contains two sequences involved in interacting with AF-2 in the C terminal ligand binding domain [45], which are located at coordinates 23FQNLF27 and 433WHTLF437 (Fig. 2.2). (2) A DNA binding domain (DBD), encoded by exons 2 and 3 between residues 556 and 623. (3) A hinge region regulating nuclear translocation, encoded by the N-terminal portion of exon 4 between amino acids 624–665. (4) A ligand binding domain (LBD), encoded by exons 4–8 between amino acids 666 and 919. Besides regulating ligand binding, this region contains a hormone-dependent transcriptional activation function named AF-2. Unlike the other nuclear receptors where it serves the function of interacting with nuclear coregulators and controlling transcription, AR AF-2 binds the two aforementioned regions of the NTD. The function of this interaction, known as N/C interaction, is to protect the ligand within the ligand binding pocket and to prevent its dissociation from the receptor. N/C interaction is physiologically an important process that will be discussed in section B7. In recent years, an additional surface pocket, called binding function 3 (BF3), was discovered in the LBD [46]. This site is distinct from the androgen binding and AF-2 sites and is believed to allosterically regulate AF-2 function [47]. The various domains of AR have maintained a high degree of conservation throughout evolution, with the DBD being the most conserved segment (for instance human and rat AR DBD are 100% conserved) [40], followed by the hinge region and the LBD. There is a significant degree of homology also between AR and other class I NR; for instance, the DBD of AR is 79%, 76%, and 56% identical to that of PR, GR, and ER-, α, respectively [40].

DNA Binding Domain (DBD) (Residues 555–623)

With an evolutionary rate estimated at 0.04 substitutions per site per billion years, it has been estimated that the DBD of AR is among the most slowly evolving molecules known [48]. DBD is the signature domain of AR, providing this protein with the ability to interact with specific DNA sequences located all over the genome at variable distance from AR target genes. Interaction with these sequences is associated with induction or inhibition of transcription of AR-dependent genes.

The AR DBD general structure contains three helices consisting of two zinc fingers (Fig. 2.2d), each organized around four cysteine residues that chelate a single zinc ion, and a C-terminal extension (CTE, amino acids 625–636) segment. In each steroid receptor gene, the two zinc fingers are functionally and structurally different and are encoded by separate exons. The α helix of the first (N-terminal) zinc finger (known as recognition helix) binds the HRE/ARE through the P(roximal) box which contains three amino acids common to class I nuclear receptors AR, GR, MR, and

PR. The AR coordinates for these three residues are Gly577, Ser578, and Val581 (Fig. 2.2d). The second zinc finger contains a region of five amino acids (the D-box, residues 596–600) responsible for dimerization between AR monomers (49) (Fig. 2.2d). In addition to these motifs, residues in the CTE extend the interface between AR and target DNA and provide the AR DBD with the ability to recognize AREs that are specific for AR (50).

The traditional view holds that upon ligand binding, AR translocates into the nucleus where it binds regulatory sequences on the proximal promoter region of target genes and initiates a cascade of events leading to induction or repression of transcription (Fig. 2.3). The classic response element common to class I nuclear receptors, also known as the canonical androgen/glucocorticoid response element (ARE/GRE) [24] consists of two hexameric palindromic half-sites (AGAACA NNN TGTTCT), separated by 3 bp's (NNN). Upon ligand binding and nuclear translocation, AR binds ARE/GRE as a head-to-head homodimer, a structure consisting of two AR monomers where the two second zinc fingers make reciprocal contact through residues located in the D-boxes, and the two first zinc fingers bind through the P-Boxes the two half-sites of the ARE/GRE [51–53]. The two AR molecules involved in homodimerization make contact with each other through the following residues of the D-Box: A596-T602, S597-S597, and T602-A596 [54] (Fig. 2.3d).

Because all members of class I nuclear receptors share the same ARE/GRE [24] and regulate transcription of reporter genes driven by this element [55], the mechanism used by this group of transcription factors to achieve distinct DNA target specificity has been an open question for many years. The question of target specificity is further compounded by the observation that AR and GR share common regulatory sequences positioned in the same loci of chromatin [56], and GR induction of AR-dependent genes is involved in mediating prostate cancer survival when AR activity is pharmacologically blocked [57]. Years of investigations have partially answered this matter, and mechanisms contributing to maintain target specificity of class I nuclear receptors include: (1) Different level of expression of steroid receptors and coregulators in the target cells. (2) Presence of different level of steroid hormones in the tissue. This phenomenon is due to the presence of the enzymes responsible for steroid hormone synthesis and metabolism not only in classic steroidogenic organs, but also in peripheral organs. (3) Diversity in histone modification and DNA methylation patterns regulating NR access to the chromatin of target cells [58]. (4) The discovery that in addition to the canonical androgen/glucocorticoid response element ARE/GRE, AR binds selective AREs arranged as two direct repeats of the AGAACA hexamer separated by a spacer of 3 bp [59–61]. Among class I NR, only AR recognizes these selective response elements. Similar to the interaction described between class I NRs and ARE/GRE, also the AR-AR homodimer binds direct repeats in a head-to-head conformation.

Thanks to technological advances represented by ChIP-on-chip and ChIP sequencing, there has been rapid progress in understanding how gene transcription

is regulated. Studies conducted using these technologies made possible to pinpoint the entire set of cis-acting targets (named cistrome) of trans-acting factors on a genome wide scale. These studies revealed that AR occupies thousands of regulatory regions within the genome with limited similarity to classic AREs [62–64], and that these loci are for the large majority far away from proximal promoters. Indeed, approximately 86–96% of AR binding sites identified in prostate cancer cell lines and androgen-responsive tissues are located at non-promoter regions [65]. The importance of AR distal regulation became clear after observing that transcriptional control of the quintessential AR-dependent gene, prostate-specific antigen (PSA), occurs through the remotely located PSA enhancer, as opposed to the promoter region [66, 67]. Communication between a distant enhancer with the transcription starting site of that gene is made possible by a mechanism whereby the intervening DNA sequences are looped out. In addition to PSA, other classic AR targets, such as TMPRSS2, FKBP51, and UBEC2C are regulated from distant enhancers that interact with the promoter regions of these genes using the same looping mechanism [62, 68, 69].

ChIP seq experiments have identified and compared DNA sequences specific for AR or shared between AR and GR. Specific for AR is a response element with a fully conserved 5′ hexamer and a 3′ hexamer where the only conserved base is a G at position 11. In contrast, these ChIP seq experiments have established that the canonical ARE/GRE sequence is typically shared between AR and GR [70]. Thus, AR selective binding to the chromatin is achieved through interaction with a relaxed cis-element stringency rather than with a distinct and strict ARE sequence. Presence of these selective AR binding sites is essential to ensure selectivity of AR signaling when the cell expresses other steroid receptors in addition to AR [71].

Use of genome-wide technology also led to the observation that cis-acting elements for collaborating (or pioneer) factors colocalize with AR and other steroid receptors binding sites [63, 72, 73]. Pioneer factors, such as the fork-head FOXA-1, have the ability to open up condensed chromatin by replacing histone 1, and to make it accessible to transcription factors, including NR. This activity is a prerequisite for the large majority of NR transcriptional activity, as shown by the observation that a classic NR, GR, binds mostly to chromatin sites made accessible by pioneer factors [74], while only a minority of chromatin is opened up by hormone-induced remodeling. Pioneer factors have now emerged as essential regulators of AR physiology. For instance, pioneer factors such as FOXA1 in the prostate, Hnf4α in the kidney and AP-2α in the epididymis [75] determine occupancy of AR binding sites by AR in a tissue-specific manner. FOXA1 is also known to regulate AR and GR specificity of chromatin binding in LNCaP-1F5 and VCaP prostate cancer cells, respectively [76]. The importance of pioneer factors goes beyond regulating NR specificity of binding to nuclear chromatin. For instance, FOXA1 and HOXB13 are also involved in AR cistrome reprogrammation, the process whereby the network of AR-regulated genes changes when normal prostate epithelium undergoes malignant transformation [77].

Ligand Binding Domain (LBD, Residues 666–919)

The C-terminal LBD is responsible for establishing high affinity binding between AR and T or DHT. Also, the LBD is evolutionarily conserved, but to a lower degree than the DBD. AR LBD shares a common three-dimensional organization with all other NR family members. It is composed of 11 α-helices (numbered H1-12, because AR, unlikely other NR, is missing helix 2) and 4 short β strands (numbered β1-4) forming two anti-parallel β sheets. The 16 and 15 LBD residues with which DHT and T make contact [78, 79] form the ligand binding pocket (LBP). LBP consists in hydrophobic residues located in β1, H3, H4, H5, and H11, which interact in a non-polar fashion with the steroid scaffold of T or DHT [79]. In addition, T and DHT further stabilize their interaction with the LBP by forming hydrogen bonds with polar residues N705, T877, Q711, and R752 [79]. The presence of an unsaturated bond between C4–C5 in T, but not in DHT (Fig. 2.1), changes the geometry of its A ring within the LBP, which results in an orientation change of the C3 ketone group. This difference is one of the reasons why T shows faster dissociation rate from AR compared to DHT [79].

In the unliganded status, H12 (amino acids 893–909) is dissociated from the core of the LBD. Depending on whether the ligand involved is an agonist or an antagonist, ligand binding causes different conformational changes. For instance, the agonist DHT induces a conformational change resulting in H12 repositioning in the vicinity of H3 and H4 to cover the LBP and form AF-2 [79, 80]. AF-2 is a binding surface for coactivators containing a short leucine rich LxxLL motif [also known as nuclear receptor box] [81]. Presence of the LxxLL motif is a feature of many coactivators, including steroid receptor coactivator-1 (SRC1), TIF2/GRIP1/SRC2, and SRC3. Coactivator binding to AF-2 is a necessary step for the recruitment of RNA polymerase II at the transcription starting site and for the induction of transcription. AR does bind with high affinity LxxLL coactivators through AF2 [82], however, unique among NR, AR, AF2 has evolved to preferentially interact with phenylalanine-rich motifs located in the AR NTD in what is known as N/C interaction, which will be discussed in section B7.

The structural mechanism of AR antagonism exerted by non-steroidal antiandrogens remains unclear, however, the general mechanism of antagonism has been elucidated for other nuclear receptor, including GR [83], ER [84], and PPAR-α [85]. In these NR, binding of an antagonist causes H12 to rotate clockwise toward H3, block the coactivator binding site and induce recruitment of corepressors such as NCoR and SMRT. It is likely that the mechanism of antagonism reported for these NR applies to AR, although the tridimensional structure of antagonist-bound AR had not yet been resolved. Nevertheless, computer modeling [86] and functional studies [87] have shown that antagonists cause AR H12 to shift away from H3 and H4. In turn, this results in blockage of AF2 function and recruitment of corepressors [87].

Patients with prostate cancer can experience disease relapse after an initial response to treatment with nonsteroidal antiandrogens such as first generation flutamide and bicalutamide, or second generation enzalutamide. Interestingly, when the antiandrogen drug is discontinued, some of these patients experience an

improvement of their condition. This phenomenon, known as the antiandrogen withdrawal syndrome [88], is related to the selection of AR mutations converting AR antagonists into AR agonists that trigger tumor growth. AR mutations reversing the antagonistic features of AR blockers have been described for flutamide (mutation T877A) [89], bicalutamide (mutations W741C and W741L) [90], and enzalutamide (mutation F876L) [91]. Structural analysis has confirmed that upon flutamide [92] or bicalutamide [93] binding, helix 12 of ARs containing mutations T877A or W741C adopts a classic agonistic conformation uncovering the coactivator binding domain of AF2.

Hinge Region (Residues 624–665)

The hinge region is located between DBD and LBD and contains the large majority of the bipartite nuclear localization signal (NLS) (residues 615-**RK**CYEAGMTLGA**RKLKKL**-632) [41] (Fig. 2.3c). This sequence is composed of two clusters of basic amino acids (bold face) separated by ten residues. Under conditions of ligand deprivation, AR is inactive and anchored in the cytoplasm to immunophilins and chaperone proteins of the heat shock family (hsp90, hsp70, and hsp56) [94] (Fig. 2.3a). The pathway leading to AR nuclear import is initiated by ligand binding (Fig. 2.3b), which leads to shedding of anchoring proteins. This is followed by exposure of the NLS which, after binding the importin-α adapter and importin-β carrier proteins, translocates through the nuclear core complex and is released into the nucleus in a Ran-dependent way [95, 96] (3D). The crystal structure of AR NLS bound to importin-α has demonstrated that the second basic amino acid cluster of the AR NLS interacts with the inner surface of importin α, which in the process acquires a banana-shaped conformation [96].

AR Nuclear Export

AR is localized in the cytoplasm of the cell in the absence of ligand and translocates into the nucleus in the presence of ligand. Likewise, nuclear AR can be exported to the cytoplasm upon ligand withdrawal [97]. A signal specifying cytoplasmic localization of AR [called AR nuclear export signal (NES^{AR})] was identified by serial C-terminal deletions of AR LBD between amino acids 742-817 [98] (Fig. 2.3c). NES^{AR} is dominant over the NLS in the absence of ligand. In contrast, NES^{AR} is repressed in the presence of ligand when the NLS directs nuclear localization of AR.

Amino-Terminal Domain (NTD) (Residues 1–556)

NTD is essential for AR function, however, due to its highly disordered structure that compromises stability and prevents crystallization, there is no structural information available for this AR domain [99]. AR NTD is encoded by a large exon 1,

contains three homopolymeric sequences consisting of Q, G, and P repeats, and includes a segment known as activation function 1 (AF-1) (amino acids 142–485) that regulates AR transcription. NTD is the least evolutionarily conserved region of AR, however, sequence analysis in different animal species has revealed the presence of three areas of relative conservation between amino acids 1–30, which is important for AR N/C interaction, 224–258 and 500–541. AF-1 contains in its sequence the two transcription units TAU-1 (amino acids 101–360) and TAU-5 (amino acids 370–494) [100]. Studies have established that an average polyQ region consists of 21–23 Qs, and that repeats longer than 40 Qs are associated with spinal bulbar muscular atrophy (SBMA), a severe motor neuron degenerative disease associated with mild androgen insensitivity. The length of the poly-Q repeat region is associated with modifications in the folding, structure, and activity of NTD [101], impacts its α-helical structure and changes its ability to interact with regulatory proteins [102]. Increase in polyQ repeats is associated with decreased AR transcriptional activity, while a decrease in number of Q repeats is associated with increased AR transcriptional activity [103].

The significance of AF-1 in regulating AR transcription was first understood thanks to deletion experiments performed in the 1990s [100]. These experiments not only pinpointed the coordinates of AF-1, but also established that a fusion protein consisting of AF-1 and the LexA–DBD retained at least 70% of the transcriptional activity of the full-length NTD [104]. Other investigations revealed that NTD-DBD constructs (i.e., constructs truncated of the LBD) were transcriptionally active in a constitutive way [105], and this effect was AF1-dependent [106]. This suggested a two-step model of AR activity that depends on ligand availability. According to this model, in the absence of ligand LBD adopts a conformation preventing AF-1 of NTD from interacting with proteins stimulating AR transcriptional activity. In contrast, in the presence of ligand the inhibitory conformation of LBD that prevents NTD to interact with regulators of transcription is removed, leading to AR transcriptional activation. This model was confirmed when it became clear that AF-1 does indeed interact with several proteins regulating transcription, including coactivators of the p160 SRC family [107] or CREB-BP [108], corepressors such a SMRT [109], protein members of the pre-initiation complex such as TFIIF [110], various other transcription factors [111] and cell cycle regulators [112].

For most NR, the AF2 domain in the LBD is the primary inducer of transcriptional activity thanks to its ability to bind coactivators (for instance, members of the p160 SRC family) at their LxxLL-like motifs [113]. However, AR has evolved from other NR, and its AF-2 domain preferentially interacts with FxxLF motifs present in the AR NTD (^{23}FQNLF^{27} and ^{433}WHTLF^{437}) [80, 114]. The most important consequence of the preferential interaction between AF-2 and the C-terminus (i.e., N/C interaction) is that in AR physiology coregulator binding is largely delegated to AF-1, making NTD the primary mediator of AR transcriptional activity [80, 114, 115]. N/C interaction occurs intramolecularly between the N and C terminal portion of the same AR monomer (Fig. 2.3c), and intermolecularly between two distinct AR proteins (Fig. 2.3g). From a temporal point of view, intramolecular N/C interaction takes place in the cytoplasm immediately after hormone binding and before nuclear

translocation [54, 116] (Fig. 2.3c). Before DNA binding in the nucleus, the majority of ARs dimerize through the D-box and this drives transition from intra- to intermolecular N/C interaction [54, 116, 117] (Fig. 2.3f, g). AR DBD-DBD dimerization is *a condition sine qua* for N/C intermolecular interaction to occur. This was demonstrated in experiments where AR cDNAs containing mutagenized residues of the AR D-box essential for dimerization were at the same time unable to undergo dimerization and N/C intermolecular interaction [54]. Physiologically N/C interaction is indispensable because it delays ligand dissociation from the receptor, protects the ligand in the ligand binding pocket, and prevents receptor degradation [118]. That N/C interaction is essential in AR physiology is demonstrated by the identification of AR LBD mutations resulting in androgen insensitivity syndromes (AIS) that disrupt N/C interaction without affecting the equilibrium binding affinity for the ligand [119, 120].

Receptor Dimerization

Dimerization is physiologically relevant, as it influences nuclear localization, cofactor binding, DNA binding, and transactivation potential [121]. Types of AR homodimerization that have been discussed in sections B3 and B7 occur between sequences located in the DBD or the amino and carboxyl terminal regions of two distinct AR monomers. DBD-mediated dimerization occurs constitutively [122] and is a prerequisite for AR to bind DNA and to regulate AR-dependent transcription. A third form of dimerization occurs at the level of the LBD. This dimerization is less characterized, and little is known about the specific motifs involved, except that residues 624–918 are required to form LBD-LBD homodimers, and ligand binding is not required for LBD-dependent dimerization [123]. From a temporal point of view, LBD dimerization occurs before DNA binding and DBD-mediated dimerization does not occur unless LBD dimerization has already taken place [124]. LBD-LBD dimerization has been resolved through crystallography for the majority of nuclear receptors but not for AR. Nevertheless, a high level of conservation has been detected by homology modeling in the amino acids forming the dimer interface between GR monomers and spatially equivalent residues in AR [124].

Coregulatory Proteins

Regulation of AR transcription is structured around completion of two general tasks: (1) Change in chromatin conformation to provide or deny AR and component of the general transcriptional machinery access to regulatory sequences of target genes. (2) Recruitment of RNA polymerase II (Pol II) and general transcription factors (GTF) to these sites, where they form a pre-initiation complex (PIC) and engage into transcription and/or elongation. Coregulators are central regulators of chromatin reorganization recruited directly or indirectly to DNA by transcription factors. 320 NR coregulators have been identified as of 1/31/2016 (https://www.nursa.org/

nursa/molecules/index.jsf). Coregulators can stimulate or repress transcription, and are designated accordingly coactivators or corepressors. Unlike general and specific transcription factors, they do not significantly alter the basal transcription rate and do not possess DNA binding capabilities. They are part of a complex of protein that regulate chromatin structure and bridge various components of the transcriptional machinery to the site of transcription through enzymatic modifications of histone tails. Based on the enzymatic mechanism of action used to remodel chromatin, coregulators are classified as nucleosome remodeling ATPase's and histone modifiers. Nucleosome remodeling ATPase's use energy derived from ATP hydrolysis to modify in a non-covalent manner nucleosome organization [125]. This process leads to loosening and opening of tightly coiled chromatin for transcription factor binding or to condensation of chromatin structure to promote gene repression [126]. Histone modifiers catalyze reversible covalent modifications of histone tails including acetylation, methylation, phosphorylation, ubiquitination, ADP ribosylation, glycosylation, sumoylation, and others [127]. In most cases these enzymatic modifications are taking place on specific amino acids located in N-terminal histone tails, and result in net charge change within the nucleosome that generates histone codes associated with loosening or tightening of the DNA-histone complex. Acetylation and deacetylation are the two most studied post-translational modification occurring in histone tails. These activities are associated with an active or inactive chromatin state, respectively, and will be described in this review. We refer the reader to previously published outstanding articles for additional information on the other classes of coactivators [71, 128]. AR-mediated transcription has been associated with acetylation of well-characterized residues of histone 3 [128]. For instance, acetylation of K9 (acH3K9) or 14 (acH3K14) by coactivators with histone acetyltransferase (HAT) activity are typical modifications associated with AR-mediated transcription. Acetylation of positively charged residues such as K or R removes the positive charge and rescinds interaction with the negatively charged phosphate groups of DNA leading to chromatin opening and enabling transcription. On the contrary, histone deacetylation (HDAC) activity leads to closure of the chromatin and inhibition of transcription. The best characterized coactivators with HAT activity inducing AR transcription are the two homologous members of the p160 SRC family SRC-1 [129] and SRC-3 [130]. These proteins are organized around four structural domains; (1) An N-terminal helix-loop-helix domain for interaction with other proteins. (2) An LxxLL mid-region for interaction with NRs (nuclear receptor binding box). (3) Two C-terminal transcriptional activation domain (AD1 and AD2). AD1 and AD2 act as a scaffold platform recruiting additional coactivator to the multiprotein complex. AD1 binds the histone acetyl transferases p300 and CBP, which are essential for SRC-mediated transcriptional activation, and for the acetylation of AR itself at three lysine residues in the hinge region. AD2 binds with the histone methyltransferases coactivator-associated arginine methyltransferase 1 (CARM1) and protein arginine methyltransferase 1 (PRMT1). (4) A HAT domain that is also located in the C-terminal end. The features of the p160 family members are such that they function as a base for the recruitment of additional coregulators and general transcription factors. This in turn results in chromatin remodeling,

assembly of general transcription factors, and recruitment of RNA Polymerase II for transcriptional activation. Importantly, p160 SRC coactivators are overexpressed in several cancers, including prostate cancer [131, 132] and their expression correlates with clinical and pathological variables of aggressiveness. Because inhibition of AR acetylation is associated with decreased malignancy of PCa cancer cells [133], molecules targeting pharmacologically these proteins are actively searched for therapeutic purposes.

AR in Clinical Medicine

AR PolyQ Repeat

The polymorphic polyQ tract in AR NTD consists of 9–39 repeats in the normal population. Laboratory-based studies have established that larger Q repeats are associated with decreased AR transcription, and shorter repeats with increased AR transcription. Based on this, investigators have conducted epidemiological studies to identify if a longer or shorter Q repeat is associated with the risk of developing certain conditions. These studies have shown that longer Q repeats are associated with increased risk of infertility [134]. In contrast, shorter Q repeats are associated with anovulation in PCOS patients with low androgens [135], improved response to TRT in hypogonadal patients [136], male pattern baldness [137], and symptomatic BPH [138]. Shorter Q repeats have also been identified to increase the risk of higher grade and advanced stage of prostate cancer at diagnosis, and of metastasis and mortality from the disease [39].

Expansion of the polyQ tract from 40 to more than 60 repeats is responsible for SBMA also known as Kennedy's disease [139, 140], a disorder of motor neurons of the brainstem and spinal cord. Age at onset is in the early 40s, and manifestations include muscle cramps, fasciculations, and progressive generalized weakness beginning in the muscles of the lower limbs and progressing to involve the bulbar musculature with difficulty in chewing, swallowing, and speaking [141]. As the disease progresses, disability increases until the patient is wheelchair bound. Because AR with larger Q repeats is less active, these patients also exhibit mild androgen insensitivity, manifested by gynecomastia, erectile dysfunction (ED), subfertility, and testicular atrophy. Interestingly, loss of AR function in AIS does not cause motor neuron degeneration, hence the repeat expansion must be responsible for a toxic gain of function that results in neurologic damage, most likely with a mechanism shared by other disorders associated with poly-glutamine expansion, such as Huntington's disease and Spino-Cerebellar Ataxia (SCA). There is a significant correlation between the number of glutamines and both age at onset and symptoms [141].

Autopsy studies of these patients have shown loss of large, medium, and small motor neurons. Indeed, an expanded polyQ tract changes the structure of the androgen receptor, which consequently forms intracellular aggregates [142] believed to

sequester proteins indispensable for cell survival. Although these inclusions are a neuropathologic finding in SBMA, their precise role in the disease remains unclear.

Transgenic animals carrying an AR with an expanded polyQ region have a phenotype that overlaps the human disease, confirming the central role played by AR in the physiopathology of this condition [143, 144]. Based on the presence of the neural inclusions described above, the clinical presentation of SBMA has been assumed to result from AR activity in motor neurons, however, over the last 15 years there has been a growing appreciation of how non-neuronal cells maintain neuron function, thus contributing to the pathogenesis of neurological diseases. For instances, mouse models expressing wild-type AR or polyQ-AR only in skeletal muscle [145, 146] have an androgen-dependent SBMA phenotype. The observation that the phenotype in one of these models was reversed by conditionally terminating expression of the polyQ-AR construct added more emphasis to the concept that SBMA could be initiated in the muscle.

SBMA has been considered an incurable disease. One of the few therapeutic options consists in targeting mutant AR degradation, which in the CNS is not a viable option because associated with significant side effects [146]. Hence, the option to target AR in the muscle opens an attractive alternative. Other endocrine avenues recently attempted to decrease the toxic effect of polyQ AR in SBMA include the use of microRNA298 (miR-298) [147], antiandrogens [148], GnRH agonists [149], pioglitazone [150], and disruption of SUMOlyation [151].

Androgen Insensitivity Syndromes (AIS)

The critical role played by AR in regulating development of the male phenotype is exemplified by the syndromes of androgen insensitivity (AIS). Patients affected by these syndromes have an intact enzymatic machinery to produce T, DHT, and E_2, but carry a variety of abnormalities in the coding sequence of AR leading to different levels of AR protein inactivation. Thus, the primary physiopathologic mechanism leading to AIS consists in end organ insensitivity to the action of androgens, as demonstrated by lack of any discernible clinical response to 50 mg/day of methyltestosterone in a cohort of such patients treated by Dr. Wilkins in the 1940s [152]. The first review of this syndrome appeared in 1953 in a seminal paper by Dr. Morris [153] who described 82 cases from the literature and his own practice.

Clinical presentation varies from a completely feminized external phenotype in 46-XY pseudo-hermaphrodites affected by complete androgen insensitivity (CAIS or complete androgen insensitivity syndrome), to various levels of abnormal virilization in patients affected by the phenotypes of partial androgen insensitivity (PAIS or partial androgen insensitivity syndrome), to patients with impaired spermatogenesis [154] and/or other minor abnormalities of virilization such as gynecomastia (MAIS or mild androgen insensitivity). Generally speaking, there is a parallel between the degree of AR inactivation and the clinical presentation. For instance, a CAIS phenotype is invariably present in patients with non-sense mutations generating a truncated and completely inactive protein. However, different impairments of

virilization are seen in individuals from different kindreds affected by the same missense mutation [155], possibly due to the involvement of accessory proteins regulating AR activity. At least one case was described where the AR coding sequence was intact, and the syndrome was ascribed to inactivation of an AR coregulator [156]. After isolation and sequencing of the AR cDNA, large numbers of missense, non-sense, and splice-site mutations associated with the syndrome have been described (http://androgendb.mcgill.ca/AR23C.pdf). Excellent reviews describing these mutations and their mechanisms to impair AR activity have been published over the years [157–159].

A system, known as the Quigley's scale, is used to grade the external genitalia of AIS patients in conjunction with the traditional three categories of AIS. Grade 1 (corresponding to the MAIS phenotype) represents fully virilized external male genitalia. Grades 2–5 (corresponding to the PAIS phenotype) represent increasingly feminized genitalia based on defective virilization occurring in the penis, scrotum, testicles, labia and vaginal opening. Grades 6–7 (corresponding to the CAIS phenotype) represent normal female external genitalia and can be differentiated in grade 6 vs. 7 based on the presence of pubic hair after puberty [158, 159]. Patients with AIS have undescended testes, usually retained in the inguinal canal or abdominal cavity, and lack Mullerian derivatives due to the normal testicular production of AMH. CAIS patients with grade 6/7 external genitalia have a small, blind ending vagina.

Compared to normal males, the endocrine milieu of AIS patients stands up for two reasons; first, gonadotropins are elevated (LH > FSH) and second, due to the lack of negative feedback, *T* is also elevated. Estrogens, produced from androgen precursors in the gonads or peripheral tissues, are normal to elevated and work unopposed due to lack of androgen effect in the target tissue. As a consequence, CAIS patients develop normal feminine shape, breast enlargement, and acne-free complexion at puberty. Many CAIS patients are taller than average females. Because the risk of developing gonadoblastoma in the undescended testes is high, castration is performed after puberty to prevent development of malignancies [160], and estrogen replacement therapy (ERT) is given with the goal of maintaining secondary sexual characteristics and protecting the skeleton. Bone mineral density is low regardless of timing of gonadectomy [161], and thus, in addition to ERT, CAIS patients should be given CA and Vit D supplements. Other hormonal treatments, for instance, with testosterone, are not recommended unless given on a trial base (see below) because by definition these patients do not respond to this form of therapy.

Gender assignment is relatively straightforward in patients with CAIS and MAIS. Since these two conditions are at the end of the spectrum of presenting phenotypes, CAIS patients are typically raised as females and MAIS patients as males. The decision of gender assignment is much more complex in PAIS patients and should be taken with the aid of a multidisciplinary team after considering the appearance of the external genitalia, potential for virilization later on in life, future surgical options, and projected gender identity of the child. There are anecdotal reports of PAIS patients who responded to treatment with androgens [162, 163], and therefore a trial to predict potential responsiveness to androgens at puberty is

warranted. Other elements to consider in deciding sex of rearing include family history or the type of AR mutation responsible for the syndrome. Family history was instrumental is leading to the decision of treating a young patient whose uncle naturally virilized at puberty [163, 164]. Other authors are in support of utilizing an external masculinization score to predict virilization at puberty. Overall, this is a very difficult and flawed decision as demonstrated by the fact that up to 25% of PAIS patients are dissatisfied with their assigned gender [165].

AR and the Prostate

AR plays an active role in the events leading to prostate embryogenesis and carcinogenesis. Targeting AR activity with androgen depletion treatments has been the mainstay of prostate cancer treatment since the 1940s [166]. AR plays a central role in the relapse of prostate cancer to the castration resistant phenotype after failure of androgen depletion therapy (ADT) [167].

Prostate Embryogenesis

Prostate homeostasis and growth depends on androgens interacting with AR during fetal life, pubertal development, and adulthood. During fetal life, prostate organogenesis originates from the embryonic urogenital sinus, which contains urogenital sinus epithelium and mesenchyme. Tissue reconstitution studies have demonstrated that embryonic mesenchymal cells determine the fate of epithelial differentiation into prostatic acini, and prostate growth is mediated by DHT acting through the stromal AR [168]. Conditions of impaired activity of the androgen-AR axis during these developmental phases are associated with atrophy of the prostate throughout life. For instance, men with SRD5A2 deficiency who lack DHT [3] have prostates of approximately 1/10 the size of normal controls, with rudimental histology characterized by fibrous connective tissue and smooth muscle, and no identifiable epithelial tissue [169]. AR plays an essential role in the physiology of the prostate as shown by the observation that androgen regulate up to 4% [170] of the 10,570 to 23,448 polyadenylated RNAs expressed by the AR-dependent LNCaP cell line [171, 172].

Early AR-Dependent Events in Prostate Carcinogenesis

An important early event in prostate cancer consists in the appearance of various chromosomal rearrangements involving the AR-responsive TRPM2 promoter at the 5′ and ETS transcription factor family members, ERG or ETV1, at the 3′ [173]. AR acting on the TRPM2 promoter will induce overexpression of these two oncogenes and an aberrant stimulation of growth in tissues harboring these rearrangements. In the original paper reporting this observation, ERG1 or ETV1 overexpression was

detected in 57% of patients affected by prostate cancer. The TMPRSS2-ERG1 or -ETV1 fusions were detected in 90% of the cases in tissues showing ERG or ETV overexpression [173]. Successive observations not only confirmed these earlier findings [174], but also established that androgens can facilitate these recombination events [175]. Presence of the TMPRSS2-ERG1 or -ETV1 fusions is not only a feature of malignancy; these fusion proteins can also be detected in benign prostatic tissue after treatment with androgens [176], implying that their appearance may be an early event that plays a central role in prostatic malignant transformation.

Role of AR in Castration Resistant Prostate Cancer

Castration resistant prostate cancer (CRPC) usually arises after patients with prostate cancer (PCa) fail androgen deprivation therapy (ADT) [177]. The most sensitive biochemical sign of CRPC relapse is an increase of the AR-dependent protein, prostate-specific antigen (PSA). This event indicates that AR is still signaling despite patients receiving ADT have anorchid levels of serum androgens [178–180]. The question of how PCa progresses to the CRPC phenotype has fascinated generations of scientists. Of the many theories put forward, some considered the possibility that PCa progression may be regulated by signaling pathways independent from the androgen-AR axis. However, all evidence supports the notion that PCa progression to the CRPC phenotype requires AR activation, and that the tumor requires an active AR to stay alive. ADT and castrate T levels induce PCa to develop within itself alternative mechanisms leading to AR activation, which include AR [181, 182], steroidogenic enzymes [183] or coactivators [184] overexpression, selection of constitutively active AR variants [185–188] or AR mutations [91, 189], gain of function mutations of steroidogenic enzymes [190] or activation of pathways shared between GR and AR [57].

AR overexpression during ADT was one of the first genetic abnormalities detected in prostate cancer [182]. The functional consequence of this phenomenon is that AR remains active even in the presence of low androgen concentrations and maintains the ability to induce growth and promote antiapoptotic effects [181]. Activation of AR within the setting of anorchid androgen levels is achieved with the de novo synthesis of T or DHT within the tumor [191] by overexpression of key steroidogenic enzymes such as CYP17A1, HSD3B2, AKR1C3, CYP11A1, and SRD5A1 and 2 [183, 192], or through mutational activation of one of these enzymes, as described with HSD3B2 [190]. Overexpression (or increased activity) of these enzymes results in induction of the classic steroidogenic pathway shown in Fig. 2.1, where T is the metabolite proceeding DHT. Two additional pathways can be activated that utilize 5α-androstane-3α,17β-diol [193] or 5α-androstanedione [194] as immediate precursors of DHT.

The role played by AR mutations in PCa relapse after ADT has been formulated since the 1990s, with the discovery of the T877A AR mutation in LNCaP cells [89]. Most AR point mutations found in CPRC map within codons forming the hydrophobic pocket of the LBD and generate promiscuous receptors that binds with high

affinity ligands other than T or DHT. The role of AR mutations in PCa relapse consists in inducing inappropriate agonist responses to nonandrogenic ligands such as progesterone and estradiol [89], androgenic precursors such as DHEA [195] or antagonists such as flutamide [89], bicalutamide [90] or enzalutamide [196]. By inhibiting CYP17A1, Abiraterone Acetate (AA) prevents conversion of P into 17OH-P (Fig. 2.1). Hence, CYP17A1 inhibition is associated with decreased synthesis of androgens and accumulation of the precursor P in prostate cancer tissue. Because the T787A AR mutation causes P to become a powerful activator of AR, scientists have hypothesized that resistance to AA is associated with accumulation of this this particular mutation in the CPRC tissue and found that this is the case in 17% of the cases [197]. Other consequences of inhibiting CYP17 are decreased synthesis of cortisol, increase in ACTH from loss of negative feedback and accumulation of P, which cannot be converted into 17OH-P (Fig. 2.1). This new endocrine milieu is responsible for increased concentrations of MR agonists that use P as a substrate for their synthesis; hence these patient are at risk for developing hypertension and hypokalemia, which is prevented by the concomitant and FDA-mandated use of prednisone, usually given at the dose of 10 mg. In this regard, as glucocorticoid are expected to suppress ACTH and steroidogenesis, clinical trials have been conceived where prednisone or dexamethasone were given to PCa patients with the goal of inhibiting testosterogenesis. Treatment with these agents was partially successful and associated with PSA responses ranging between 10.1% and 24% [198, 199] in the case of prednisone, and 31% [200] in the case of dexamethasone. In keeping with the intricacies of the basic biology of PCa, it should also be considered that glucocorticoids have been associated with PCa progression. For instance, an AR carrying mutations L701H and T877A [201] has been described in a CRPC cell line [202] that is activated by glucocorticoids such as cortisol, prednisone, and dexamethasone [203]. In addition, wild-type GR activated by glucocorticoid hormones activates a transcriptional program similar to that of AR that has been associated with disease progression under castrate conditions [57].

Outlaw activation of AR in CRPC can occur also through the selection of constitutively active AR variants (AR-Vs) that lack the ligand binding domain or skip certain exons encoding the LBD. We have been aware that lab-generated steroid receptors truncated of the LBD are constitutively active since the 1980s [204] and 1990s [105]. However, it was not until several years later that this phenomenon was reported to have clinical relevance in prostate cancer [205]. Twenty different AR-Vs [205–216] have been isolated to date (Fig. 2.4), however, this number is destined to change as new members of this family of molecules are continuously identified. In this review we will focus on AR-V7 [208, 209] and AR^{v567es} [212], because these are the two transcriptionally active AR-V reproducibly found in human CRPC primary tumors, xenografts, and metastases. The common structural denominator of AR-Vs consists in the presence of exons 1, 2, and 3, plus a C-terminus tail of different size, which derives from cryptic intronic sequences localized in AR introns 2 or 3. Notable exception to this general structure is AR^{v567es} (also known as ARV-12, Fig. 2.4), an AR-V derived from the deletion of exons 5, 6, and 7 [212]. As discussed earlier, in the absence of ligand LBD adopts a conformation preventing NTD

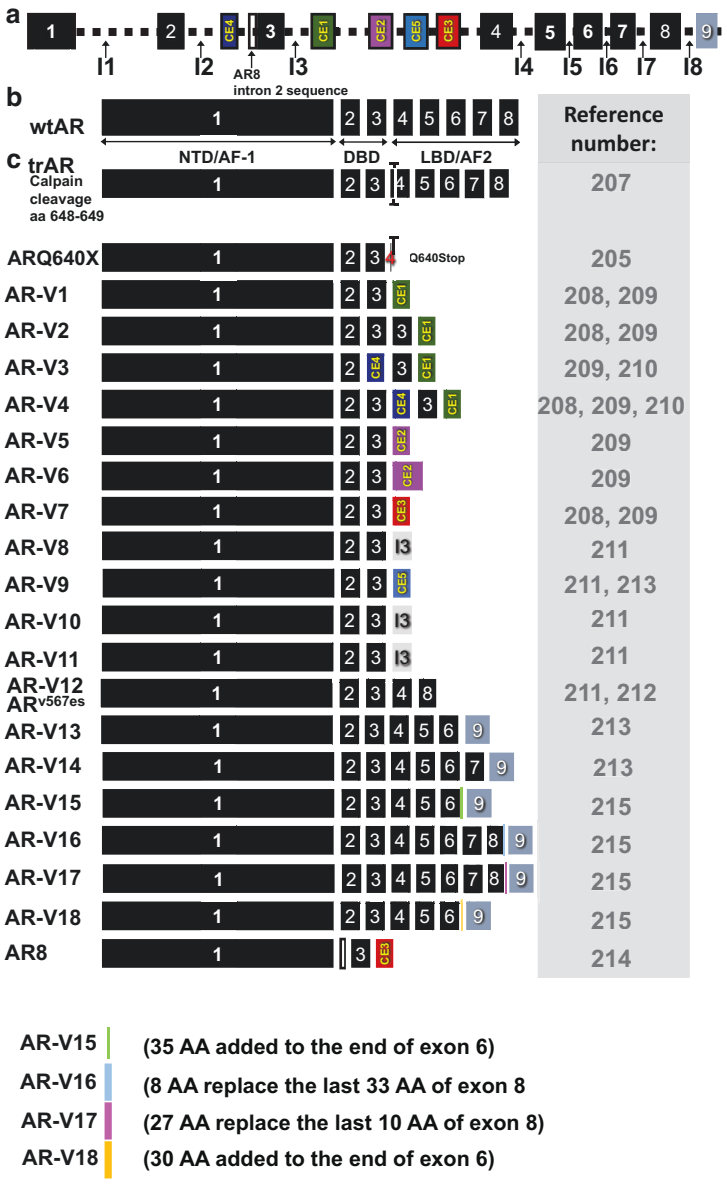

Fig. 2.4 (**a**) The *AR* gene locus containing 8 exons, 8 introns (I1–I8), and 6 cryptic exons (including CE1-5 and exon 9). (**b**) Wild-type AR-FL mRNA structure showing eight exons encoding three functional domains. (**c**) Alternatively spliced AR-Vs in prostate and breast cancer. The exons shared between wild-type AR and AR-Vs are in black. Cryptic exon maintain the same color code as in (**a**). C-terminal sequences of AR-V8, AR-V10, and AR-V11 are derived from DNA sequences within I3. Colored bars at the bottom of the figure represent sequences found in AR-V15-18. References are listed on the right side of the figure

from interacting with proteins stimulating AR transcriptional activity (Fig. 2.3a). If LBD is missing, AF1 freely interacts with these transcriptional activators and induces gene transcription through the intact DBD. From a clinical point of view several studies except one [217] have demonstrated that AR-V7 and AR^{v567es} expression increases in conditions of castration resistance and predicts risk of recurrence. A study performed in CRPC metastatic to the bone marrow showed presence of AR-V7 and AR^{v567es} in 100% and 23% of the cases, respectively [186]. In addition, this study demonstrated that increased AR-Vs expression was associated with shorter time to death [186]. Another study correlated presence of AR-V7 with increased postsurgical risk of biochemical recurrence [209]. Reliable antibodies for AR-V7 are not widely available, however, one of such antibodies was used in a group of PCa specimens, and demonstrated AR-V7 presence in 44% of CRPC samples. In addition, this study demonstrated that high AR-V7 staining is associated with increased risk of recurrence [208]. To date it is still unclear whether the growth advantage conferred by AR-V7 to the cell occurs under all circumstances or only after castration. This is a relevant question, because AR-Vs have been detected mostly in tissues of patients undergoing ADT. The observation that transgenic models overexpressing AR-V7 or AR^{v567es} have both been associated with prostatic carcinogenesis [218, 219] supports the notion that a growth advantage is conferred under all hormonal circumstances.

Once it became clear that AR transition to CRPC is mediated by AR reactivation, second generation ADT drugs were developed and approved by the FDA. They included the CYP17A1 inhibitor abiraterone acetate (AA) [220] and the second generation AR antagonist enzalutamide [221]. Although yielding an average increase in life of a few months, AA and enzalutamide were limited in effect, as some patients manifest de novo resistance, while others relapsed after a median of 8–10 months of treatment [198, 199, 222]. First and second ADT drugs target the AR LBD either by preventing the synthesis of the natural ligand DHT (i.e., GnRH agonists and AA), or by preventing AR from acquiring a transcriptionally competent conformation (Bicalutamide and Enzalutamide). In addition, Enzalutamide has been described to prevent AR translocation to the nucleus [223], while AA was shown to inhibit 3βHSD and SRD5A and to antagonize AR after conversion to Δ(4)-abiraterone (D4A) [224]. By critically looking at the mechanisms used by AR to remain active after transition to CRPC, one would predict that a major mechanism of resistance to currently available drugs consists in the selection of AR-Vs in the cancerous tissue. A pivotal study demonstrated this important concept by analyzing circulating tumor cells (CTC) for the presence of AR-V7 in patients receiving second generation ADT [187]. This prospective study enrolled 62 patients with metastatic CRPC; 31 were receiving AA and 31 Enzalutamide. AR-V7 was present in 39 and 19% of the patients receiving Enzalutamide and AA, respectively. PSA response rate was 0% in AR-V7 (+) patients, 53 and 55% in AR-V7(-) patients receiving Enzalutamide and AA. Progression-free survival was 2.1 vs. 6.1 months and 2.3 vs. 6.3 months in Enzalutamide and AA treated patients who were AR-V7(+) or (−) at the outset. While none of the AR-V7(+) patients converted to an AR-V7(−)

status, 14% of the AR-V7(−) converted to an AR-V7(+) status. This and other studies [188, 225] have demonstrated that: (1) AR-V7 expression provides CRPC with a powerful mechanism to resist LBD-directed drugs. (2) Treatment with second generation ADT drugs increases the likelihood of converting PCa to an AR-V7(+) status. (3) There is an association between AR-V7(+) status and a history of having been treated with many prior therapies.

Clinical trials have demonstrated that Docetaxel is the only chemotherapeutic agent that prolongs survival in CRPC [226]. Important recent work suggests that the microtubule network of prostate cells is critical for AR nuclear translocation and activity [227], hence taxane-based chemotherapy has been studied in detail. A clinical trial described 37 taxane-treated patients with CRPC [228]. 46% of these patients were AR-V7(+) at baseline. The overall PSA response rate was 54% and there was no difference between AR-V7(+) or (−) patients (i.e., 41 vs. 65%, 95% CI: −13 to +60, $P = 0.19$). In this study outcome differences between AR-V7(+) or (−) patients treated with taxanes of second generation ADT (from a parallel trial) were compared. Among AR-V7(−) patients there was no difference in the two groups (response rate 65% taxane-treated vs. 64% second generation ADT). In contrast among AR-V7(+) patients the response rate was 41% in taxane- vs. 0% in second generation ADT-treated patients. Interestingly, 58% of the AR-V7(+) patients treated with taxane-based chemotherapy converted to negative. The main message of these results is that AR-V7 detection is not an absolute negative predictor of outcome in patients receiving taxane chemotherapy; in contrast presence of AR-V7 appear to be associated with primary and acquired resistance to second generation ADT. Xenografts- or cell lines-based experiments evaluating the effect of taxane chemotherapy have not fully confirmed the results of this trial. Thadani-Mulero et al. demonstrated that xenografts carrying AR^{v567es} were sensitive to taxane chemotherapy [229], whereas xenografts carrying AR-V7 were resistant. Another group reported that ectopic expression of AR-V7 and AR^{V567es} but not the full-length AR reduced sensitivities to taxanes in LNCaP cells [230].

The common AR target of first and second generation ADT drugs is the carboxyl terminal ligand binding domain (LBD). Current medications targeting the LBD are not expected to antagonize C-terminally truncated AR variants, therefore research should be aimed at the identification of drugs that successfully inhibit AR action by targeting AR NTD. The prediction is that these drugs will be effective against full-length AR as well as AR-Vs. Medications with the ability to target AR NTD are the EPI drugs (EPI-001, EPI-002, and EPI-506) that have shown promising therapeutic effects [231, 232]. Other drugs that inhibit AR-V7 and flAR about to be tested in clinical trials are niclosamide [233], an antihelmintic agent, and galeterone [234], a compound with a multifaceted mechanism of action that include CYP17 inhibition, AR signaling inhibition, and degradation of the full-length and AR-V7 proteins. Other agents that successfully target AR-V7 are LY294002 [235], an inhibitor of the phosphatidylinositol 3-kinase (PI3K)-AKT-FOXO1 signaling pathway, and CUDC-101 [236], an inhibitor of HER2/NEU, EGFR, and HDAC.

AR and Breast Cancer

AR is expressed in 80–90% of all breast cancers, including 55% of ERα (−) and 35% of those classified as triple-negative breast cancer (TNBC) [i.e., ERα (−), PR (−), and HER2/NEU (−). ERα (−)]. TNBC are unresponsive to conventional treatments targeting ERα signaling, E_2 synthesis, and HER2/NEU activity, thus their prognosis is very poor. AR role in breast cancer is contingent to the ERα status and molecular subtype. In ERα (+) luminal breast cancer, AR expression is usually associated with a better clinical prognosis. In these tumors AR functions as mediator of an anti- proliferative signaling pathway by binding estrogen responsive elements (EREs) and preventing ER-mediated transcription [237]. In contrast to what observed in ERα (+) luminal breast cancer, treatment of MCF-7 and MDA-MB-453 ER(−) AR (+) cell lines with an AR agonist induces proliferation [238]. In addition, certain histologically defined "molecular apocrine" AR(+) and ER(-) breast cancers display signature microarray profiles very similar to those of prostate cancer cells stimulated with androgens [239]. ChIP seq analysis in MDA-MB-453 cell line, an in vitro model of molecular apocrine tumor, demonstrated that the AR cistrome has a profile that overlaps that of ERα in MCF-7 cells [240]. These exciting data suggest that in certain tumors, such as AR(+) and ER(−) molecular apocrine breast cancer, AR may have complementary activity to ER, and therefore be responsible for tumor growth. This hypothesis is further supported by the observation that antiandrogens inhibit growth of laboratory models of AR(+) TNBC [241], and this paradigm is now been tested in clinical trials where women with resistant and metastatic AR(+) TNBCs are treated with ADT. Although these clinical trials are not completed, an important question raised by scientists is whether breast cancer, like PCa, can develop resistance to ADT and whether the mechanisms are overlapping. At this purpose a recent study [242] demonstrated that AR-V7 and other AR-Vs are expressed in primary non-malignant and malignant breast tissues and in AR(+) ER(−) cell lines, such as MDA-MB-453, MFM-223, and ZR-75-1. In these models, AR-V7 expression is upregulated by treatment with the AR antagonist Enzalutamide and regulates a network of genes predictive of aggressive metastatic disease [242]. At this juncture the importance of these observations is theoretical, as it is not known whether ADT is a useful therapy for women with metastatic ER(−) AR(+) breast cancer. However, should the ongoing clinical trials demonstrate that ADT is a viable option for this condition, these data will identify the same potential mechanism of resistance that has been identified in CRPC failing second generation castration therapy.

AR and Bone Health

CAIS provides a model to determine the role of androgens and AR in bone health. Marcus studied a mixed population of CAIS and PAIS patients [243] and reported an average height of 174 cm (68.5 in.) for adults of this cohort compared with an average height in adult American women of 162.3 cm, reflecting that calcification

of the epiphyses in these patients is delayed, possibly because estrogen levels are lower than in normal women. BMD Z score was significantly decreased in CAIS women compared to control, however, good compliance to ERT—given to these patients after gonadectomy—was associated with better BMD Z scores compared to noncompliant patients, reflecting a component of inadequate ERT rather than androgen lack alone. The ARKO mouse provides an easier model to study the effects of AR on the bone, as these animals have low androgen levels and do not receive exogenous estrogens. The potential weakness of this model is that unlike their human CAIS counterpart, ARKO mice usually release low concentrations of T.

ARKO^{EX1} males are affected by high-turnover osteopenia with increased bone resorption, reduced trabecular bone mass and cortical thickness and volume, suggesting that AR function is essential for the development of a normal bone phenotype. The bone phenotype of male ARKO^{EX1} mice was partially rescued by treatment with T, but nor with DHT, which is non-aromatizable [244]. This indicates that some AR functions are indispensable for male-type bone formation and remodeling, whereas others are mediated by E_2 that is formed locally from T aromatization. To elucidate the direct target of androgen-AR signaling in the microenvironment of the bone, models have been generated where AR is overexpressed or deleted in proliferating or mature osteoblasts [245, 246]. Both overexpression models displayed a phenotype of reduced bone turnover leading to increased trabecular bone volume. The model overexpressing AR in proliferating osteoblasts had larger bones compared to controls due to increased periosteal mineral apposition. Mice with selective AR deletions displayed complementary phenotypes, suggesting that androgens activate AR in mineralizing osteoblasts to maintain trabecular and cortical bone, and in proliferating osteoblasts to induce an anabolic effect on cortical bone and the periosteum. ARKO models where AR inactivation is targeted to the osteoclast have been reported only in abstract form several years ago. These animals contain increased number of osteoclasts in the lumbar spine, suggesting that their expression is inhibited by androgens expression via the AR.

Development of ARKO Mice

Naturally occurring animal models of AR inactivation have been described in mice [also known as testicular feminization mouse (*tfm*)] [247], rats [248], dogs [249], cats [250], pigs [251], and several other species. The identified genetic defects in rodents consist in a single base deletion followed by an early stop codon in the mouse [252, 253], and a single base replacement causing an amino acid substitution in the rat [254]. The phenotype of *tfm* mice recapitulates the phenotype observed in humans affected by the CAIS phenotype. The main difference between mice and humans (and rats) is that in *tfm* mice 17α-hydroxylase activity is absent because the expression of this enzyme is exquisitely AR-dependent [255]. As a consequence, mice models of CAIS (naturally occurring or lab-generated) are androgen deficient and resistant at the same time.

Sophisticated recombinant technologies including conditional gene knock out using Cre-LoxP technology have made it possible to generate global AR knockout (ARKO) mice and models where AR inactivation is directed in specific target cells [256]. Cre-LoxP technology involves the use of transgenic mice expressing the bacterial Cre enzyme that excises the DNA located between loxP sites, referred to as "floxed." Five models of global ARKO mice have been generated by crossing transgenic mice carrying loxP sites surrounding exon-1 ($ARKO^{Ex1}$) [257–259], -2 ($ARKO^{Ex2}$) [256, 260, 261] or -3 ($ARKO^{Ex3}$) [262, 263] with different transgenic Cre mice, where the Cre recombinase enzyme is driven ubiquitously by different promoters such as CMV [257, 259] and Sycp1 [258] (used in the two published $ARKO^{Ex1}$ models), β-actin [256], and PGK [261] (used in the two published $ARKO^{Ex2}$ models) and again CMV [262, 263] (used to generate the $ARKO^{Ex3}$ model). The strategies involved with the generation of $ARKO^{Ex1}$ and $ARKO^{Ex2}$ mice introduced premature stop codons in the AR sequence with complete lack of AR protein expression in these strains. In contrast, the cloning strategy for $ARKO^{Ex3}$ resulted in expression of an AR protein of 900 amino acids missing exon 3. A careful review of these animal models has been instrumental to shed further light on the importance played by AR in male physiology. Global ARKO mice share the same phenotype of the *tfm* mouse, characterized by normal female external genital and somatic appearance, sterility, atrophic intra-abdominal testes, and absence of male or female internal organs.

Specific Conditional Knock-Out of AR in the Testes

The testes are responsible for T production from the interstitial Leydig cells and spermatogenesis from the seminiferous tubules. In addition to Leydig cells, the interstitial space also contains macrophages, perivascular smooth muscle cells, and vascular endothelial cells. In addition to germ cells at various stages of maturation, the seminiferous tubules contain Sertoli cells, which provide structural and nutritional support for germinal cell development by secreting a variety of proteins, releasing tubular fluid and maintaining the blood-testis barrier (BTB). Normal tubular morphology is also maintained by a layer of cells located at the base of the tubules, known as peritubular myoid cells. These compartments and cell types are in physical and functional communication to ensure normal T production and spermatogenesis. AR is expressed in almost each testicular cell type, and functional inactivation of testicular AR in a cell-specific fashion has added much to our understanding of testicular physiology.

Sertoli Cell-Specific ARKO Models

The AMH (anti-Mullerian hormone) [258, 264–266] and Abp (androgen binding protein) [266] promoters have been used to target cre-recombinase expression specifically in Sertoli cells. AR inactivation was achieved by deleting floxed exon 1

[258], exon 2 [264, 265] or exon 3 [266]. The common denominator of these models consisted in normal development of the male phenotype, suggesting that AR activity in Sertoli cells is not necessary for testicular differentiation and descent, virilization of the internal and external genitalia and development of the accessory organs. The main phenotype of these mice consisted in reduced testicular size by 25–60%, followed by sperm arrest at the pachytene spermatocyte stage with no sperms present in the epididymis. In contrast with what observed in global ARKO mice, Sertoli cell were not decreased in number but dysfunctional in appearance, suggesting that AR is important for Sertoli cell function but not differentiation. Testosterone was mildly decreased in one of the models [264], normal others [265, 266] and increased together with LH in mice with exon 1 deletion [258]. These differences reflected either different analytical methods, or differential leaking of Cre inactivation in Leydig cells [264] or in the hypothalamic regions [258].

Leydig Cell-Specific ARKO Male Mice

Leydig cell-specific ARKO males (LC-ARKO) were generated by crossing male mice carrying Cre recombinase driven by the anti-mullerian hormone receptor 2 (Amhr2) promoter with females carrying exon 2 floxed AR [267]. The resulting phenotype requires confirmation because AR was not knocked out from all Leydig cells, and the AMHr2-cre recombinase leaked in the seminiferous tubules, causing some of the Sertoli cells to not express AR. LC-ARKO mice exhibited normal male appearance with descended testes, preserved mating behavior and copulatory plug formation. Testes and epididymis were atrophied, whereas seminal vesicles and prostate weights remained similar to wild-type controls [267, 268]. Spermatogenesis was arrested at the pachytene stage, and no sperms were detected in the epididymis. The endocrine milieu of LC-ARKO mice was significant for hypogonadotropic hypogonadism. Low T was attributed to decreased expression of steroidogenic enzymes involved in T synthesis, in particular 17βHSD3 and CYP17 [269]. These results suggest that AR expressed on Leydig cells may have an effect on normal T production, spermatogenesis, and male fertility.

Peritubular Myoid (PTM) Cell-Specific ARKO Mice

AR (+) peritubular myoid cells are stromal cells part of the basement membrane where they contribute to maintain normal morphology of the seminiferous tubules. In other androgen-dependent tissues, such as the prostate, AR signaling through the stromal cells influences organ development and epithelial cell function. If similar stromal-epithelial interactions occur in the testis, one would predict stromal PTM cells to play an essential role in mediating effects of androgens on epithelial Sertoli cell function and spermatogenesis. To determine if deletion of AR from these cells is associated with a testicular phenotype, female mice carrying exon 2-floxed AR were crossed with males carrying Cre recombinase driven by the transgelin

promoter [270] or smooth muscle myosin heavy chain promoter (smMHC) [271] to generate PMCARKO and PTM-ARKO mice, respectively. Reliability of the phenotypes generated with these promoters is an issue, since Welsh et al. published that transgelin does not affect AR expression in peritubular cells [271]. In addition, although the smMHC promoter drives Cre recombinase expression and ablates AR expression, it does so only in 40% of PTM cells [271], and, to make things even more complicated, is active also in extra-testicular tissues such as seminal vesicles [272] and prostate [273].

PMCARKO mice were fertile, developed normal internal and external sexual organs, normal T, FSH, and LH. Abnormalities consisted in lower epididymal sperm counts, reduction of 30% in testicular volume associated with reduced expression of Sertoli cell functional marker genes, such as epidermal fatty acid-binding protein and androgen binding protein [270]. Based on this phenotype, it was concluded that the lack of AR in peritubular myoid cells affects the nursery function of Sertoli cells. This in turn leads to decreased germ cell differentiation and maturation, and to a decreased number of sperms in the epididymis. The PTM-ARKO mouse [271] exhibited normal virilization and gonadal descent, however, testicular, seminal vesicle, ventral prostate, and seminiferous tubule volume were reduced [271]. Spermatogenesis was severely impaired and associated with progressive infertility. Immunocytochemistry-based experiments indicated that the morphology and function of some Leydig cells was abnormal in PTM-ARKO males. In the population of Leydig cells scored as abnormal, AR expression was decreased, leading to the conclusion that in peritubular myoid cells AR works as paracrine modulator of adult Leydig cell function.

The described differences in the phenotypes of PMTARKO and PMCARKO mice could results from the inefficiency of the models used, and a distinctive function of the AR localized in peritubular myoid cell cannot be conclusively established until a third peritubular myoid specific ARKO mice is generated.

Germ Cell-Specific ARKO Male Mice

Despite it is still matter of controversy whether AR is expressed in germ cells [274–278], a germ cell-specific ARKO mouse (GARKO) has been reported [267]. Males carrying CRE recombinase driven by the synaptonemal complex protein 1 gene promoter (Sycp1-Cre) were crossed with homozygous females carrying AR with a floxed exon 2. Virilization, spermatogenesis, T level, and mating behavior were normal in GARKO mice. Given that AR expression in germinal cells is controversial, these results should be interpreted with caution.

Function of AR in the Prostate and Accessory Glands

The male reproductive tract develops from two embryonic anlagen: the Wollfian ducts (WD) and the urogenital sinus (UGS), which are of endodermal and mesodermal origin, respectively. During embryologic development the epididymis, vas deferens, and seminal vesicle are generated from the WD, while the bladder, prostate, bulbourethral glands, and urethra derive form the upper and pelvic portions of the urogenital sinus. Epididymis is a storing organ where sperms are collected and undergo final maturation. Prostate and seminal vesicles are located along the vas deferens and contribute the large majority of seminal fluid together with nutrients such as proteins, sugars, and zinc that prepare sperms for fertilization. All male accessory organs contain an epithelial compartment (consisting of basal and luminal cells), surrounded by a stromal compartment composed of a variety of cell types including fibroblasts, dendritic, smooth muscle, and endothelial cells. AR plays a major role in the development of all organs derived from the WD (i.e., epididymis, vas deferens, and seminal vesicle) [279], the prostate [280], bulbourethral gland [281], and the ventral portion of the urethra [282]. Interestingly, at the beginning of sexual differentiation AR expression is concentrated in the mesenchyme of urogenital anlagen and is absent from the epithelia [283], suggesting, as established by the work of Cunha [284, 285], that mesenchymal androgen signaling plays a major role in directing tissue differentiation by providing signaling for epithelial morphogenesis. During adult life androgens are thought to mediate different effects in each cell compartment; for example, prostatic epithelial AR regulates epithelial secretory functions [286] and inhibits proliferation [287]. In contrast, stromal AR regulates the fate of the epithelium by regulating epithelial cell differentiation, apoptosis, and proliferation [284, 288]. In order to understand the functions of stromal vs. epithelial AR during embryologic development and adult life, a variety of groups have generated a number of ARKO models resulting from selective inactivation of AR in various compartments and cell types of the male reproductive tract.

AR Functions in Prostatic Stroma

Smooth muscle myosin heavy chain (smMHC)-Cre was used to selectively ablate AR from prostatic smooth muscle (SM) cells [273] and to generate PTM-ARKO mice. During adulthood this mouse revealed a 44% reduction in prostate size compared with controls. In addition, the prostates of these animals showed histological changes consisting in hyperplasia, inflammation, fibrosis, and reduced expression of epithelial, smooth muscle, and stem cell markers (for instance *p63* was reduced by 27% and *PTEN* by 31%). The smMHC-Cre model also provided evidence that the absence of SM AR is associated with an 8.5-fold greater increase in prostate weight than controls in response to estradiol.

Two additional types of prostate stromal AR KO mouse models were developed by the same group by using strains of C57BL/6 male mice carrying Cre-recombinase driven by fibroblast-specific protein 1 (FSP-ARKO) [289] or transgelin/smooth muscle 22α promoters (SM-ARKO) [290], and crossing them to (C57BL/6) female mice with floxed AR. FSP-ARKO displayed deletion of AR in fibroblasts located in the ventral prostate, and was associated with underdevelopment of this lobe of the gland. Furthermore, the FSP-ARKO displayed reduced prostatic epithelial differentiation at later adult stages. SM-ARKO mice displayed deletion of AR from smooth muscle cells located in the anterior prostate and was associated with decreased epithelial in-folding and epithelial cell proliferation. This study also demonstrated that defective development of the prostate in SM-ARKO may be due to lack of IGF-1.

Male FSP-AR and SM-AR double KO mice [also known as double stromal androgen receptor knockout (dARKO)] [291] with deleted androgen receptor (AR) in both stromal fibroblasts and smooth muscle cells showed reduced size of the anterior prostate (AP) lobes as compared to those from wild-type littermate controls. The reduction in prostate size of the dARKO mouse was accompanied by impaired branching morphogenesis and partial loss of the infolding glandular structure. Further experiments found decreased proliferation and increased apoptosis of prostatic epithelium in the anterior prostate of dARKO mice. The molecular pathways affecting epithelial development were mediated by a number of stromal growth factors. For instance, IGF-1, placental growth factor, and secreted phosphoprotein-1 controlled by stromal AR were differentially expressed in prostate stromal cells immortalized form dARKO mice vs. controls. The common denominator of PTM-ARKO, SM-ARKO, FSP-ARKO, and dARKO mice confirmed that stromal AR is important as a positive regulator of prostatic epithelial cell proliferation and survival, and that these effects are mediated by stromally expressed growth factors.

AR Functions in Prostatic Epithelium

Both mouse lines carrying a deletion of AR specifically in prostatic epithelium were generated using a probasin-Cre strain [292] crossed with mice carrying exon 2 floxed AR (also known as pes-ARKO mouse) [287] or exon 3 floxed AR (also known as PEARKO mouse) [286, 293]. The phenotypes of pes-ARKO and PEARKO were different due to distinctive tissue specificity in Cre expression which resulted in AR inactivation in the ventral and dorsolateral prostate of pes-ARKO mice [287], and all prostate lobes and accessory glands of PEARKO mice [293]. As a result of these differences, pes-ARKO mouse was fertile with a normal external male phenotype, demonstrating that the ventral prostate (VP) does not play a major role in fertility in this model. The main phenotype recognized in the VP of pes-ARKO mice consisted in decrease epithelial height, loss of glandular infolding, and increase in luminal epithelial cells apoptosis, suggesting that AR is an important survival factor for luminal epithelial cells [294]. In contrast, PEARKO males with epithelial AR inactivation in all prostate lobes, seminal vesicles, and epididymis displayed reduced weight of these androgen-responsive organs and reduced fertility.

These mice displayed normal sperm production but abnormal kinetic of epididymal passage, abnormal flagellar morphology, and decreased fertilization rate of oocytes recovered from wild-type females after mating [293]. Due to these abnormalities only five of 15 PEARKO males (33%) were fertile, with only one of 15 siring a second litter within a 90-days mating trial.

A basal prostatic epithelium-specific ARKO (pbes-ARKO) model has been generated using a cytokeratin 5-Cre construct. The phenotype of this model revealed that AR decreases proliferation of basal epithelial cells and exerts a positive role in their differentiation into luminal epithelial cells [294].

Taken together, these studies suggest that epithelial AR regulates functions of prostatic epithelium and stroma related to proliferation, survival, and differentiation. The PEARKO model implies that androgen action on male accessory glands is a requirement for acquisition of sperm maturation and motility independently from normal testicular function.

AR Functions in Seminal Vesicles (SV)

The function of AR in SV smooth muscle was studied using PTM-ARKO [271] and PEARKO mice [293]. PTM-ARKO mice were originally generated to study the consequence of ablating AR in PTM cells, however, further analysis demonstrated that in this model AR is ablated also in SV smooth muscle cells. PTM-ARKO mice had smaller SV with thinner layer of smooth muscle, reduced epithelial cell height, decreased epithelial cell proliferation, and production of seminal proteins.

AR ablation was observed also in SV smooth muscle cells of PEARKO mice, although it is not clear why in this strain the probasin promoter induced Cre-recombinase expression in an extra-epithelial location [293]. In the proximal region, epithelial cells of PEARKO seminal vesicles were low, cuboidal and with very little cytoplasm. The lumen was filled with acidophilic fluid, similar to that present in the normal SV. In the distal region, the epithelium had a more normal morphology with rare foci of hyperplastic epithelial cells, smaller acini, and thinner smooth muscle layer. Gene expression studies demonstrated reduced mRNA expression of SVS2 and SVP99, two androgen-dependent markers of epithelial function in seminal vesicles.

These observations implied an impairment of epithelial cell function in the seminal vesicles of PTM-ARKO and PEARKO mice, and argued that that smooth muscle cells play a vital role in androgen-driven stromal-epithelial interactions in the SV.

AR Functions in the Epididymis

The epididymis is essential for sperm maturation and storage. The primordium of the epididymis is the mesonephros, which arises as a part of the transient kidney to form the Wolffian ducts (WD). Its stability and differentiation are regulated by growth factors and sex hormones, including androgens. WD are present in females,

but the female hormonal milieu is associated with WD regression. However, regression of WD in females is prevented by the presence of androgen secreting subcutaneous testicular grafts [295]. In human, the process of epididymis development consists in the formation of a 6 m duct, coiled and packed into a three-dimensional organ of approximately 10 cm in length [296] and composed by a differentiated epididymal epithelium consisting in principal, clear, narrow, basal, and dendritic cells throughout the duct. In mouse embryos AR is expressed in the periductal mesenchyme starting at E12.5. Subsequently AR is expressed in larger amount in both the epithelium and mesenchyme during WD development between E16.5 and E18.5 [297]. From later stages of development to the adult stage, AR expression in the epithelia is greater than in the mesenchyme.

To elucidate whether the mechanism responsible for WD stabilization and maturation is dependent on epithelial vs. mesenchymal AR, WD epithelium-specific AR KO mice were generated by mating activating enhancer binding protein 2-alpha (AP2α-Cre) promoter-driven Cre males with exon-1 floxed AR female mice [297]. In support for the essential role played by mesenchimal and not epithelial AR for the morphogenesis/stabilization of the WD, these animals revealed normal WD stabilization, elongation, and coiling at E18.5. Postnatal analysis revealed that principal and basal cell differentiation was perturbed in epithelia-specific AR KO mice, and associated with reduced expression of p63, a protein essential for differentiation of basal cells in the epithelium of the epididymis. Several growth factors, including FGF and EGF, are believed to mediate androgen function in the WD and to reproduce in this organ the type of epithelial/mesenchymal interaction described in the prostate [298].

Other epithelial AR KO mice have been reported, and their phenotype consists in hypoplastic epididymis with dysfunctional epithelial cell differentiation leading to impaired spermatogenesis [293, 299, 300]. In two of these models, Cre recombinase was placed under the control of promoters derived from ribonuclease 10 (Rnase10-Cre) [299] and fork-head box G1 (FoxG1-Cre) [300]. These two strains revealed lack of AR expression restricted to principal cells, epithelial cell hypoplasia in the proximal region of the epididymis and ductal obstruction, indicating the requirement for AR in the epididymal epithelial principal cells for proper development and function of the proximal epididymis.

Function of AR in Testicular Descent

Testicular descent occurs in two phases, each under the control of testicular hormones. The first phase, called transabdominal, occurs between 8 and 15 GW when insulin-like hormone 3 (Insl3) stimulates the gubernaculum to grow and to anchor the testes to the area of the body that will give raise to the future inguinal canal. The second, or inguinoscrotal phase, occurs between 25 and 35 GW, when the gubernaculum bulges out of the external inguinal ring, reaches the scrotum where it gives raise to the processus vaginalis, a peritoneal pouch inside the scrotum, and leads the testes to migrate inside the scrotal cavity. The inguino-scrotal phase occurs under T

control, which is believed to act through AR and to induce production of calcitonin gene-related peptide (CGRP) [301] by the genitofemoral nerve (GFN). The notion that testicular descent may be an AR-dependent process is supported by the fact that AR is widely expressed in various portions of the gubernaculum [302] as well as in the GFN [303], and prenatal use of antiandrogens is associated with cryptorchidism [304]. The theory that CGRP mediates AR action to induce testicular descent is controversial, as the genetic targeting of this peptide was not associated with cryptorchidism [305].

Testicular descent was investigated in a number of ARKO models. The PMC-ARKO (peri-tubular myoid cells) [270] and SM-ARKO (vascular smooth muscles) [306] mice revealed normal testicular descent, suggesting that presence of AR in fibroblasts and smooth muscle cells of the gubernaculum is not necessary for normal testicular descent. More recently, a gubernaculum-specific ARKO (GU-ARKO) mice was generated by crossing male mice carrying retinoic acid receptor 2 promoter-driven Cre (*Rarb-Cre*) with female mice carrying homozygous exon 2-floxed AR [307]. GU-ARKO mice exhibited presence of Cre activity not only in the gubernaculum but also Leydig cells, cauda epididymis and vasa deferentia, suggesting leakage of the *Rarb-Cre* construct. GU-ARKO mice were affected by cryptorchidism. There was a normal male phenotype and hormonal milieu (i.e. normal testosterone and LH) except smaller testes and epididymis. These cryptorchid animals produced viable sperm and were able to sire pups until 3 months of age. After 3 months of age these animals became infertile and showed abnormal seminiferous tubules, arrested spermatogenesis, and vacuolization of Sertoli cells. The mutant gubernaculum failed to give raise to the processus vaginalis, leaving the testes in a low abdominal position. GU-ARKO also showed abnormal development of the cremaster, possibly indicating that AR plays a role in the differentiation of this muscle. However, conditional ablation of AR from striated or smooth muscle cells, was associated with normal testicular descent [307].

Taken together these data demonstrate that the gubernaculum is an essential target of androgen signaling in testicular descent, however, the mechanism downstream of AR activation is still matter of controversy. As stated above, in GU-ARKO mice AR expression is ablated in Leydig but not Sertoli cells. Unlike LC-ARKO mice, GU-ARKO has normal levels of T and LH, raising the possibility that AR ablation in Leydig cells does not, in fact, affect testosterone production.

Functions of AR in Females

A naturally occurring human model with biallelic inactivation of AR would be valuable to understand the physiologic role played by AR in females, however, such model is unavailable because it would require a hemizygous father carrying an inactive AR allele, and this condition is associated with infertility. Animal models have therefore been instrumental to understand the physiopathology of AR in females. An early study utilized a total of seven androgen resistant female mice carrying biallelic inactivation of AR (i.e., *Tfm*/*Tfm* females bread from males chimeric for the

T*fm* gene with heterozygous AR^{Tfm} females) and demonstrated that biallelic AR inactivation is associated with infertility and accelerated ovarian aging [308]. Large numbers of homozygous AR-/- (ARKO) female mice could be generated for sustainable analysis only after Cre-LoxP technology became available [256, 259, 260, 263]. Three models or ARKO female have been generated, with loxP sites surrounding exon 1 (ARKO^{Ex1}) [259], exon 2 (ARKO^{EX2}) [260], or exon 3 (ARKO^{Ex3}) [262, 263]. Of these, ARKO^{Ex1} and ARKO^{EX2} mice produced no AR protein, in contrast ARKO^{Ex3} mice were conceived to produce an AR protein that contains an in-frame deletion of the second zinc finger and is expected to be unable to bind DNA and to retain AR non-genomic functions. All ARKO females exhibit dysfunctional ovulation leading to reduced fertility, longer estrous cycle with characteristically decreased litter numbers and sizes. In addition, ARKO^{Ex1} and ARKO^{Ex2} but not ARKO^{Ex3} females developed premature ovarian failure with reduced number of follicles/corpora lutea and increased follicular atresia [259, 260, 263]. These differences between models indicate that AR activities not requiring DNA binding rescue the phenotype of premature ovarian failure observed in ARKO^{Ex1} and ARKO^{Ex2} mice.

AR is expressed in many cellular subtypes of the ovary, including oocytes and granulosa cells (GC). Recent studies have characterized the phenotype of GC-specific and oocyte specific ARKO mice (Grc-ARKO and Oo-ARKO) [309]. There was normal fertility and estrous cycle in Oo-ARKO females; in contrast Grc-ARKO mice demonstrated premature ovarian failure, subfertility with longer estrous cycle, and decreased ovulation. These experiments established that it is the AR expressed in GC that regulates ovarian development and reproductive functions [309]. AR-dependent signaling pathways involved in this process include induction of the microRNA miR-125b, which suppresses expression of proapoptotic proteins involved in follicular atresia, and of the FSH receptor, which stimulates FSH-mediated follicle growth and development [310]. Additional factors regulating GC-oocytes paracrine interaction that are found downregulated in microarray experiments of ovaries derived from ARKO^{Ex1} include bone morphogenetic protein 15 (Bmp-15), KIT ligand, and growth differentiation factor 9 (Gdf-9) [259]. These observations are potentially relevant, as inactivating mutation of GDF9 and BMP15 have been associated with premature ovarian failure [311]. Further support for the importance of the entire AR locus in ovarian function include the observation that 50% of women with deletions in Xq11, the region of the X chromosome harboring the AR gene, have premature ovarian failure and the other 50% are affected by amenorrhea [312, 313].

AR and Breast Development

Breast development occurs at puberty primarily under influences from female sex hormones, which include estrogen-dependent growth of adipose tissue and lactiferous ducts, and progesterone-dependent lobular growth and alveolar budding. The breast is subjected to several additional hormonal influences not only restricted to sex hormones, however, sex hormones acting through AR, PR, and ERα have

fundamental and sometimes opposing roles. In particular, clinical observations support the notion that androgens, acting through AR (androgen->AR axis) oppose the stimulatory effect of estrogens acting through ERα (estrogen → ERα axis). That the androgen → AR axis blunts the effect of the estrogen → ERα axis is observed in several clinical models. For instance, the basic physiopathology of gynecomastia consists in an imbalance favoring estrogenic over androgenic activities at the level of the male breast, resulting in abnormal breast growth in males. 46XY male CAIS patients carrying inactivating mutations of AR develop female size breasts, and this process is mediated by unopposed ERα. At the opposite end of the spectrum are females with hyperandrogenic states such as PCOS, or receiving androgens for gender dysphoria disorders, who exhibit impaired breast development or breast atrophy, respectively.

Female ARKO models have generated valuable but discrepant information on how AR inactivation affects the breast phenotype. While at puberty ARKO^{Ex3} mice females exhibit accelerated mammary ductal growth and increased number of terminal end buds compared with WT female [314], at 4 weeks of age ARKO^{Ex2} animals display reduced ductal extension and decreased size and number of terminal end buds compared with wild-type animals [315]. The signaling pathways responsible for the phenotype observed in ARKO^{Ex3} animals include upregulation of ERα, activation of Wnt/β-Catenin signaling and increased expression of cyclin D1 [314]. As discussed above, the reason for these discrepant results has to do with the fact that an AR protein is translated in ARKO^{Ex3}. Although speculative at this time, one could hypothesize that the Ex3(−) AR protein maintains the ability to interact with coregulators or other nuclear receptors, thus affecting the phenotype of these animals.

AR and Uterus

AR is expressed in the uterus of various species, however, a specific role for AR in uterine physiology is unclear. A clear association between AR signaling and uterine physiology was reported in a study where the non-metabolizable androgen, DHT, was given to ovariectomized female rats and shown to stimulate uterine growth [316]. This observation is in line with data where ARKO^{Ex2} females demonstrated thinner uterine walls and endometrium compared to wt animals at the estrous stage and after gonadotropin stimulation [260]. Overall ARKO^{Ex2} females exhibited decreased reproductive potential, with decreased production of oocytes, corpora lutea, and litter size which was more evident with aging. There were little uterine and reproductive differences between wt controls with the ARKO^{Ex1} and ^{Ex3} models [259, 263], however, a subsequent study with ARKO^{Ex3} mice found that androgens have a direct effect on the growth and development of the uterus, with uteri showing increased horn length but reduced uterine diameter and total uterine area in this model [317]. Future studies with tissue-specific ARKO restricted to the uterus may be helpful in solving some of these discrepancies.

AR and PCOS

PCOS is a hyperandrogenic condition found in up to 6% of women and represents the number one cause of female infertility worldwide. While the hyperandrogenic state present in PCOS has been recognized for decades, a precise role for AR in the etiology of this condition has been hypothesized only recently, after observing an association between a shortened polyQ repeat and two AR splice variants with PCOS [318, 319]. The observation that the AR expressed in granulosa cells of 62% of Southeastern Han Chinese women with PCOS contains two alternative splice variants (AR-ASV) is relatively recent, and very intriguing. The first ASV results in the insertion of 69 a bp fragment between exons 2 and 3, the second causes skipping of exon 3. These two ASV's are associated with higher serum and follicular androgen levels, and with a longer menstrual cycle and greater number of antral follicles compared to PCOS women not expressing ASV's, or normal controls. Functionally, the two AR-ASVs demonstrate altered nuclear translocation and transcriptional activity, and, compared to wt AR, a change in the network of transcripts regulated in response to DHT. For example, AR ASV's induce upregulation of CYP17A1 and reduced binding efficiency to the androgen response element (ARE) located in the promoter region of the CYP19 gene, resulting in decreased aromatase expression, impaired conversion of androgens into estrogens, and consequent hyperandrogenism both in the general circulation and follicle fluid. Despite the novelty and potential importance of this discovery, it is unclear whether these ASV are the cause or consequence of PCOS. AR deleted of exon 3 is known to be associated with CAIS via germline mechanisms in humans [159, 320] and mice [262], and to protect against PCOS when female $ARKO^{Ex3}$ mice are treated with DHT in an experimental model of PCOS [321]. Based on this it has been argued that the AR ASV associated with exon 3 deletion is the consequence rather than the cause of PCOS [322].

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Physiology of Male Gonadotropic Axis and Disorders of Sex Development

3

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Introduction

Pulsatile secretion of GnRH by neurons of medium basal hypothalamus region is a crucial element of the reproductive cascade. GnRH binds to its receptor (GnRHR) on the gonadotrophs surface initiating the synthesis release of pituitary gonadotropins. In turn, LH and FSH stimulate testicular hormones secretion that induces pubertal development, and gametogenesis [1]. Normal testicular physiology results from the integrated function of the tubular and interstitial compartments.

Fetus testes differentiate by the end of the fifth embryonic weeks, before the gonadotrophs are functionally active. The hypothalamic-pituitary-testicular axis is activated at the third trimester of intrauterine life and in the neonatal period. However, GnRH deficiency does not affect male sexual differentiation that occurs in the first trimester of pregnancy because in this phase the Leydig cells' function is regulated by the placental chorionic gonadotropin (hCG). Conversely, in the second half of gestation, fetal luteinizing hormone (LH) and follicle-stimulating hormone (FSH) become major regulators of testicular physiology [2]. FSH stimulates Sertoli cells proliferation, inhibin B and the Anti-Müllerian hormone (AMH) secretion responsible for the regression of the Müllerian ducts during embryonic development. Fetal LH stimulates the production of androgens and insulin-like factor 3 (INSL3) by Leydig cells, leading to penile and scrotum growth and testicular descent [3]. Toward term, a decline in pituitary and testicular hormones is observed. These physiological events explain the occurrence of micropenis and cryptorchidism and lack of genital ambiguity in male newborns with congenital hypogonadotropic hypogonadism.

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After birth gonadotropins, testosterone, and AMH levels are transiently low, increase, and remain high for 3–6 months. Thereafter, gonadotropins levels decrease, resulting in a fall of testicular testosterone secretion to low or undetectable levels during infancy and childhood. Sertoli cells persist active, producing AMH, and inhibin B during infancy and childhood [3].

Control of Male Gonadotropic Axis Function

GnRH Secretion

Although the hypothalamic secretion of GnRH has been considered the key player in the to control of the male hypothalamic-pituitary-testicular axis, a number of other important players in the GnRH neuronal network have been identified. Stimulatory (kisspeptin) and inhibitory (MKRN3) pathways have been described in the control of GnRH secretion [4, 5].

The discovery of the crucial role of kisspeptin in human puberty completely changed current knowledge of the neuroendocrine regulation of human reproduction [6, 7]. Kisspeptin is a modulator that acts upstream of GnRH and is sensitive to sex steroid feedback. This peptide is now recognized as a critical regulator of the puberty onset, sex hormone-mediated secretion of gonadotropins, and fertility [8]. Kisspeptin is a potent stimulator of the hypothalamic-pituitary-gonadal axis and acts directly to GnRH neurons through its receptor, KISS1R (GPR54) to release GnRH into portal circulation, which in turn stimulates the secretion of LH and FSH (Fig. 3.1). The same

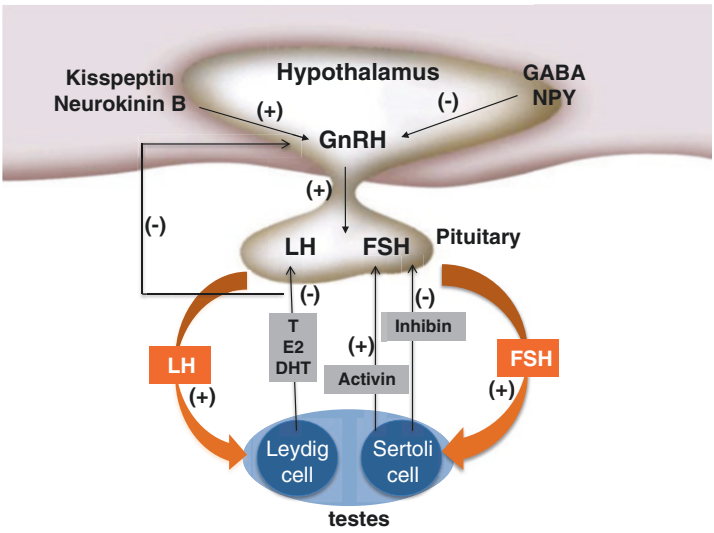

Fig. 3.1 Hormonal control of male gonadotropic axis. T = Testosterone; E2 = Estradiol; DHT = Dihydrotestosterone

neuronal network co-secretes Kisspeptin, neuroKinin B, and dynorphin, an opioid inhibitor. Neurokinin B, via neurokinin B receptor, stimulates the pulsatile Kisspeptin secretion whereas, dynorphin acting in its kappa opioid peptide receptor, inhibits kisspeptin secretion [9].

LH Secretion

It is well established that testosterone, estradiol, and dihydrotestosterone inhibit LH secretion [10] (Fig. 3.1). Santen and Bardin [11] demonstrated that testosterone acts at the hypothalamic level by decreasing GnRH pulse frequency without a change in pulse amplitude in portal blood. However, the action of estradiol appears to be predominantly at the pituitary where it decreases LH pulse amplitude without changing pulse frequency. In addition, these studies demonstrated that treatment with estradiol lowered LH levels by decreasing LH pulse amplitude without altering GnRH secretory patterns in portal blood [12]. However, administration of anastrozole, a selective aromatase inhibitor, in male caused an increase in LH pulse amplitude and pulse frequency. The authors also related increased testosterone concentrations accompanied by an increase in FSH levels. The investigators concluded that estradiol exerted a negative feedback by acting at the hypothalamus decreasing GnRH pulse frequency and at the pituitary decreasing the responsiveness to GnRH [13].

More recently, it has been shown that Kisspeptin also stimulates the secretion of both LH and FSH in the human. While kisspeptin stimulates LH release two- to threefold in most circumstances, the stimulatory effect on FSH levels is much smaller and is less consistent [14]. This differential effect of Kisspeptin on LH and FSH secretion is concordant with studies in rodents [15, 16]. The capacity of kisspeptin to enhance LH pulsatility has also been demonstrated in human reproductive disorders, including male hypogonadism associated with type 2 diabetes [14] and in hypogonadism due to neurokinin B signaling defects [17].

FSH Secretion

It is well established that testosterone and estradiol are capable of suppressing FSH levels in males [18]. However, inhibin, a glycoprotein hormone, exerts a specific negative feedback inhibition on FSH secretion at the pituitary level was also isolated [19]. Two forms of inhibin have been isolated, the inhibin A and inhibin B [20]. Both inhibins have the capacity to specifically inhibit FSH secretion by pituitary cells in culture. In contrast, the dimers of the β subunit, termed activins (activin A, activin B, and activin AB) all have the capacity to stimulate FSH secretion by pituitary cells in culture [21, 22] (Fig. 3.1). Activin, inhibin, and anti-Müllerian hormone belong to the TGF-β protein superfamily.

Finally, a protein termed follistatin has the capacity to suppress FSH secretion specifically by pituitary cells in culture [23]. This action is due to the capacity of follistatin to bind and neutralize the actions of activin [24].

The inhibin is produced by the Sertoli cell and the predominant form of inhibin secreted by the testis is inhibin B [25]. The levels of inhibin B in males are inversely related to the levels of FSH [26]. It has been demonstrated that FSH predominantly stimulates inhibin α-subunit production and does not alter the β-subunit message [27]. Corroborating to this, a clear increase in these glycoproteins under the stimulation of elevated FSH levels has been shown [28].

In men, testosterone at amounts equivalent to or greater than its production rate can suppress both FSH and LH [10], with greater suppression of LH secretion in contrast to the actions of inhibin [18]. The observation of an increase in FSH levels in men treated with a selective aromatase inhibitor raised the possibility that estradiol exerts a negative feedback action on FSH especially since the treated men experienced a concomitant significant increase in testosterone [13].

There is evidence that the Sertoli cells, Leydig cells, and peritubular myoid cells can produce activin that exerts local actions within the testis such as the stimulation of spermatogonial mitosis [29]. Moreover, activin A is responsible for the stimulation of Sertoli cell mitosis during the development of the testis in both rats and mice [30, 31]. Additionally, receptors for activin are present on primary spermatocytes, round spermatids and Sertoli cells [32]. However, our knowledge on activin functions in male gonadotrophic axis is still emergent.

Follistatin is also produced by the Sertoli cells, spermatogonia, primary spermatocytes, and round spermatids in the testis [33]. However, castration does not result in a clear decrease in follistatin levels in the circulation suggesting that the testis does not contribute significantly to the circulating levels of follistatin [34].

The absence of changes in activin and follistatin levels, whereas the inhibin levels in the circulation decreased to undetectable levels after castration, strongly suggest that the gonadal feedback signal on FSH secretion is inhibin. Furthermore, in several species the infusion or injection of recombinant human inhibin caused a specific fall in FSH secretion 6 h following administration [35]. In addition, normal levels of inhibin A in castrate rams suppressed FSH levels into the normal range in the absence of testosterone [36].

There is substantial evidence that activin and follistatin can exert a paracrine role directly in the pituitary gland. It is, therefore, likely that the actions of inhibin are predominantly exerted through secretion from the testis and transport via the peripheral circulation whereas the actions of activins and follistatin on FSH secretion occur through paracrine actions at the level of the pituitary gland. Further evidence supporting the stimulation of FSH by activin secretion emerges from the decline in FSH levels in mice with targeted disruption of the activin type II receptor gene [37].

The increase of gonadotropin pulse amplitude and frequency drives pubertal development of the testis. FSH induces a new Sertoli cell proliferation and LH stimulates the maturation of Leydig cells again. The increase of testosterone concentration into the testis incites Sertoli cells maturation [38] and down-regulation of AMH

levels [39]. It is noteworthy that intratesticular testosterone levels regulate spermatogenesis. Indeed, the administration of exogenous testosterone results in elevated serum testosterone levels, but without reaching intratesticular testosterone concentration to induce spermatogenesis. Moreover, testosterone levels associated with an adequate expression of the androgen receptor in Sertoli cells are necessary for meiosis [40]. Young et al. demonstrated that mean serum AMH levels in untreated men with hypogonadotropic hypogonadism were significantly higher than in normal men and were similar to those previously reported in pre-pubertal boys. hCG treatment in these patients induced an increase of plasma T associated with a dramatic decrease of serum AMH. The similar increase in plasma T levels was obtained in those patients treated with exogenous T, but a lesser decrease of serum AMH. These data suggest that Intratesticular testosterone concentration can be estimated by measuring serum AMH [41].

Inhibin B secretion during puberty is regulated by FSH and germ cells [42]. Adult levels of inhibin B are achieved in coincidence with the increase in serum LH and intratesticular testosterone levels.

46,XY Disorders of Sex Development

The term disorders of sex development (DSD) comprises congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical. The 46,XY DSD are characterized by atypical or female external genitalia, caused by incomplete intrauterine masculinization, in the presence or absence of Mullerian structures. Complete absence of virilization results in normal female external genitalia and these patients generally seek medical attention at pubertal age due to the absence of breast development and/or primary amenorrhea [43].

Male phenotypic development can be viewed as a two-step process: (1) testis formation from the primitive gonad (sex determination) and (2) internal and external genitalia differentiation due to factors secreted by the testis (sex differentiation) [43, 44].

At the beginning of gestation, embryos of the two sexes differ only for their karyotypes. Specific genes lead to the determination of the bipotential gonad into a testis or an ovary. In turn, the hormonal production of the fetal gonad will induce the anatomical and possibly psychological differences, leading to different behaviors that are ultimately influenced by the social environment. This pool of factors will determine the individual sex.

At 6–7 weeks of gestational the paramesonephric duct (Müllerian duct) develops next to the mesonephric duct (Wolffian duct). If a testis develops, AMH, a glycoprotein secreted by the Sertoli cells, acts on its receptor in the Müllerian ducts to cause their regression. Testosterone secreted by the testicular Leydig cells acts on the

androgen receptor in the Wolffian ducts to induce the formation of epidydimis, deferent ducts, and seminal vesicles. Testosterone is further reduced to dihydrotestosterone (DHT), which acts on the androgen receptor of the prostate and external genitalia to cause its masculinization. If testes do not develop normally, and his hormones are absent or insufficient, the mesonephric duct does not grow and eventually degenerates, whereas the paramesonephric duct proliferates and the fallopian tube, uterus, and upper third of the vagina develop [45].

Testosterone mediates three main functions in male physiology: regulation of the LH secretion from the anterior pituitary, virilization of the wolffian ducts in the male embryo, and regulation of spermatogenesis. The other androgen action during embryogenesis and intrauterine life is mediates by DHT [46]. Testosterone and DHT act via a single androgen receptor, and dihydrotestosterone binds more tightly to the hormone-binding domain of the receptor due to a decreased rate of dissociation of the DHT-receptor complex; the consequence of similar association rates but different dissociation rates is that, in the steady state, dihydrotestosterone occupies most receptor sites, even when testosterone is the predominant steroid in the cell [47].

Testosterone is the principal androgen synthesized by both fetal and mature testes. Testosterone secretion begins just prior to the onset of virilization of the male embryo and promotes the conversion of the wolffian ducts into the eipidiymides, vasa deferentia, seminal vesicles, and ejaculatory ducts. Dihydrotestosterone, in turn, causes the development of the prostate in the urogenital sinus, midline fusion, elongation, and enlargement of the urogenital tubercle and the urogenital folds, eventuating in the development of the scrotum and phallus [46].

46,XY DSD result from decreased production of testosterone, decreased conversion of testosterone into DHT or from impairment of their peripheral action. At histological analysis, testicular tissue in 46,XY DSD patients can be absent, partially or completely dysgenetic, or almost normal [45].

Taking in account testosterone levels, the etiology of the 46,XY DSD can be classified into two large groups:

1. Low testosterone secretion
   (a) Defects in the formation of the testes
   (b) Enzymatic defects in testosterone synthesis
2. Normal or high testosterone secretion
   (a) Defects in the conversion of testosterone in DHT
      - 5α reductase 2 deficiency
   (b) Defects in testosterone action
      - Androgen receptor defects

46,XY DSD Due to Low Testosterone Secretion

Defects in Formation of the Testes

46,XY Gonadal Dysgenesis

46,XY gonadal dysgenesis includes a variety of clinical conditions in which the fetal testes development is abnormal. This group encompasses both complete and partial forms, embryonic testicular regression syndrome and testicular agenesis. The complete form is characterized by female external and internal genitalia, lack of secondary sexual characteristics, normal or tall stature without somatic stigmata of Turner syndrome, and the presence of bilateral dysgenetic gonads. On the other hand, the partial form of this syndrome is characterized by impaired testicular development that results in patients with ambiguous external genitalia with or without Mullerian structures [43].

46,XY gonadal dysgenesis is a genetic heterogeneous disorder associated with alterations in a number of genes involved in the male gonad development. SRY and NR5A1/SF1 mutations are the most frequent cause of nonsyndromic 46,XY gonadal dysgenesis [48]. Considering that molecular diagnosis is established in just 20% of DSD patients, aCGH or whole-exome or -genome sequencing evaluation may enable molecular diagnosis involving known genes and novel candidate genes for 46,XY gonadal dysgenesis [45].

Table 3.1 summarizes the genes that determine abnormalities in testis development and may be associated or not with other syndromic signs.

The dysgenetic testes showed disorganized seminiferous tubules and ovarian stroma with occasional primitive sex cords devoid of germ cells; primordial follicles are sometimes observed in the streak gonad in the first years of life [49].

The laboratorial diagnosis is based on the 46,XY karyotype and high levels of LH and FSH with a predominance of FSH. Basal testosterone levels are within prepubertal range and fail to increase after hCG stimulation.

Table 3.1 Causative Genes of abnormalities in testis development

Gene		Locus	Protein	Protein function	Human phenotype
Symbol	Name				
ARX	Aristaless-related homeobox	Xp22.13	ARX	Transcription regulation	46,XY gonadal dysgenesis, epilepsy, psychomotor impairment
ATRX	X-linked α-thalassemia and mental retardation	Xq13	ATRX (ou XNP)	Transcriptional regulation and chromatin remodeling	46,XY gonadal dysgenesis, several body malformations, thalassemia, mental retardation
CBX2	Chromobox homolog 2	17q25	CBX2	Transcriptional repression	46,XY gonadal dysgenesis, female external genitalia and ovaries
DHH	Desert hedgehog	12q12-13.1	DHH	Signaling activity	46,XY gonadal dysgenesis, polyneuropathy
DMRT1	Double sex, Mab3, Related transcription factor 1	9p24.3	DMRT1	Transcription regulation	46,XY gonadal dysgenesis (deletions of 9p region)
DSS locus (*DAX1*)	Dosage sensitive sex reversal, Adrenal hypoplasia, X chromosome 1	Xp21.3	DAX1 (NR0B1A)	Transcription regulation	46,XY gonadal dysgenesis, cleft palate and dysmorphic face associated or not with mental retardation (*DSS locus duplication*)
FOG2/ ZFPM2	Friend of GATA 2/Zinc finger protein multitype 2	8q23.1	FOG2	Modulation of GATA family activity	46,XY gonadal dysgenesis, hipogonadismo hipergonadotrófico com defeito cardíaco congênito
GATA4	GATA-binding protein 4	8p23.1-p22	GATA 4	Transcription regulation	46,XY gonadal dysgenesis associated or not with congenital heart defects
MAMLD1/ CXORF6	Mastermind-like domain containing 1/chromosome X open reading frame 6	Xq28	MAMLD1/ CXORF6	Transcriptional co-activation	46,XY gonadal dysgenesis, hypospadias
MAP3K1	Mitogen-activated protein kinase 1	5q11.2	MAP3K1	Kinase	46,XY gonadal dysgenesis

Gene		Locus	Protein	Protein function	Human phenotype
Symbol	Name				
NR5A1/SF1	Nuclear receptor subfamily 5 group A member 1/ Steroidogenic Factor 1	9q33	NR5A1/SF1	Transcription regulation	46,XY gonadal dysgenesis with or without adrenal insufficiency
SOX9	SRY-related, HMG-box gene 9	17q24.3-25.1	SOX9	Transcription regulation	46,XY gonadal dysgenesis and campomelic dysplasia
SRY	Sex-determining Region-Y chromosome	Yp11.3	SRY	Transcription regulation	46,XY gonadal dysgenesis
WNT4	Wingless-type MMTV integration site family, member 4	1p35	WNT4	Signaling activity	46,XY gonadal dysgenesis (gene duplication)
WT1	Wilms' Tumor 1	11p13	WT1	Transcription regulation	46,XY gonadal dysgenesis—Frasier syndrome Denys–Drash syndrome and WAGR syndrome

Defects of Testosterone Production

LH/hCG Insensibility: Leydig Cell Hypoplasia

Leydig cell hypoplasia is an autosomal recessive disorder. The inability of Leydig cells to secrete testosterone in 46,XY DSD results in the failure of intrauterine and pubertal virilization. Both hCG and LH act by stimulating a common G-protein coupled receptor (LHCGR) and mutations in this gene cause Leydig cell hypoplasia. Affected patients with complete form have female external genitalia leading to female sex assignment, absence of sexual characteristics at puberty, primary amenorrhea undescended testes slightly smaller than normal with relatively preserved seminiferous tubules and absence of mature Leydig cells, presence of rudimentary epididymis and vas deferens and absence of uterus and fallopian tubes, low testosterone levels despite elevated gonadotrophin levels, with LH levels predominant over FSH levels, testicular unresponsiveness to hCG stimulation, and no abnormal step up in testosterone biosynthesis precursors [50, 51]. Several different mutations in the LH receptor gene were reported in these patients [43, 50, 51].

In contrast to the homogenous phenotype of the complete, the partial form of Leydig cell hypoplasia has a broad spectrum [50–53]. Most patients have predominantly male external genitalia with micropenis and or hypospadias. Testes are cryptorchidic or topic. During puberty, partial virilization occurs and testicular size is normal or only slightly reduced, while penile growth is significantly impaired. Testosterone response to the hCG test is subnormal without accumulation of testosterone precursors. After puberty, LH levels are elevated and testosterone levels are intermediate between those of children and normal males [43].

46,XY DSD Due to Cholesterol Synthesis Defects

Smith-Lemli-Opitz is rare syndrome caused by a deficiency of 7-dehydrocholesterol reductase [54]. The first step of testosterone biosynthesis begins with the uptake of cholesterol from the extracellular space and/or the endogenous synthesis of cholesterol by Leydig cells. In both instances, the action of 7-dehydrosterolreductase is necessary for cholesterol synthesis from 7-dehydrocholesterol. The SLOS phenotypic spectrum is broad and variable—from early embryonic non-viability to varying levels of severity postnatally, including distinctive facial appearance, growth and mental retardation, autistic behavior, hypotonia, failure to feed, decreased life span and variable structural anomalies of the heart, lungs, brain, gastrointestinal tract, limbs, genitalia, and kidneys.

Enzymatic Defects in Testosterone Synthesis

Five enzymatic defects that alter the normal synthesis of testosterone from cholesterol have been described (Fig. 3.2). Three of these defects are associated with defects in cortisol synthesis leading to congenital adrenal hyperplasia associated with 46,XY DSD. All of them are rare and present an autosomal recessive mode of inheritance.

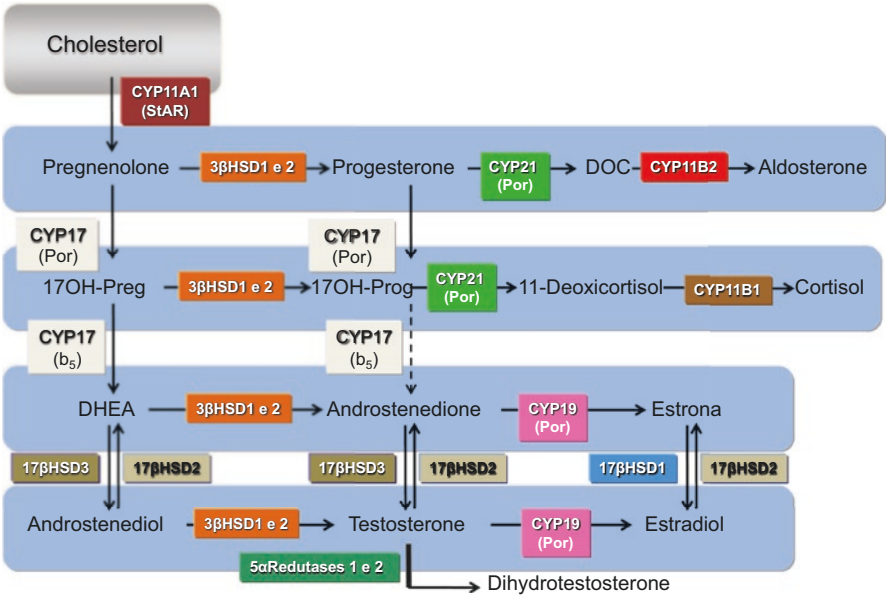

Fig. 3.2 Main biosynthesis pathways of adrenal and gonadal steroids

Congenital adrenal hyperplasia (CAH) is associated with hypoadrenocorticism or a mixed of hypo-and hyper-corticoadrenal steroid secretion. Synthesis of just cortisol or both gluco-and mineralocorticoids is impaired leading to a compensatory increase in adrenocorticotrophic hormone (ACTH) and in renin-angiotensin production. These compensatory mechanisms may return cortisol or aldosterone production to normal or near normal levels, but with an excessive production of other steroids causing undesirable hormonal effects. Defects in P45011A enzyme, also called P450scc, steroidogenic acute regulatory (StAR) protein, 3β-hydroxysteroid dehydrogenase (3β-HSD) type II, and 17α-hydroxylase cause congenital adrenal hyperplasia in 46,XY patients [43].

P45011A and StAR protein catalyze the first step in conversion of cholesterol to hormonal steroids known as cholesterol side-chain cleavage to form pregnenolone. This is the most severe form of CAH associated with 46,XY DSD. Affected subjects are, in general, phenotypic females or, sometimes, present slightly virilized external genitalia with or without cryptorchidism, underdeveloped internal male organs and an enlarged adrenal cortex, engorged with cholesterol and cholesterol esters. Adrenal steroidogenesis deficiency, when untreated, leads to salt wasting crisis, hyponatremia, hyperkalemia, hypovolemia, acidosis and death in infancy [55].

The following step in testosterone biosynthesis is the conversion of dehydroepiandrosterone (DHEA) in androstenedione by 3β-HSD type II (Fig. 3.2). Male patients with 3β-HSD type II deficiency present with atypical external genitalia, characterized by microphallus, perineal hypospadias, bifid scrotum, and a blind vaginal pouch. Defects with severe impact in enzymatic activity are associated with

salt loss. Gynecomastia is common at pubertal stage. Male subjects with 46,XY DSD due to 3β-HSD type II deficiency without salt wasting showed clinical features in common with the deficiencies of 17β-HSD 3 and 5α-reductase 2 [43].

The next step in the biosynthesis is the conversion of pregnenolone into 17α-hydroxypregnenolone and further down into DHEA by P450c17 (Fig. 3.2). The classical phenotype of 17α-hydroxylase deficiency in male patients described is a female-like or slightly virilized external genitalia with blind vaginal pouch, cryptorchidism, and high blood pressure, usually associated with hypokalemia. Differently of other forms of CAH, these patients do not present signs of glucocorticoid insufficiency due to the elevated levels of corticosterone, which has glucocorticoid effect [43].

Treatment of patients with these different forms of CAH consists of glucocorticoid and mineralocorticoid replacement in salt-losing forms and testosterone replacement in male patients.

The two last enzymatic defects in testosterone synthesis are not associated with adrenal insufficiency, the isolated 17,20-lyase deficiency (CYP17 deficiency) and 17β-HSD III deficiency (17-β-HSD 3 deficiency) (Fig. 3.2).

The 17,20 lyase activity is catalyzed by P450c17 that convert 17OH-pregnenolone into DHEA and 17OH-progesterone into androstenedione. This is a very rare form of 46,XY DSD and patients with isolated 17,20 lyase deficiency present atypical genitalia with microphallus, perineal hypospadias, and cryptorchidism [56].

The last step of biosynthesis of testosterone in Leydig cell is the conversion of androstenedione to testosterone activated by 17β-HSD III.

17β-Hydroxysteroid Dehydrogenase Type III Deficiency

This is the most common disorder of androgen synthesis. There are five steroid 17β-HSD enzymes which catalyze this reaction and 46,XY DSD results from mutations in the gene encoding the 17β-HSD 3 isoenzyme [57] that is almost exclusively expressed into testis. Patients present female-like or atypical genitalia at birth, with the presence of a blind vaginal pouch, intra-abdominal or inguinal testes and epididymides, vasa deferentia, seminal vesicles, and ejaculatory ducts. Most affected males are raised as females, but important virilization occurs at the time of expected puberty (Fig. 3.3). This late virilization is usually a consequence of the presence of testosterone in the circulation as a result of the conversion of androstenedione to testosterone by some other 17β-HSD isoenzyme in extragonadal tissue and of the secretion of testosterone by the testes when levels of LH are elevated in subjects with some residual 17β-HSD 3 function. However, the discrepancy between the failure of intrauterine masculinization and the virilization at the time of expected puberty is poorly understood. Most 46,XY patients are raised as girls during childhood and change to male gender role behavior at puberty. Hormonal diagnosis is based on elevated basal serum levels of androstenedione and low levels of testosterone. At the time of puberty, serum LH and testosterone levels increase in all affected subjects and testosterone levels may stay into the normal adult range [43].

Mutations in the *HSD17B3* gene are involved with etiology of this disorder.

Table 3.2 summarizes the characteristics of patients with 17β-HSD 3 deficiency.

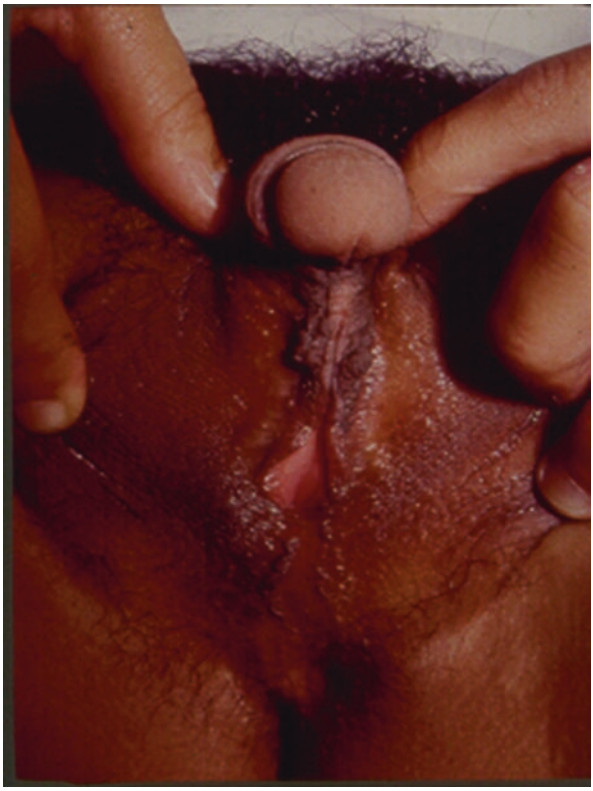

Fig. 3.3 Adult patient with 46,XY DSD due to 17β-hydroxysteroid dehydrogenase type III deficiency

Table 3.2 Characteristics of patients with 17β-HSD 3 deficiency

Inheritance	Autosomal recessive
External genitalia	Ambiguous, frequently female-like at birth
Müllerian duct derivatives	Absent
Wolffian duct derivatives	Normally developed
Testes	Well developed, frequent cryptorchidism
Hormonal diagnosis	Low T and elevated basal and hCG-stimulated A and A/T ratio
Molecular defect	Inactivating mutation of 17β-HSD 3 gene
Puberty	Virilization at puberty; variable gynecomastia
Gender role	Most patients keep the female social sex; some change to male social sex
Treatment	Repair of sexual ambiguity; estrogen or testosterone replacement according to social sex
Outcome	Male or female behavior; in male fertility possible by in vitro fertilization

46,XY DSD with Normal or High Testosterone Secretion

Defects in the Conversion of Testosterone in Dihydrotestosterone: 5 Alpha Reductase 2 Deficiency

Two different enzymes catalyze 5α-reductase reactions. The 5α-RD2 isoenzyme promotes the conversion of testosterone to DHT, the main active metabolite of testosterone, responsible for masculinization of external genitalia in male fetus. It has been demonstrated that the 5α-reductase 1 activity is normal in these subjects [58] and that the disorder is due either to homozygous or compound heterozygous loss-of-function mutations of the steroid 5α-reductase 2 gene [59].

Male affected patients present with ambiguous external genitalia, micropenis, normal internal male genitalia (Fig. 3.4b), prostate hypoplasia, and testes with normal differentiation, usually located in the inguinal region and normal or reduced spermatogenesis. Virilization and deep voice appear at puberty, along with penile enlargement and muscle-mass development without gynecomastia. These patients present scarce facial and body hair and absence of temporal male baldness, acne, and prostate enlargement. The majority of the patients are reared in the female social sex due to female-like external genitalia at birth (Fig. 3.4a), but many patients who have not been submitted to orchiectomy in childhood undergo male social sex change at puberty. In our cohort, all subjects were registered in the female social sex except for two cases—one who has an affected uncle and the other who was

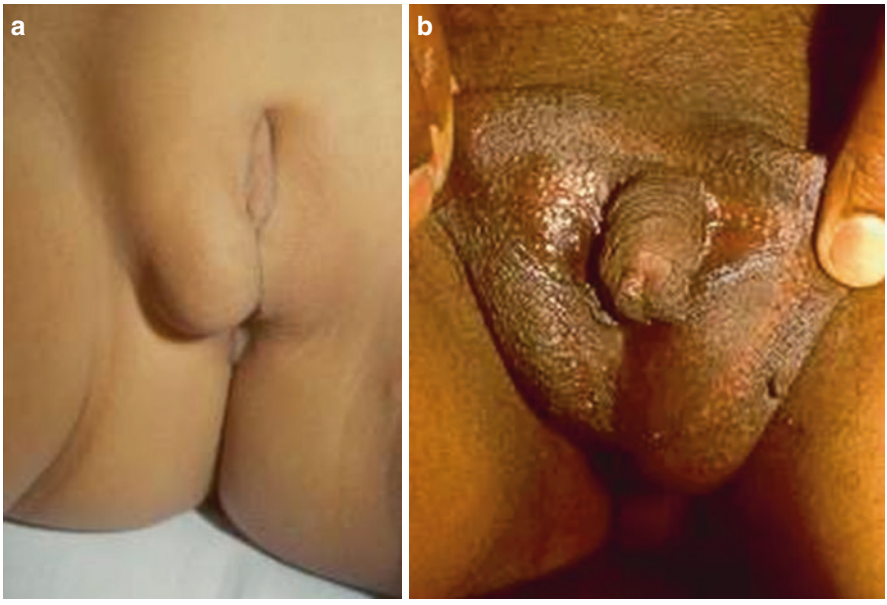

Fig. 3.4 (**a**) Female-like external genitalia at pre-pubertal age. (**b**) atypical external genitalia and micropenis at pubertal age

diagnosed before being registered [43]. Fourteen out of 30 patients changed to the male gender role. No correlation was observed between SRD5A2 mutation, testosterone/DHT ratio, and gender role change in these patients. Three cases adopted children and in two cases in vitro fertilization using the patient's sperm cells resulted in twin siblings in one family and in a singleton pregnancy in the other. None of the ten adult female patients are married but eight of them have satisfactory sexual activity [43]. The main differential diagnosis of 5α-RD2 deficiency is with 17β-HSD3 deficiency and partial androgen insensitivity syndrome although in these two disorders the gynecomastia is generally observed.

The mode of inheritance for 5α-RD2 deficiency is autosomal recessive, however, the uniparental disomy was described in two unrelated patients [60].

Affected children show lower DHT levels and elevated testosterone/DHT ratio, after hCG stimulation. Post-pubertal affected patients present normal or elevated testosterone levels, low DHT levels, and elevated testosterone/DHT ratio in basal conditions. Low DHT production following exogenous testosterone administration is also capable of identifying 5a-RD2 deficiency. Elevated 5β/5α urinary metabolites ratio is also an accurate method to diagnose 5α-reductase 2 deficiency even at pre-pubertal age and in orchiectomized adult patients [61].

There are more than 50 families with this disorder described worldwide. In a few cases of 46,XY DSD due to 5-a RD2 deficiency diagnosed by clinical and hormonal findings, no mutations were identified in SRD5A2 gene.

Small penis size is the main concern of male patients with 5-a RD2 deficiency.

Table 3.3 summarizes the characteristics of patients with 5-a RD2 deficiency.

Table 3.3 Characteristics of patients with 5α-reductase 2 deficiency

Inheritance	Autosomal recessive
External genitalia	Ambiguous, small phallus, perineal hypospadias, bifid scrotum, blind vaginal pouch
Müllerian ducts derivatives	Absent
Wolffian ducts derivatives	Normal
Testes	Normal size at inguinal or intra-abdominal region
Clinical features	Virilization at puberty, absence of gynecomastia
Hormonal diagnosis	Increased T/DHT ratio in basal and hCG-stimulation conditions in post-pubertal patients and after hCG-stimulation in pre-pubertal subjects. Elevated 5β/5α C_{21} and C_{19} steroids in urine in all ages
Gender role	Female → male in 60% of the cases
Molecular defect	Mutations in *5RD5A2*
Treatment	High doses of T or DHT for 6 months to increase penis size
Outcome	Maximum penis size in males after treatment = 7 cm; fertility is possible by in vitro fertilization

Defects in Testosterone Action: Androgen Receptor Defects

Disorders of androgen action are the most frequent cause of 46,XY DSD. Androgen insensitivity syndrome is classified as the complete form (CAIS) when there is an absolute absence of androgen action, as the partial form (PAIS) when there are variable degrees of androgen action impairment and the mild form that is reported in healthy men and boys who can present with adolescent gynecomastia or infertility in later life. Therefore, androgen insensitivity syndrome can be defined as a disorder resulting from complete or partial resistance to the biological actions of androgens in a XY subject with normal testis determination and production of age-appropriate androgen concentrations [62].

Prenatal diagnosis of CAIS can be suspected when a 46,XY fetus presents with female genitalia on prenatal ultrasound. At pre-pubertal age, an inguinal hernia in girls can indicate the presence of testes. At puberty, complete breast development and primary amenorrhea associated with reduced or absent pubic and axillary hair suggest CAIS. Adult women who have intact gonads have the endocrine profile of hormone-resistant state. Serum testosterone concentrations are either within or above the normal range for men and LH concentrations are increased. FSH and inhibin are generally normal. Serum estradiol levels arising from testosterone aromatization are higher than those observed in men. Serum gonadotropin levels increase further after gonadectomy but are only partly suppressed with estrogen substitution [62].

Gonadectomy should be performed because of the increased risk of testicular tumors, although it has been reported that tumor risk is low in CAIS patients before and during puberty [63]. On the other hand, some authors advise to postpone gonadectomy until after spontaneous breast development at puberty [64]. We are for pre-pubertal gonadectomy as soon as diagnosis is established and then induction of puberty with estrogens at the appropriate age. This approach diminishes the period of presence of an inguinal mass that is, often painful. In addition, a young child better psychologically handles the surgical procedure than an adolescent girl. In our experience, breast development is similarly obtained with endogenous estrogenization or with pharmacological replacement. Ultimately, the optimal timing for gonadectomy in CAIS patients is still controversial [43].

Whereas the clinical picture of CAIS is quite homogeneous, the phenotype of PAIS is quite variable and depends on responsiveness of the external genitalia to androgens. Atypical genitalia with microphallus, severe hypospadias, bifid scrotum, and palpable gonads is the most frequent phenotype of PAIS. The large phenotype spectrum of PAIS patients can cause misdiagnosis with several 46,XY DSDs due to defects in androgen production [62]. PAIS diagnosis is unequivocally established by the identification of a molecular defect on AR gene.

The maternal female relatives of the patient are eligible for screening the mutation identified in index case. In case of the carrier status, genetic counseling should be performed.

In AIS patients, final height is intermediate between mean normal male and female and decreased bone mineral density in the lumbar spine have been demonstrated [65].

Mild androgen insensitivity syndrome is associated with a mutation of the androgen receptor gene and is infrequently reported. It presents in men as infertility but is not associated with genital anomalies [66]. The product of serum LH and testosterone concentrations as an index of possible mild androgen insensitivity syndrome in infertile men could be a useful screening test for the presence of a mutation in the androgen receptor gene [65].

AR mutations are found in the great majority of CAIS patients and in several patients with PAIS [67, 68]. In our experience, selecting patients with normal basal and hCG-stimulated testosterone and steroid precursors levels, gynecomastia at puberty, and a family history suggestive of X-linked inheritance, results in the identification of mutations in 89% and 77% of the families with post-pubertal patients with CAIS and PAIS, respectively [68].

More than 800 different AR mutations have been entered in the Cambridge database of androgen receptor genes as of September, 2011.

Patients with CAIS were raised as females and maintained female gender. Most of the patients with PAIS who were raised as females maintained a female social sex after post-pubertal age, despite clitoral growth and partial virilization. In our experience, all cases with PAIS kept the female social sex [68]. This is in distinct contrast to other forms of 46,XY DSD such as 5-reductase 2 deficiency and 17β-hydroxysteroid dehydrogenase III deficiency in which several affected individuals raised as females undergo a change to male social sex at puberty [61, 69].

Table 3.4 summarizes the characteristics of patients with PAIS.

Table 3.4 Characteristics of patients with partial androgen insensitivity syndrome

Inheritance	X-linked recessive
External genitalia	Broad spectrum from female with mild clitoromegaly to male with micropenis and/or hypospadias
Müllerian duct derivatives	Absent
Wolffian duct derivatives	Broad spectrum from absent or male
Testes	Eutopic, inguinal or intra-abdominal, normal or slightly subnormal size
Puberty	Gynecomastia
Hormonal diagnosis	High or normal serum LH and T levels, normal or slightly elevated FSH levels
Gender role	Female or male
Molecular defect	Mutations in *AR* gene
Treatment	Females: surgical feminization, gonadectomy, replacement with estrogens at the time of puberty, vaginal dilation (if necessary)
	Males: repair of hypospadias, bifid scrotum; high doses of T or DHT to increase penis size
Outcome	Infertile, female or male gender role

Hormonal treatment: High doses of testosterone esters (250–500 mg twice a week) are used to increase DHT levels and consequently penis size and male secondary characteristics. Maximum penis enlargement is obtained after 6 months of high doses and then the normal dosage is reinstituted. The use of topic DHT gel is also useful to increase penis size with the advantage of not causing gynecomastia and promoting a faster increase of penis size as it is 50 times more active than testosterone. DHT is not aromatized, allowing the use of higher doses than testosterone during pre-pubertal age and consequently attaining a higher degree of virilization.

Management of Patients with 46,XY DSD

The treatment of 46,XY DSD patients requires an appropriately trained multidisciplinary team. Early diagnosis is important for good outcome of the patients and should start with a careful examination of the newborn's genitalia at birth.

Psychological evaluation is of extreme importance in the treatment of DSD patients. Every couple who giving birth a child with atypical genitalia must be assessed and counseled by an experienced psychologist, specialized in gender identity, who must act as soon as the diagnosis is suspected.

The physician and psychologist must inform parents about normal sexual development. A simple, detailed, and comprehensive explanation about what to expect regarding integration into social life, sexual activity, requirement of hormonal, and surgical treatment and the possibility of fertility according to the sex of rearing, should also be discussed with the parents, before the attainment of final social sex.

The determination of social sex must take into account the etiological diagnosis, penis size, ethnic traditions, sexual identity, and the acceptance of the assigned social sex by the parents. In case parents and health care providers disagree over the sex of rearing and psychological support was not able to change parents' choice, their opinion should always prevail to avoid ambiguous sex of rearing. The affected child and his/her family must be followed throughout life to ascertain the patient's adjustment to his/her social sex [43].

Hormonal Therapy in DSD Patients with Male Social Sex

We start testosterone replacement between 10 and 11 year of age, simulating normal puberty according to the child's psychological evaluation and height. The initial dose of short-acting testosterone esters is 25–50 mg/month intramuscular via. The maintenance dose in adult patient is 200–250 mg every 2 weeks for injectable short-acting testosterone, 1000 mg every 3 months for long-acting testosterone, and 50–60 mg every day for transdermal testosterone.

In those patients with androgen insensitivity, higher doses of testosterone esters (250–500 mg twice a week) are used to increase penis size and male secondary characteristics. Maximum penis enlargement is obtained after 6 months of high doses and after that, the normal dosage is reinstituted [61].

The use of topic DHT gel is also useful to increase penis size with the advantage of not causing gynecomastia and promoting faster increase of penis size as it is 50 times more active than testosterone. Considering that DHT is not aromatized, one would expect it to have no effect on bone maturation, allowing the use of higher doses than testosterone and consequently attaining a higher degree of virilization [43].

Surgical Procedures in DSD Patients with Male Social Sex

The aims of surgical treatment are to provide an adequate external genitalia and removal internal structures that are inappropriate for the social sex. Patients must undergo surgical treatment preferably before 2 years of age, which is the time when the child becomes aware of his/her genitals and social sex. Only skilled surgeons with specific training in the surgery of DSD should perform these procedures [43].

Surgery consists of orthophaloplasty, scrotumplasty with resection of vaginal pouch, proximal and distal urethroplasty and orchidopexy when necessary. Surgeries were performed in two or three steps in the patients with perineal hypospadias. The most frequent complication is urethral fistula in the penoscrotal angle and urethral stenosis that can occur several years after surgery. The aesthetical and functional results of surgical correction are good, in our and other series [70].

Most of our patients present satisfactory sexual performance as long as they present a penis size of at least 6 cm [70]. New approaches, such as the use of donor-grafting tissue to elongate the urethra and penis, may help these patients in the future.

Ultimately, patients with 46,XY DSD present a large phenotype spectrum, and the etiological diagnosis sometimes cannot be determined through conventional techniques available in the clinical routine. The molecular tools have added up new possibilities in the investigation of these patients [45]. The majority of DSD patients present atypical genitalia and their sex assignment may be a complex procedure. The choice of male sex of rearing in 46,XY babies with ambiguous genitalia is a challenge situation. The participation of a multidisciplinary team is essential in this process and the fast identification of a molecular defect causative of the disorder might collaborate in this decision [43].

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Utility and Limitations in Measuring Testosterone

4

Mathis Grossmann

Introduction

In men, testosterone, the principal circulating androgen, has essential reproductive functions in establishing and maintaining the male phenotype. It also plays important anabolic roles in somatic tissues, such as on muscle, fat mass, and bone.

Organic hypogonadism due to structural hypothalamic-pituitary testicular (HPT) axis pathology is an important diagnosis not to be missed. It is an important differential to consider not only in the man presenting with low libido or infertility but also with non-reproductive features, for example, otherwise unexplained weakness, anemia or osteoporosis. Hypogonadism is primarily a clinical diagnosis. Men who present with features suggestive of androgen deficiency should have a thorough history and physical examination to determine the degree of clinically significant androgen deficiency. Verification of the clinical impression by confirming low circulating testosterone concentrations is an essential component of the diagnosis. Accordingly, the Endocrine Society recommends making a diagnosis of androgen deficiency only in men with consistent symptoms and signs as well as unequivocally and repeatedly low serum testosterone concentrations [1]. While the diagnosis, once considered, is relatively straightforward in young otherwise healthy men, it is more difficult in older, obese men with comorbidities. Even low libido, the most specific sexual symptoms can be caused by many other conditions such as vascular disease or depression, and the physical exam can be nonspecific. In the European Male Ageing Study (EMAS), the prevalence of sexual symptoms ranged from 27.5% to 39.9% in community dwelling men, yet only 2.1% met the definition of late onset hypogonadism, i.e., the syndromic combination of symptoms with low testosterone [2]. Given this nonspecificity of clinical features in the absence of a

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biological gold standard of male hypogonadism akin to cessation of menses in females, accurate biochemical confirmation is important. This can be fraught with difficulties and pitfalls, discussed in this chapter. This chapter will focus on testosterone measurements in adult men, and not discuss women and children. While some of the biologic actions of testosterone are, at least in part, mediated via aromatization to its downstream product estradiol, measurement of estradiol as part of the routine biochemical work-up for male hypogonadism is currently not recommended.

Measurements of estradiol and of other reproductive steroids (such as dihydrotestosterone, and adrenal androgens) and further work-up of low testosterone is beyond the scope of this contribution.

Serum Testosterone As a Measure of Androgen Status

In adult men, the most common reason for measuring circulating testosterone is to confirm the clinic suspicion of androgen deficiency based on history and physical examination. Other indications may include monitoring the adequacy of testosterone therapy, or of androgen deprivation therapy in men with prostate cancer. In general, there is no indication for testosterone measurement in men without clinical evidence of androgen deficiency. However, a measurement in the absence of symptoms may be justified in specific situations, for example, in the work-up for secondary osteoporosis or unexplained anemia. While there is debate regarding routine measurement of testosterone in men with diabetes or the metabolic syndrome, there is no currently no proven glycemic or symptomatic benefit with testosterone treatment in men with established diabetes [3]. However, T4DM a study in 1007 men at high risk of diabetes has recently reported that testosterone treatment over 2 years reduces the prevalence of type 2 diabetes by 40% compared to placebo, above and beyond the effects of a lifestyle program alone [4]. Further evidence to support routine screening this population is required.

Testosterone is the main circulating androgen in men and its measurement is considered a surrogate of tissue androgenization. However, this is an oversimplication because firstly, the concentration of circulating testosterone does not necessarily reflect local hormone concentration and biological effects in target tissues, which also depend on uptake into and clearance from target tissues, interactions with receptors and their coactivators. Androgenic action may also be modulated by polymorphisms of the androgen receptor, sex hormone binding globulin (SHBG) or steroid metabolizing enzymes, although their clinical significance remains contentious. Secondly, testosterone is both a hormone and a prohormone. Testosterone is converted to dihydrotestosterone, a more potent androgen receptor agonist allowing local amplification of androgen actions, for example, in the prostate gland and in the skin. Testosterone is also aromatized to estradiol, and there is increasing evidence that biological actions traditionally attributed to testosterone may in fact be mediated by estradiol, such as regulation of bone mass, fat distribution, and insulin

resistance [5]. Thirdly, a single point in time measurement may not be representative because of biological variability in testosterone concentrations, and because of technical assay limitations, both further discussed below.

Biological Variability of Circulating Testosterone Concentrations

Due to the pulsatility of hypothalamic gonadotropin releasing hormone (GnRH) secretion, testosterone is secreted in a pulsatile fashion, but in part due to buffering effects of its carrier proteins SHBG and albumin, pulse frequency is rapid and amplitude relatively low, with moment to moment fluctuations of less than 10–15% [6]. There is circadian rhythmicity, and in men 30–40 year old, testosterone concentrations are 20–25% lower at 1600 h than at 0800 h [7]. In fact, up to 15% of healthy men can have abnormally low concentrations within a 24 h period [8]. While some data suggest blunting of circadian rhythmicity with age [7], a signification proportion of men older than 65 years with low afternoon testosterone will have normal concentrations in the morning [8]. Testosterone concentrations should therefore be measured before 10 am in the morning irrespective of age. This also allows drawing a sample in the fasted state. While current guidelines do not specify fasting [1], there is recent evidence that food intake can reduce testosterone concentrations abruptly. In one study of 66 healthy men, glucose ingestion was associated with a 25% decrease in mean T concentrations (delta = −4.2 ± 0.3 nmol/L, $p < 0.0001$), reducing testosterone concentrations to below the reference range in 15% of study subjects [9]. Consistent with these findings, in an observational study of repeated testosterone measurements, overnight fasting increased testosterone concentrations by 9% ($p < 0.001$). This fasting effect was less pronounced but still significant ($p < 0.05$) in men with a higher body mass index [10]. There is also significant day-to-day variability in testosterone concentrations. In a longitudinal study of community dwelling men, the intra-individual variability of testosterone was up to 28% when two measurements were made on a subject [11]. In men with [12] or without [13] diabetes, more than 30% of individuals with low testosterone will have normal concentrations when retested a few months later.

This biological variability, further compounded by testosterone assay shortfalls (see below) contributes to clinical observations that, in general there are no consistent circuiting testosterone threshold concentrations below which hypogonadal symptoms and signs appear, neither with respect to individual tissues nor between different individuals. For example, testosterone concentrations below which increases in fat mass have been reported to range from 6.1 to 13.9 nmol/L in different studies [5, 14]. Similarly, in one study of healthy older men receiving GnRH analogs to suppress endogenous testosterone production given graded testosterone add-back, self-reported sexual desire and erectile function decreased only at testosterone concentrations of 3.5–6.9 nmol/L [15], yet in aforementioned T4DM, older men randomized to testosterone treatment had sexual benefits despite a baseline

serum testosterone of 13.4 nmol/L [4]. However, in a cohort of hypogonadal men receiving testosterone replacement implants, despite a wide range in thresholds for androgen deficiency symptoms between individuals, testosterone threshold concentrations at the time of return of androgen deficiency symptoms were highly reproducible within individuals [16]. So overall the serum testosterone threshold below which hypogonadal features manifest varies across individuals but may be relatively constant for individual men.

Effect of Illness on Testosterone Concentrations

Any acute illness or chronic illness, medications (e.g., glucocorticoids or opioids), obesity or malnutrition, and excessive exercise can decrease testosterone concentrations [17, 18]. Obesity decreases testosterone by 30%, and in the presence of at two or more comorbidities, the prevalence of late onset hypogonadism is increased by tenfold [2]. Conversely, weight loss and optimization comorbidities may lead to HPT axis recovery [19]. Conditions such as type 2 diabetes mellitus, depression, obstructive sleep apnea, chronic kidney disease or anorexia nervosa are associated with decreases in testosterone concentrations of between 2 and 10 nmol/L depending on their severity [20]. Any illness can cause nonspecific symptoms resembling those seen with true androgen deficiency. There is good evidence that the age-related accumulation of chronic disease and especially obesity can accelerate the age-related decline in testosterone concentrations [21]. Healthy ageing by itself may not inevitably be associated with marked decreases in testosterone concentrations [10]. In general, a repeatedly low testosterone concentration is more indicative of hypogonadism the younger, healthier, and leaner the man is, but much less predictive in older obese men with chronic disease and nonspecific symptoms.

Serum Testosterone: What To Measure?

Because of the variability in testosterone concentrations, Endocrine Society guidelines recommend making a biochemical diagnosis of androgen deficiency only based on repeatedly low testosterone concentrations [1]. Chronic comorbidities and nutritional status should be optimized, and offending medications ceased. If this is not possible, it should be recognized that these conditions be associated with a decrease in testosterone concentrations. Testosterone concentrations should be drawn in the morning (before 10 am), in the fasted state, in a medically stable patient without current or recent acute illness. Low testosterone should be confirmed at least once, while a clearly normal concentration (see below) does generally not need to be confirmed.

Total Testosterone

Total testosterone is the mainstay of biochemical diagnosis of androgen deficiency and is recommended as the initial diagnostic test [1]. Indeed, in an international survey among 943 mostly adult endocrinologists, more than 90% of participants requested a total testosterone, drawn in the morning as the initial diagnostic for work-up of suspected androgen deficiency [22]. In practice, a normal fasting early morning total testosterone concentration (somewhat arbitrarily defined as ≥12 nmol/L) is generally consistent with eugonadism, and usually does not need to be repeated. If the total testosterone is ≥12 nmol/L, non-specific symptoms will generally not be due to androgen deficiency, unless SHBG is markedly elevated (e.g., in men with liver cirrhosis or with certain anticonvulsant medications), or if there is androgen resistance, a very rare condition.

A low total testosterone, however, needs confirmation because a falsely low concentration due to, for example, unrecognized intercurrent illness or assay imprecision at the lower range especially if measured with immunoassay (see below) is more likely than a falsely normal concentration. Therefore, a diagnosis of androgen deficiency should never be based on a single low testosterone concentration. Up to 35% of men with a low testosterone will have a normal testosterone on repeat testing [12, 13]. Even among endocrinologists, however, re-testing is unfortunately not universal practice; in the afore mentioned international survey, 25% of respondents did not confirm a low testosterone but offered testosterone treatment based on a single low concentration [22], contrary to guideline recommendations [1].

Free Testosterone

Circulating testosterone is largely plasma protein bound, 60% tightly to sex hormone binding globulin, and 38% loosely to albumin, while 0.5–2% circulates as free testosterone. The combination of free and albumin-bound testosterone is referred to as bioavailable testosterone. While the free hormone hypothesis is debated [23], quantification of free (or of bioavailable) testosterone may be helpful when total testosterone is borderline and/or the clinical picture does not agree with the total testosterone measurement. SHBG abnormalities are the most common reason. For example, free testosterone can be useful to exclude hypogonadism in men where low total testosterone is due to low SHBG because of insulin resistance in obesity or diabetes [24]. In this context, a normal free testosterone can be reassuring that nonspecific symptoms are not due to androgen deficiency. However, the age-related decline of free testosterone is steeper than that of total testosterone, because of the age-associated increase in SHBG. A low free testosterone should be used with caution to confirm hypogonadism in older men, as the risk of overdiagnosis is substantial given assay reference ranges are usually based on young men.

An extreme case of a "falsely low" total testosterone was described in a man who presented with a very low total testosterone but essentially normal masculinization. SHBG concentrations were undetectable, due to a missense mutation in the SHBG gene, and dialyzable free testosterone was in the reference range [25].

Rarely, men can be androgen deficient despite a normal total testosterone. This usually occurs if SHBG is markedly elevated most commonly in the setting of antiepileptic treatment or chronic liver disease, but these men usually have elevated gonadotropin concentrations and a clearly low free testosterone.

Testosterone Measurement: Which Method?

The Endocrine Society Council has established a "Sex Steroid Assays Reporting Task Force" to address necessary performance criteria that should be met for any testosterone measuring method used for clinical and research studies. The task force emphasized requirements for minimal analytical validity including standards of accuracy, precision, sensitivity, specificity, reproducibility and stability [26]. In order to be acceptable for clinical and research use, assays should be validated and of high quality so that they provide accurate results (results representing the true value as determined by a gold standard or reference method) and meet the performance criteria required for their intended use. For adult men, most testosterone assays have reasonable clinical utility but are relatively inaccurate [27]. While assay quality, validation and suitability for the clinical need or research in question are more important than assay technology, mass-spectrometry-based assays are increasingly replacing automated immunoassays [28]. Despite improving technologies for testosterone measurements, measurement variability within and across laboratories is still an issue [29]. The Centre or Diseases Control (CDC) has initiated a Hormone Standardization (HoSt) Program to improve the accuracy and precision of sex steroid assays, providing measurement traceability to CDC reference methods, and to assist laboratories to improve analytical assay performance (http://www.cdc.gov/labstandards/hs.html) [29]. Traceability of assays to a "gold standard" allows comparability of results across different methods and laboratories and should facilitate the establishment of reliable, age-dependent reference ranges for circulating testosterone.

Immunoassays

Advantages of immunoassays include their relative technical simplicity, speed, and low cost, and they are currently in routine use in many clinical laboratories. Given they are usually direct, automated platform-based assays and do not employ an extraction step, they can be prone to cross-reactivity and analytical interference. For example, DHEA, present in male serum in micromolar concentrations can interfere with immunoassay testosterone measurements especially at testosterone concentrations of <10 nmol/L [30]. Testosterone immunoassay reference ranges are generally

not well validated, and assays are not standardized, so that results and reference ranges are method dependent and cannot be compared across different platforms. Validation of performance and standardization of commercial assays is manufacturer-dependent, given reagents are proprietary, but is improving, perhaps driven by progress and increasing availability of mass spectrometry-based assays [31]. Immunoassay sensitivity is limited, with deteriorating accuracy and positive bias at total testosterone concentrations of less than 10.4 nmol/L [27]. In one study comparing immunoassays with a mass spectrometry-based method, at testosterone concentrations below 8 nmol/L, methods disagreed by up to fivefold, and immunoassays generally overestimated testosterone concentrations [32].

There is no question that current immunoassays, due to the lack of precision and accuracy leading to bias especially at the lower reference range are not suitable to accurately quantify low testosterone concentrations in women, children, or men with prostate cancer receiving androgen deprivation therapy. In one study, measuring testosterone by different immunoassays, over 60% of the samples (with testosterone concentrations within the adult male range) were within ±20% of those reported by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The authors concluded that immunoassays are capable of distinguishing eugonadal from hypogonadal men if adult male reference ranges have been established in each individual laboratory [33]. This is critical given that in another study of 124 reproductively healthy young men, lower reference intervals provided by eight different immunoassays ranged from 7.5 to 12.7 nmol/L, with deviations by as much as 30% from the LC-MS/MS lower limit of 9.8 nmol/L [34]. Unfortunately, clinical laboratories commonly use manufacturer-supplied reference ranges rather than intervals validated in a local reproductively healthy population. A large study of more than 3000 men reported a high correlation ($R = 0.93$, $p < 0.001$) between testosterone measured by a rigorously validated, CDC-traceable immunoassay and mass spectrometry [35]. However, the correlation at testosterone concentrations of less than 11 nmol/L was lower ($R = 0.72$, $p < 0.001$), and when mass spectrometry was used as the comparator method, sensitivity and specificity of the immunoassay to ascertain total testosterone of less than 11 nmol/L was 75% and 96%, respectively [35].

Mass Spectrometry Assays

Due to logistic advantages, LC-MS/MS assays have largely superseded gas chromatography-mass spectrometry assays and are increasingly available, especially in research laboratories. Compared to immunoassays, they offer, provided they are carefully validated using stringent performance criteria, less interference, improved specificity and dynamic range, lower intra- and interassay variability, and the ability to multiplex (a panel of sex steroids can be measured in a single run) [28]. They offer improved analytical sensitivity and are the method of choice for accurately quantifying testosterone circulating at low concentrations. While sample throughput, costs and technical demands are improving, issues limiting widespread availability of LC-MS/MS assays include the need for relatively expensive

equipment and maintenance, and the requirement for adequately trained staff. Just like immunoassays, mass spectrometry assays need to be validated to yield accurate and reproducible data, including calibration and regular quality control [29]. While an earlier publication reported interassay coefficients of variations for different mass spectrometry assays of up to 14% at total testosterone concentrations of less than 10.4 nmol/L even in reference laboratories [36], implementation of the CDC HoSt program has, from 2007 to 2011, led to a 50% decline in mean bias between different mass spectrometry assays [29].

Free Testosterone Methods

Laboratory equilibrium dialysis (ED) is the gold standard for free testosterone measurements, but not widely available due to assay complexity and cost [27]. Importantly, the free androgen index is inaccurate in men and should not be used, and free analogue displacement using direct free testosterone (analogue) assay is analytically invalid and should not be used [27]. In practice, free testosterone is usually calculated by empiric formulae. The original equations suggested good agreement with ED but were evaluated in single laboratories using relatively small number of human samples [37, 38]. A relatively large-scale evaluation in more than 2000 serum samples found that the accuracies of five different formulae (two based on equilibrium binding, three empirical) commonly used to calculate free testosterone were suboptimal, and tended to overestimate free testosterone relative to the ED measurement [39]. There is currently no universally accepted formula that accurately reflects the interaction between plasma protein bound and free testosterone. In addition, these formulae are critically dependent (80% variance) on the accuracy of the total testosterone and SHBG assays and may augment errors in their measurement.

Bioavailable testosterone generally yields information comparable to that of free testosterone. It is measured by ammonium sulfate precipitation. While technically relatively simple, the technique can be inaccurate, is not easily automated and not widely available [27].

Testosterone bioassays offer the intuitive advantage of measuring the total androgenic activity in a serum sample rather than quantitating sex steroids immunologically. Most are artificial cell-based recombinant assays expressing a transgene linking an androgen receptor response element that controls reporter gene expression [40]. They remain experimental.

Quantitative biomarkers reflecting tissue-specific androgen sufficiency useful in the confirmation of hypogonadism remain elusive. Hematocrit and prostate specific antigen have been proposed but remain nonspecific with high interindividual variation [41].

While guidelines [1] stress the importance for practitioners to be familiar with locally available assays, this is in reality not the case, even among endocrinologists. When 943 endocrinologists were asked how they measure testosterone, 55% of those measuring total and 47% of those measuring free testosterone indicated they

would use "whatever my laboratory uses" [22]. In 2014, respondents from Northern America reported the highest access to mass spectrometry (19.1%) and equilibrium dialysis (12.6%) [22].

Testosterone Concentration: What Is Low?

In contrast to, for example, bone density, where age-dependent reference ranges are quite well defined, there is no general agreement on the acceptable normal range of testosterone, especially in older men. This is because there have been relatively few large population-based studies of healthy older men. A panel of US experts was divided on the total testosterone concentration below which to consider testosterone treatment, with opinions ranging from 6.9 to 10.4 nmol/L [1, 42]. Joint recommendations from the International Society of Andrology and associated societies consider total testosterone concentrations between 8.0 and 12.1 nmol/L to represent a gray zone, whereas concentrations above 12.1 nmol/L are considered normal and <8.0 nmol/L low [43]

Bhasin et al. using a CDC-certified LC-MS/MS assay reported a 2.5th percentile for total testosterone of 12.1 nmol/L for healthy young men [44]. Population based studies in healthy Australian men reported lower limits for total testosterone of 9.8 nmol/L for healthy young men [34], and of 6.4 nmol/L for older men reporting excellent health [45], measured by LC-MS/MS assay.

The US Federal Drug administration uses a testosterone threshold of 10.4 nmol/L the value used by to define hypogonadism for clinical trial purposes, without reference to age. This is the threshold concentration most commonly (in 43%) chosen by Endocrinologists to offer treatment to older man presenting with symptoms compatible with androgen deficiency [22]. This stresses the importance of providing robust reference ranges for older men, to avoid overtreatment. The T-trials, a 12-month trial comparing testosterone treatment with placebo, enrolled men with clinical features of androgen deficiency and a serum testosterone of <9.54 nmol/L [46]. In this trial, testosterone improved sexual function, anemia, and bone strength, although an increase in coronary artery plaque volume was noted [46, 47].

Summary

Testosterone measurements play an important role in the confirmation of androgen deficiency because the clinical picture can be nonspecific. Clinical utility can be improved by relatively simple strategies to minimize biological variability and taking into account comorbidities affecting gonadal axis function. Rigorous internal and external quality control and proficiency testing can improve analytical shortcomings of testosterone assays. Assay validity and optimization to its clinical purpose is more important than assay technology. Assay standardization is important to facilitate the generation of robust age-dependent reference ranges, which will in turn inform clinical practise and contribute to a better characterization of the risks

and benefits of testosterone therapy in men without pathological hypogonadism. While mass spectrometry assays offer improvements over immunoassays and are increasingly available, whether and how quickly immunoassays become obsolete remains unknown.

Moreover, more research is needed to better define the clinical utility of free testosterone, and to determine the best way to measure it. Whether measurement of estradiol or of dihydrotestosterone is of value either in the diagnosis of hypogonadism, or in monitoring response to testosterone treatment [48] likewise requires further study.

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Male Puberty: What Is Normal and Abnormal?

5

David W. Hansen and John S. Fuqua

Introduction

In the adolescent years, young men may be anxious about whether they are developing normally compared to their peers. These questions are often relayed to their trusted primary care physician. When the physician is comfortable with such an assessment, he or she can better put a family at ease by providing reassurance or appropriately referring the patient to a pediatric endocrinologist.

Although the development of pubic hair in boys is often viewed as the beginning of puberty, in actuality, the first physical manifestation of centrally-mediated puberty is testicular enlargement. This phase of puberty is often overlooked, but it is crucial to evaluate this finding in any patient with pubertal concerns. Testicular size is best measured with an orchidometer [1], which is a series of ellipsoid beads ranging in volume from 1 to 25 mL. The beads are compared to the testis in order to assess testicular volume. More obvious than testicular enlargement is pubarche, which refers to the development of pubic hair. Pubarche is typically preceded by adrenarche, which refers to the physiologic increase in adrenal androgen production. Adrenarche is a biochemical phenomenon, and this term is sometimes incorrectly interchanged with pubarche. Adrenarche occurs separately from hypothalamic-pituitary-gonadal (HPG) axis maturation, the first clinical manifestation of which is gonadarche, or testicular enlargement. Both low-potency adrenal androgens and testosterone from testicular Leydig cells play an important role for

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pubarche in boys. The most commonly used measure for pubarche is pubic hair Tanner staging. Although developed decades ago [2], this method continues to be used throughout the world as the standard system for measuring pubic hair development. There are five Tanner stages. Stage 1 is prepubertal and is defined by the lack of pubic hair. Tanner stage 2 consists of fine, lightly pigmented hair, usually at the base of the penis. Tanner Stages 3–5 indicate further progression through puberty, as indicated in Fig. 5.1. Tanner Stage 5 is considered to be fully developed adult pubic hair, with extension to the medial thighs and inferior abdomen.

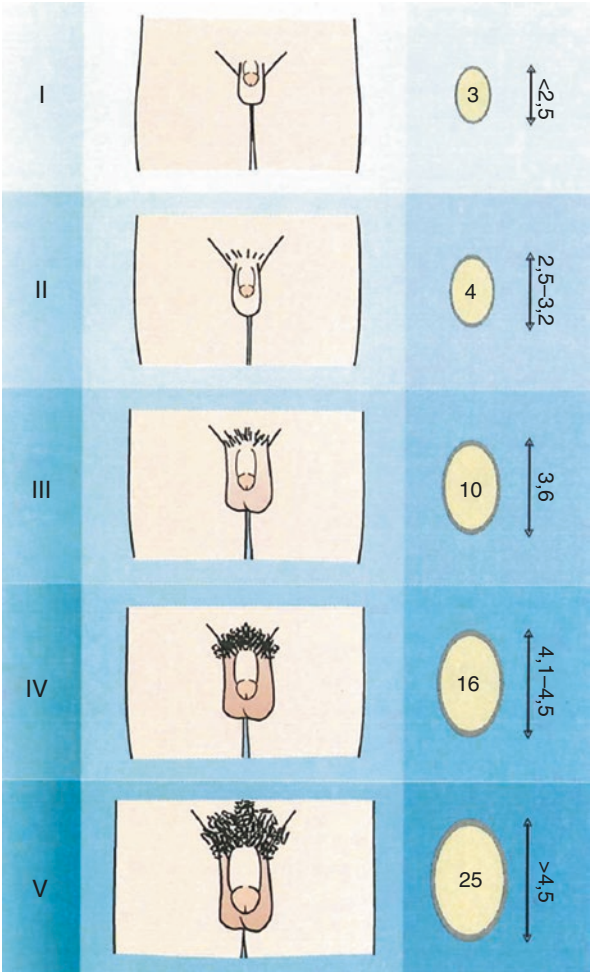


Fig. 5.1 Tanner staging of pubic hair and genitalia in boys, with representative testicular volumes (mL) and long axis lengths (cm) at each stage. (Figure courtesy of Michal Komorniczak)

A basic understanding of endocrine function during puberty will help physicians better understand and explain the physical changes of puberty to their patients. Control of pubertal timing remains poorly understood. There is a strong genetic component to this control, and it is thought that 50–80% of the variance of pubertal timing is based on genetic factors [3]. There appear to be multiple layers of genetic control, with many of the higher-level networks being largely inhibitory and influenced by epigenetic mechanisms [4]. An example of an inhibitory input is makorin ring finger protein 3, which is a hypothalamic factor that maintains prepubertal suppression of gonadotropin releasing hormone (GnRH) secretion [5]. Epigenetic mechanisms influencing the onset of puberty include DNA methylation, histone modification, and microRNA effects, all of which have been demonstrated in rodents and non-human primates [6]. The multiple layers of regulation alter the pulsatile release of GnRH by the hypothalamus, beginning about 1–2 years before any physical signs of puberty. Pulsatile GnRH secretion is controlled by a group of neurons in the arcuate nucleus of the hypothalamus. These neurons secrete a neuropeptide called kisspeptin as well as the stimulatory neurotransmitter neurokinin B and the inhibitory neurotransmitter dynorphin. This combination of secretory products led to the moniker KNDy neurons [7]. Acting in an autocrine fashion, neurokinin B and dynorphin stimulate and inhibit kisspeptin release in a cyclic pattern [8], with a frequency of about 1/h in adult humans. The developmental physiology of this process is not well understood. Early in puberty, pulsatile kisspeptin secretion from the hypothalamus increases, resulting in activation of GPR54, the kisspeptin receptor [9]. Pulsatile activation of the kisspeptin receptor alters the release of GnRH, leading to increased pulse amplitude and frequency of GnRH release and increased gonadotropin production. At the beginning of puberty, anterior pituitary gonadotroph release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in response to GnRH pulses is minimal. However, as the GnRH pulses become stronger and more regular, LH and FSH responses increase [10]. Circulating LH binds to its receptor in the testicular Leydig cells, and FSH binds to its receptor in the testicular Sertoli cells. The Leydig cells produce testosterone in response to LH stimulation. Testosterone has many effects, including increased muscle mass, deepening of the voice, Sertoli cell maturation, and spermatogenesis. In response to FSH, Sertoli cell numbers increase. This, along with the increase in size of these cells, is responsible for testicular enlargement (gonadarche). In addition, the increase in serum testosterone concentrations result in lower sex hormone binding globulin (SHBG) concentrations, leading to more bioavailable free testosterone in the circulation [11]. The increased free testosterone permits more testosterone to be converted to dihydrotestosterone (DHT), which is more potent due to its slower dissociation from the androgen receptor [12]. This is important for continued development of secondary sexual characteristics, especially sexual hair growth.

With the processes that precede the normal start of gonadarche and adrenarche described, this chapter will now focus on when these processes vary from the expected timeline.

Precocious Puberty

Definition

The onset of puberty in boys is defined clinically as a testicular volume ≥4 mL. Traditionally, this stage of testicular enlargement has been thought to occur on average between 11 and 12 years of age. Precocious puberty in boys is classically defined as testicular volume ≥4 mL occurring before 9 years of age. This timing is based on studies performed between 1950 and 1970 in racially and ethnically uniform populations in the USA and in a group of boys from lower socioeconomic strata living in a children's home in England. These studies found a mean age at the start of genital enlargement of 11.64 years and a lower limit of 9.5 years [2]. These studies also defined an upper limit of normal of about 14 years, after which puberty is considered to be delayed.

More recently, newer and more exacting studies demonstrate that the age of onset of puberty may be decreasing, perhaps by a few months and similar to the well-documented trend in girls. A European study of 142 Swiss boys conducted between 1954 and 1980 found that the mean age at attainment of Tanner stage 2 genital development was 11.2 years [13]. A longitudinal study sponsored by the U.S. National Institutes of Health (NIH) conducted in various centers in the U.S. from 2000 to 2006 assessed 427 boys between ages 9.5 and 15.5 years. The mean age at Tanner stage 2 genital development was 10.4 ± 0.6 years for White boys [14]. A study from the cross-sectional U.S. Pediatric Research in Office Settings (PROS) network conducted from 2005 to 2010 assessed the pubertal status in 4131 healthy boys of different races and ethnicities [1]. The mean age of attainment of Tanner stage 2 genital development in non-Hispanic White boys was 10.14 ± 2.18 years. The explanation for the apparent decline in the age of onset of puberty in boys is uncertain. However, comparison between studies is difficult, as some studies use global Tanner stage assessments and others use differing testicular volumes (≥3 mL vs. ≥4 mL) to define the onset of puberty. The increasing prevalence of obesity is clearly a contributing factor in the declining age of puberty in girls [15, 16]. In boys, however, the role of increased body weight is more controversial, with older reports associating obesity with both earlier and later onset of puberty [16–22]. Most recent studies indicate that overweight and obesity either accelerate or do not affect the timing of puberty in boys.

There is also significant ethnic variability in the timing of normal puberty. In the NIH study [14], the mean age at Tanner stage 2 genital development was 10.4 ± 0.6 years for White boys and 9.6 ± 0.8 years for Black boys. In the PROS study [1], the mean ages of attainment of testicular volume ≥4 mL were 11.46 years, 11.75 years, and 11.29 years for non-Hispanic White, African-American, and Hispanic boys, respectively. Other studies show that for boys in urban China the mean age for testicular volume ≥4 mL is 10.55 years, and for Danish boys, 11.6 years [23, 24]. In the USA, the mean age for white, African-American and Hispanic boys at attainment of Tanner stage 2 pubic hair is 11.47 years, 10.25 years,

and 11.43 years, respectively [1]. Thus, one must consider the racial and ethnic makeup of the population under study when interpreting the appropriateness of pubertal timing.

Although variation does exist, until more conclusive studies are conducted for a variety of ethnicities, the age range of 9.5–13.5 years for normal gonadarche is a good guide, and many clinicians continue to define abnormally early puberty in boys as onset occurring before 9 years of age [25–27].

Variations of Normal Puberty

Premature Adrenarche

Premature adrenarche is a common variation of normal in which production of adrenal androgens, predominantly DHEA-S, begins at an unusually early age. It is considered a benign condition in boys, without any long-term sequela. In contrast, girls with premature adrenarche appear to be at increased risk for insulin resistance and other features of the metabolic syndrome in adulthood [28] and may have a higher incidence of polycystic ovary syndrome, at least in some populations [29]. The cause of the early onset of adrenal androgen production is not well understood.

The prevalence of premature adrenarche varies depending on how it is defined. When defined as serum DHEA-S concentration ≥1 μmol/L (≥37 μg/dL) plus any clinical evidence of androgen action in girls less than 8 years and boys less than 9 years, the prevalence has been estimated to be 8.6% and 1.8%, respectively [30].

The typical first sign of premature adrenarche is the development of apocrine sweating with adult body odor, followed over a varying time frame by pubic and/or axillary hair growth, sometimes accompanied by mild facial acne. The time course for these findings is often quite long, sometimes spanning several years. Unlike boys with pathologic precocious puberty, boys with premature adrenarche typically do not have an increase in height velocity. Most children with premature adrenarche have body mass indexes above the average for age and sex. Most boys presenting with this condition have relatively small amounts of sexual hair growth, less than would be expected for the duration of symptoms. Importantly, testicular volume remains prepubertal, and there is no increase in penile size.

Radiologic and laboratory evaluation of boys with premature adrenarche typically reveal advancement of skeletal maturation that may be attributed to the increased adiposity in these patients and/or effects of the mild elevations in adrenal androgens. Bone age is usually advanced by approximately 2 years. DHEA-S concentrations are commonly increased into the range seen in boys in the early stages of normal puberty, although they may be within or minimally above the reference interval for age. Androstenedione levels may be similarly increased. Testosterone is usually normal or minimally elevated above the prepubertal norms. Despite the advancement in bone age, adult height is typically normal in boys with premature adrenarche, because as children they are usually taller than their genetic potential (target height) would suggest [31]. The physical signs of adrenarche typically

progress gradually, blending with the signs of true puberty as the child gets older. Long-term follow-up is usually not needed, although significant obesity or insulin resistance may require monitoring.

Early Normal Puberty

Early normal puberty in boys is the occurrence of testicular enlargement at an age earlier than average but after 9 years. In girls, a large body of evidence indicates that early normal puberty is associated with a variety of adverse psychosocial effects, including higher rates of adolescent depression, eating disorders, social anxiety, early sexual debut, and other high-risk behaviors. Data for boys are relatively scant but have indicated variable associations between age at pubertal onset and social anxiety, depression, alcohol and illegal drug use, violent behavior, and sexual activity before age 16 [32–34]. These results have been attributed to a mismatch between the emotional and cognitive status of the young pubertal child and to increased likelihood of associating with older peers, although those destined to enter puberty earlier than average may demonstrate more problems with behavior and psychosocial adjustment years before the onset of puberty [35].

A number of studies have associated early normal puberty in girls with adverse cardiometabolic outcomes, including adult obesity, hypertension, and dyslipidemia. Data for boys are more variable, at least in part stemming from the lack of an easily recalled milestone of puberty. Prentice and Viner reported a meta-analysis of studies in an attempt to relate the timing of puberty to these outcomes in men and women [36]. In boys, the difference between definitions of pubertal onset was sufficient to render meta-analysis impossible. The investigators did note that the majority of the analyzed studies reported an inverse association between the age of puberty and BMI in adulthood, although three of eight included studies did not identify such a relationship. Some studies identified an increased prevalence of hypertension in men with earlier puberty, although this also was not a consistent finding. Data were insufficient to exclude confounding by childhood obesity. A more recent analysis of data from the UK Biobank that included nearly 200,000 men demonstrated an association with earlier age at puberty and hypertension, angina, and type 2 diabetes after controlling for socioeconomic status and adiposity. However, this study was limited by the use of historical recall of voice break as a measure of pubertal onset [37]. Thus, it remains uncertain whether early normal puberty in boys leads to adverse cardiometabolic outcomes.

Precocious Puberty

Precocious puberty is the result of increased androgen action. The causes of sexual precocity may be separated by the regulatory mechanism of androgen secretion, dividing them into gonadotropin-dependent and gonadotropin-independent etiologies.

Gonadotropin-Dependent Precocious Puberty

Gonadotropin-dependent and GnRH-dependent precocious puberty are more frequently known as central precocious puberty (CPP), indicating that androgen secretion from the testes is under the influence of pituitary-derived LH and FSH, which are in turn driven by hypothalamic GnRH. Children with CPP present with typical changes of puberty, such as apocrine sweating, pubic and axillary hair growth, accelerated linear growth, facial acne, and genital enlargement. Dental development may be advanced for chronological age. Particularly in boys, it may be difficult to determine when these changes began, as they develop gradually and parents (or the patient) may not be aware of genital changes. Assessment of testicular growth is particularly important in the evaluation. Boys with CPP have testicular sizes commensurate with the degree of pubertal maturation, indicating normal gonadotropin secretion. If the testicular size is small for the stage of genital development or if testicular growth is asymmetric, the clinician should suspect a non-gonadotropin mediated cause of precocity (see below).

Skeletal maturity as assessed by bone age is advanced in cases of precocious puberty, usually by 1–3 years. The more advanced the pubertal maturation, the more advanced is the bone age. Laboratory studies to assess suspected CPP may include measurement of serum LH, FSH, and testosterone concentrations. Testosterone levels are increased compared to the prepubertal norms and are also usually commensurate with the stage of maturation. Pubertal boys have a significant diurnal variation in testosterone secretion, which is highest overnight and in the early morning hours. Thus, an afternoon testosterone level may be low in the early stages of puberty and not an accurate indicator of overall circulating amounts, and it may be more helpful to obtain an early morning (8:00 AM) sample. There is a large overlap between prepubertal LH concentrations and those occurring in the early stages of puberty. Thus, LH levels measured by standard immunoassays may be reported as normal, even in cases of CPP. One approach to avoid this pitfall is to measure LH using an ultrasensitive assay, such as an electrochemiluminometric (ECL) assay. Such assays are available with puberty-related normal ranges, and this may be helpful in distinguishing the early stages of CPP from pubertal changes arising from other causes. However, the standard approach to biochemically confirming suspected cases of CPP is the GnRH stimulation test, in which gonadotropin concentrations are measured serially after intravenous administration of synthetic GnRH or a GnRH analog. Peak concentrations of LH >5 U/L or a ratio of peak LH/peak FSH >0.66 are commonly used cutoffs to diagnose CPP [38]. Once CPP is diagnosed, a search for anatomic abnormalities should include magnetic resonance imaging of the central nervous system (CNS).

CPP arises due to a variety of disorders of the central nervous system and genetic abnormalities, as summarized in Table 5.1.

Table 5.1 Causes of precocious puberty in boys

Gonadotropin-dependent	Gonadotropin-independent
Genetic disorders	Adrenal conditions
Activating mutation of kisspeptin and its receptor	Premature adrenarche
Loss of function mutation of *MKRN3*	Congenital adrenal hyperplasia
Loss of function mutation of DLK1	
CNS disorders	Virilizing adrenal tumor
Tumors	Glucocorticoid resistance
Hypothalamic astrocytoma	Testicular source of androgen
CNS germinoma	McCune-Albright syndrome
Optic pathway tumors in NF1	Familial male-limited precocious puberty
Others	Androgen-secreting testicular tumor
Hydrocephalus	hCG-secreting tumors
Post-trauma	Non-testicular germ cell tumor
Metabolic disease	Hepatoblastoma
Post-infectious	Exposure to exogenous androgen
Cerebral palsy	Primary hypothyroidism (testicular enlargement only)
Hypothalamic hamartoma	–
Tuberous sclerosis	–
Sturge-Weber syndrome	–
International adoption	–
Following prolonged androgen exposure	–
Idiopathic	–

Genetic Disorders Causing CPP

Kisspeptin and its receptor, GPR54, were identified as playing key roles in the regulation of puberty in 2003, when an inactivating mutation in the receptor was first reported in a patient with hypogonadism [39]. More recently, an activating mutation in the receptor gene (*KISS1R*) was reported in an 8-year-old Brazilian girl with slowly progressive breast development since birth, advanced skeletal maturation, and a GnRH-stimulated LH of 6.4 U/L. Genetic analysis revealed an Arg386Pro mutation in *KISS1R*, and functional studies showed prolonged activation of the mutant protein relative to wild-type [9]. The same investigators subsequently reported a boy with clinical evidence of CPP at age 12 months. At age 17 months, his testicular volume was increased, and his bone age was advanced. His basal LH concentration was elevated at 11.5 U/L, and his GnRH-stimulated LH was 47.2 U/L. Molecular analysis of his *KISS1* gene revealed a mutation leading to substitution of a conserved amino acid (Pro74Ser). Further studies showed that the mutated kisspeptin protein was resistant to normal degradation, resulting in increased GPR54 stimulation [40]. The same authors reported two unrelated girls

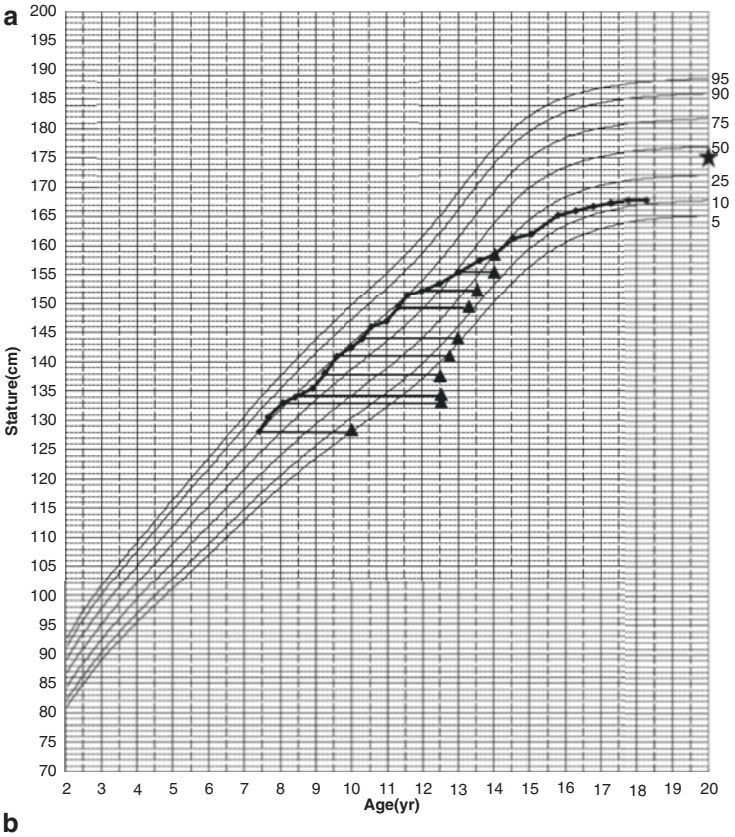
with CPP who had homozygous His90Asp mutations in the *KISS1* gene. However, functional studies suggested that this alteration can be considered a rare SNP. Other studies have found similar variants [41]. Hence, mutations in the kisspeptin system appear to be rare causes of CPP. The *KISS1* and *KISS1R* genes have been studied in several additional cohorts around the world, but no other pathogenic mutations have been identified to date [42–45].

In a search for additional genes that play roles in pubertal regulation, whole exome sequencing was employed to analyze 32 individuals with precocious puberty from 15 families [46]. The investigators identified three frameshift mutations and one missense mutation in the *MKRN3* gene, encoding the makorin RING finger protein 3. *MKRN3* is a maternally imprinted gene located on chromosome 15q11.2 in the Prader-Willi syndrome critical region. The makorin family of proteins is thought to be involved in ubiquitin-ligase activity. The exact function of MKRN3 protein is unknown, but expression in the hypothalamus is high before puberty, and the decline in expression correlates with the onset of puberty, suggesting that the MRKN3 protein acts as an inhibitory input to suppress puberty [47]. A number of mutations in this gene have subsequently been reported, and it appears that *MKRN3* mutations are relatively common causes of familial cases of CPP [48]. In these cases, CPP is inherited in an autosomal dominant fashion but is transmitted only from the father, consistent with the gene's maternal imprinting [47].

Using whole exome sequencing, Dauber, et al. identified a complex heterozygous defect in the *DLK1* gene that affected the translational start codon in two sisters and two paternal half-sisters in a large multigenerational kindred [49]. DLK1 protein was undetectable in the serum of the affected family members. Similar to *MRKN3*, *DLK1* is also a maternally imprinted gene, consistent with this pattern of inheritance from the father only. Since then, *DLK1* mutations have been reported in six other individuals [50]. Interestingly, most of those identified with *DLK1* mutations have a metabolic phenotype of obesity with an increased occurrence of dyslipidemia, glucose abnormalities, and polycystic ovary syndrome. *DLK1* is located in a chromosomal region that has been implicated in Temple syndrome, which is also associated with similar metabolic changes as well as CPP. DKL1 protein is expressed in the hypothalamus in mice and has also been identified as an adipogenesis gatekeeper that normally inhibits preadipocyte differentiation [51, 52].

CNS Disorders Causing CPP

A wide variety of disorders of the central nervous system may cause CPP (Table 5.1, Fig. 5.2). Children with CPP due to one of these disorders may present with accompanying symptoms and signs, such as headache, hemianopsia, papilledema, or characteristic dermatologic findings. It is thought that the more generalized disorders


a
Stature(cm)
Age(yr)
95
90
75
50
25
10
5
b
1.5T GEMSow0
Ex: 3825
SAG T1 +C
C: MAGNEVIST
Se: 15/15
Im: 6/13
Sag: L3.7 (COI)
Mag: 3.5x
ET: 0
TR: 450.0
TE: 21.0
HEAD
3.0thk/0.1sp
Id:DCM / Lin:DCM / Id:ID
W:905 L:452

interfere with normal inhibitory inputs that prevent progression of puberty during the quiescent period between infancy and the age of normal puberty. An exception are hypothalamic hamartomas, which are benign congenital ectopic foci of GnRH-secreting neurons that function independently of the normal inhibitory inputs. Patients with CPP due to hypothalamic hamartomas usually present at a very young age. They may also have gelastic seizures, a rare type of epilepsy that mimics uncontrollable laughing.

Other Conditions Causing CPP

There is an increased incidence of CPP in children adopted from underprivileged areas of the world and brought to more wealthy countries [53, 54]. Although the great majority of reported cases occur in girls, boys may also be affected. The pathophysiology underlying CPP in this setting is incompletely understood, but it appears to be related to nutritional deprivation during early life and subsequent improved nutrition with growth acceleration after arrival in the adoptive country. The timing of puberty is these children is significantly earlier than that of non-adopted children in both the native and the adoptive countries [55].

Some children who have had significant long-term exposure to sex steroids from adrenal or gonadal disease (see below) may enter central puberty abnormally early, typically after treatment or withdrawal of the underlying cause. The reason for this is not well understood, but is thought be due to accelerated maturation of the hypothalamus induced by androgens or estrogens.

In the great majority of girls, the cause of CPP is not discovered, and it remains idiopathic in 95% of cases [56]. By contrast, idiopathic CPP occurs less often in boys, historically making up only about 50% of cases. Although some reports indicate this figure may be higher [57, 58], it may depend on the extent of the initial evaluation and length of follow-up. Hence, all boys with CPP should undergo careful physical, laboratory, and radiologic investigation, including magnetic resonance imaging of the CNS, as serious undetected pathology is often found.

Fig. 5.2 This patient presented at age 7.3 years with a 3-month history of pubic hair. He had no growth acceleration, axillary hair, apocrine body odor or acne. He had no history of headache or vision disturbances. He had pervasive developmental delay with autistic features. His height was on the 75th percentile, and his midparental height was at the 45th percentile. (Panel **a**) His physical exam was remarkable for Tanner stage 3 pubic hair and testicular volumes of 10 mL bilaterally. His bone age was advanced to 10 years. Baseline laboratory studies included a serum total testosterone concentration of 56 ng/dL, LH of 1.9 μU/mL, and FSH of 0.8 μU/mL. His 17-hydroxyprogesterone level was 262 ng/dL. An ultrasensitive LH concentration was elevated into the pubertal range (1.3 μU/mL, normal for prepubertal boys 0.20–0.3). An ACTH stimulation test was normal, excluding congenital adrenal hyperplasia. An MRI of the brain revealed a poorly-defined hypothalamic mass with increased T2 signal and contrast enhancement with mild hydrocephalus, consistent with a low-grade astrocytoma. (Panel **b**, arrow) Additional tests of pituitary function were normal. Treatment with depot leuprolide acetate was initiated. After advancing during the first 6 months of treatment, his bone age stabilized and his pubertal maturation did not progress. Treatment was continued until age 12.0. At age 15.5 he had tumor growth and received proton beam irradiation. He was later diagnosed with hypogonadotropic hypogonadism and central hypothyroidism. Triangles represent bone age; star indicates midparental height

Gonadotropin-Independent Precocious Puberty

Clinical and biochemical findings of gonadotropin-independent precocious puberty (peripheral precocious puberty, PPP) are generally similar to those of CPP, with a few notable exceptions. Testicular size remains small and symmetric in the absence of gonadotropin stimulation of Sertoli and Leydig cell growth. Thus, testicular asymmetry or testicular size that is not consistent with the degree of pubertal development should prompt the practitioner to consider causes of PPP. Laboratory studies will reveal suppressed or prepubertal LH and FSH concentrations at baseline and upon GnRH stimulation.

Adrenal Disorders Causing PPP

Premature adrenarche (see above) is by far the most common cause of early pubertal maturation in boys. Other causes of overproduction of androgens from the adrenal glands include virilizing forms of congenital adrenal hyperplasia (CAH), androgen-secreting adrenal tumors, and rarely the syndrome of familial glucocorticoid resistance.

CAH is most commonly caused by mutation of the *CYP21A2* gene leading to deficiency of the 21-hydroxylase enzyme. Severe loss of function mutations impair both glucocorticoid and mineralocorticoid synthesis and lead to adrenal insufficiency with hypoglycemia, salt wasting, volume loss, shock, and death in the neonatal period if untreated. Milder mutations may allow for adequate mineralocorticoid activity and sufficient cortisol production to avoid symptomatic glucocorticoid deficiency. Boys with mild CAH may not present until mid-childhood with symptoms and signs of precocious puberty. Testicular volume will be small, and laboratory studies will reveal elevated serum concentrations of 17α-hydroxyprogesterone. Many countries have universal newborn screening for 21-hydroxylase deficiency that will detect mild cases. Other causes of virilizing CAH, such as 11β-hydroxylase deficiency, are very rare.

Adrenocortical tumors are rare in children. They may present with virilization, features of Cushing syndrome, or both [59]. The tempo of virilization tends to be more rapid than in CPP, and testicular volume remains small. Tumors may be small and non-palpable. Large tumors (>5 cm diameter) are more likely to be malignant [60]. Treatment requires surgical removal.

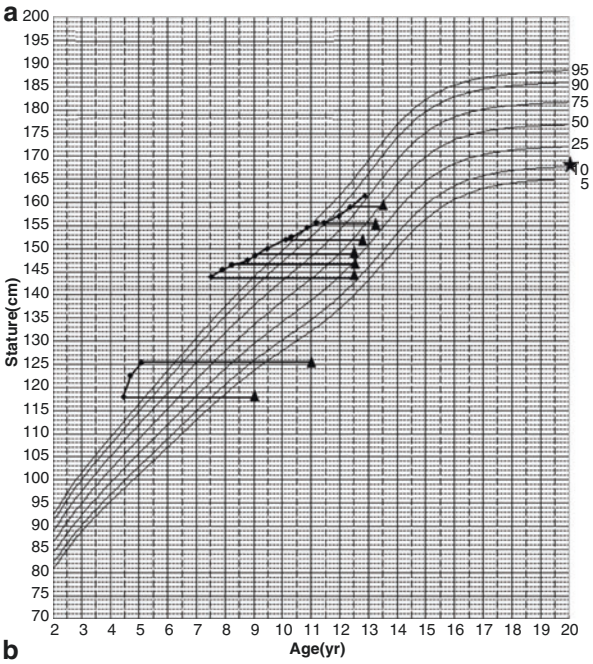
Familial glucocorticoid resistance is a rare condition caused by mutation of the glucocorticoid receptor. Affected individuals are able to overcome the resistance by increasing production of ACTH and cortisol. Due to the glucocorticoid resistance, Cushing syndrome is not present. However, hypersecretion of ACTH leads to overproduction of adrenal androgens and mineralocorticoid excess. Children with this condition may present with precocious puberty that mimics premature adrenarche, although they also may have hypertension and hypokalemic metabolic alkalosis [61].

Testicular Disorders Causing PPP

Familial male-limited precocious puberty (FMPP) was identified in 1993 as being caused by activating mutations in the LH receptor [62] (See Fig. 5.3). The condition is inherited in an autosomal dominant but sex-limited fashion. Thus, the condition is inherited in successive generations but is only manifested in boys. Boys with FMPP present with signs of sexual precocity, and there may be a small amount of testicular enlargement due to proliferation of Leydig cells. However, testicular size remains proportionally smaller than expected for the degree of pubertal maturation. Testosterone concentrations may be very elevated, but LH and FSH concentrations are suppressed.

McCune-Albright syndrome results from post-zygotic mutations in the *GNAS* gene encoding the alpha subunit of the Gs protein. These mutations prevent deactivation of the G protein-coupled seven-transmembrane-domain receptors, leading to a variety of manifestations depending on the affected cell type. Frequent findings include fibrous dysplasia of bone, café au lait macules of the skin, and precocious puberty, usually in girls. Approximately 10–15% of boys with McCune-Albright syndrome have signs of precocious puberty. Testicular size may be asymmetric but sometimes bilateral enlargement may be present. Affected boys may have ultrasonographically hyper- or hypoechoic testicular lesions as well as testicular microlithiasis [63].

Tumors secreting human chorionic gonadotropin (hCG) may result in gonadotropin-independent precocious puberty through stimulation of the LH receptor. These tumors include CNS and mediastinal germ cell tumors and hepatoblastomas. Patients with hCG-induced precocious puberty have mild testicular enlargement because Leydig cell growth is stimulated by hCG, but the testes are smaller than would be expected for the degree of precocity, because Sertoli cell growth is limited. Testicular Leydig cell tumors may secrete testosterone in an unregulated fashion and usually present with testicular asymmetry and suppressed gonadotropin levels.


a
Stature(cm)
200
195
190
185
180
175
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
95
90
75
50
25
10
5
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Age(yr)
b

Other Causes of GnRH-Independent PPP

There have been many case reports of PPP arising from long-term exposure to exogenous sex steroids, often following exposure to transdermal androgens used by a caretaker. Affected patients may have pubic hair, genital development, growth acceleration, and advancement of skeletal maturation. After the exposure stops, serum testosterone concentrations decline and signs of virilization may regress [64–66].

Some cases of severe primary hypothyroidism in children may be complicated by gonadotropin-independent precocious puberty, a condition known as Van Wyk-Grumbach syndrome. The pathophysiology is poorly understood, but precocious puberty may result from cross reactivity of TSH at the FSH receptor or stimulation of FSH secretion by hypothalamic thyrotropin releasing hormone. The pubertal changes appear to be related to increased FSH action, with breast development and vaginal bleeding in girls and testicular enlargement without pubic hair development in boys. In keeping with the coexisting severe hypothyroidism, the rate of linear growth is slow and skeletal maturation is delayed [67].

Treatment of Precocious Puberty

Treatment of CPP

The treatment of choice for children with CPP is GnRH analogs (GnRHa). Exposure of pituitary gonadotrophs to tonically high levels of GnRH activity overrides the normally pulsatile secretion of GnRH, halting gonadotropin production and decreasing Leydig and Sertoli cell activity, thus limiting testicular growth and testosterone secretion. Clinically, some features of puberty regress, such as facial acne, while other signs stabilize, such as pubic and axillary hair growth. Testicular size

Fig. 5.3 This 4.5-year-old boy presented for evaluation of pubic hair that began at 3.5 years of age as well as mild acne and apocrine body odor. He had aggressive behavior and was occasionally noted to masturbate. His father began puberty at age 6, and the paternal uncle also began puberty abnormally early. The child's height and weight *Z*-scores were +2.9 and +2.3, respectively (Panel **a**). He was Tanner stage 2 for pubic hair and his stretched phallic length was 13 cm. Testicular volume was between 2 and 3 mL bilaterally (Panel **b**). His serum total testosterone concentration was 131 ng/dL. LH and FSH concentrations were <0.2 μU/mL and 0.5 μU/mL, respectively. 17-hydroxyprogesterone was 105 ng/dL. His bone age was advanced to 9.0 years. He was diagnosed with familial male-limited precocious puberty. Treatment with bicalutamide and anastrozole were initiated. Adherence to the medication regimen was poor, and his height velocity remained above normal and bone age advanced rapidly. He was seen at another institution for 2.5 years, during which central precocious puberty was diagnosed and treatment with depot leuprolide acetate was initiated. Soon after re-establishing care at our institution, he was removed from his parents' care for neglect. He was maintained on bicalutamide, anastrozole, and leuprolide, and height velocity slowed and bone age did not progress. At age 11.7 years, bicalutamide and leuprolide were discontinued. Testicular volume had increased to 6 mL by age 12.9, when anastrozole was discontinued. He was subsequently lost to follow-up. Triangles represent bone age; star indicates midparental height

stabilizes and may decrease, although it typically remains greater than the prepubertal norm. Height velocity normalizes, and the rate of skeletal maturation decreases.

Multiple forms of GnRHa are available [68]. Commonly used extended release preparations include every one, three, or six monthly injections of leuprolide acetate, 6-monthly injections of triptorelin [69] and a subdermal implant containing histrelin. All preparations effectively suppress central puberty [70, 71]. Although approved by the United States Food and Drug Administration (FDA) for 1 year, the histrelin implant has shown continued effectiveness for as long as 2 years [72]. More recently, every 6-month injections of leuprolide acetate were also approved by the FDA to suppress CPP.

There are few long-term outcome data for boys with CPP, as the vast majority of clinical trial subjects are girls. Adult height is often reduced in untreated boys with CPP occurring before age 6–8, and treatment may increase adult height in this group [73]. Older boys with CPP may have a reduction in adult height regardless of treatment, although this may depend on the rate of pubertal progression [74]. Very little long-term data exist to assess psychological outcomes of CPP in boys.

Treatment of PPP

Because of the varied causes of PPP, treatment differs between the etiologies. Resection of hormone-secreting tumors and adequate treatment of congenital adrenal hyperplasia or hypothyroidism leads to reductions in the associated signs of puberty. Familial male-limited precocious puberty may be effectively treated with a combination of androgen receptor blockade and aromatase inhibition, reducing the effects of testosterone and preventing aromatization to estradiol and the resulting rapid skeletal maturation [75–77]. The treatment of boys with precocious puberty due to McCune-Albright syndrome follows a similar approach. Following treatment of longstanding PPP, boys are at risk for the development of CPP. This may be recognized by recurrent signs of androgen exposure and an increase in testicular volume.

Delayed Puberty

Delayed puberty in boys occurs when no signs of pubertal maturation have occurred by age 14 years. This definition can be refined to include the lack of testicular enlargement to ≥4 mL by 14 years in order to separate the effects of adrenal androgens, which may produce small amounts of pubic and axillary hair and apocrine body odor in the absence of true hypothalamic-pituitary-testicular axis activity. Causes of delayed puberty may be sorted into two categories: etiologies limiting the ability of the testes to secrete testosterone (primary hypogonadism) and etiologies limiting pituitary gonadotropin secretion (hypogonadotropic hypogonadism, central hypogonadism) (Table 5.2). Hypogonadotropic hypogonadism is discussed extensively in **Chap. 7** of this text and is only briefly reviewed here. In this chapter, we will focus on aspects unique to the adolescent male.

Table 5.2 Causes of delayed puberty in boys

Primary hypogonadism	Hypogonadotropic hypogonadism
Klinefelter syndrome	Constitutional delay of growth and puberty
XX sex reversal	Chronic illness—inflammatory bowel disease, sickle cell disease, cystic fibrosis
Defects in testosterone biosynthesis	Multiple congenital pituitary hormone deficiency
Vanishing testes	Isolated HH (iHH, many identified genetic defects)
Acquired	Kallmann syndrome
Trauma	Normosmic iHH
Torsion	Head trauma
Radiation	CNS tumor
Infection	Craniopharyngioma
–	Prolactinoma
–	Germinoma
–	Astrocytoma
–	Primary hypothyroidism
–	Prader-Willi syndrome
–	Laurence-Moon syndrome
–	Bardet-Biedl syndrome
–	Langerhans cell histiocytosis
–	Sarcoidosis

Primary Hypogonadism

One of the most common causes of primary hypogonadism is Klinefelter syndrome, occurring in 1:1000 males and caused by an abnormal sex chromosomal complement, most commonly 47,XXY. Klinefelter syndrome is often undetected in childhood, although it may present with delays in language development. Prepubertal children have a subtle alteration in body proportions, with a decreased upper:lower segment ratio that is accentuated at the time of puberty. The onset of puberty occurs at a normal age, but because testosterone secretion may be limited, the pace of progression may be slow or may cease before maturation is complete. In later adolescence, the serum testosterone concentration may decrease as testicular failure progresses. There may be some phenotypic overlap between individuals with Klinefelter syndrome and those with 46,XX sex reversal [78].

Conditions preventing the normal testicular synthesis of testosterone in utero may lead to abnormal embryological development of the genitalia. These conditions include a variety of enzymatic defects in androgen biosynthesis, such as feminizing forms of congenital adrenal hyperplasia, 17β-hydroxysteroid dehydrogenase deficiency, and 5α-reductase deficiency. In adolescence, these enzyme defects also prevent normal virilization and may lead to abnormal progression of puberty.

Vanishing testes refers to the loss of functioning testicular tissue that occurs late in gestation, after normal male differentiation and after normal penile growth has been attained. At birth, affected patients appear normal with the exception of bilaterally non-palpable testes. Anatomic investigation usually reveals hemosiderin-stained nubbins, suggesting a prenatal vascular insult with testicular infarction.

Although this is usually detected at birth or in childhood, the resulting anorchia may not become apparent until the age at which puberty is expected.

There is a large number of etiologies of acquired testicular dysfunction, including trauma and loss of testes from bilateral torsion. Testicular germ cell tumors or infiltration of the testes by leukemia may necessitate orchiectomy. Viral testicular infections leading to loss of endocrine function are rare. Exposure to ionizing radiation or alkylating agents during cancer treatment commonly causes loss of germ cells and infertility, but endocrine function may be preserved.

Hypogonadotropic Hypogonadism

Loss of function mutations in many genes regulating testicular function have been reported and are summarized in Table 5.3. Many of these mutations are associated with anosmia and are known as Kallmann syndrome, while mutations in other genes lead to normosmic isolated hypogonadotropic hypogonadism (iHH). Many of the genetic causes of Kallmann syndrome are related to the FGF8 system, including the *FGF8* gene itself, the gene encoding the FGF8 receptor, and a number of genes that encode accessory proteins and cofactors. Additional genes have been implicated in more global defects in pituitary cell development and are associated with multiple pituitary hormone deficiencies or syndromes such as septo-optic dysplasia or holoprosencephaly.

Hypogonadotropic hypogonadism is a feature of several eponymous syndromes, including Prader-Willi syndrome (PWS), Laurence-Moon syndrome, and Bardet-Biedl syndrome. Both males and females with PWS have central hypogonadism, but data suggest there is also frequently an element of primary hypogonadism [81]. The hypogonadism is quite variable, with some individuals demonstrating significant virilization and others with little or no spontaneous maturation.

Infiltrative diseases and tumors of the pituitary and hypothalamus commonly lead to disorders of pubertal maturation by damage or destruction of tissues. These conditions include craniopharyngioma, Langerhans cell histiocytosis, and sarcoidosis. Prolactinomas may lead to delayed puberty or lack of progression of puberty through inhibition of stimulatory input from the hypothalamus.

Although primary hypothyroidism may be associated with precocious puberty (see above), it more commonly results in delayed puberty, with associated slow linear growth and delayed skeletal maturation [67]. Additional signs and symptoms of hypothyroidism may be present, although these may not be as prominent as in adult patients with a similar degree of hypothyroidism.

Table 5.3 Genetic defects associated with hypogonadotropic hypogonadism

Gene		Condition/phenotype	OMIM number
Genes associated with anosmic/hyposmic iHH (Kallmann syndrome)			
FGF8 system genes	*FGF8*	KS, cleft lip/palate, ear abnormalities, dental agenesis	600483
	FGFR1	KS or nIHH, cleft lip and palate, facial dysmorphism	136350
	ANOS1 (*KAL1*)	KS, renal agenesis, synkinesia	300836
	HS6ST1	KS or nIHH	604846
	FGF17	KS or nIHH	603725
	IL17RD	KS or nIHH	606807
	DUSP6	KS or nIHH	602748
	SPRY4	KS or nIHH	607984
	KLB	KS or nIHH	611135
	FLRT3	KS or nIHH	604808
Other genes associated with Kallmann syndrome	*NELF*	KS or nIHH	608137
	PROK2	KS or nIHH, severe sleep disorder, obesity	607002
	PROKR2	KS or nIHH	607123
	CHD7	KS or nIHH, CHARGE syndrome	608892
	WDR11	KS or nIHH	606417
	SOX10	KS, Waardenburg syndrome	602229
	WDR11	KS or nIHH	606417
	SEMA3A	KS or nIHH	603961
	SEMA3E	KS or nIHH	608166
	SEMA7A	KS or nIHH	607961
	CCDC141	KS or nIHH	616031
	FEZF1	KS	613301
	IGSF10	KS or nIHH	617351
	AXL	KS or nIHH	109135
	SMCHD1	KS	614982
Genes associated with normosmic HH only			
KISS-1		nIHH	603286
KISS1R		nIHH	604161
GNRH1		nIHH	152760
GNRHR		nIHH	138850
TAC3		nIHH	162330
TACR3		nIHH	162332
LEP		nIHH and obesity	164160
LEPR		nIHH and obesity	601007
NR0B1 (*DAX1*)		nIHH and AHC	300473
PCSK1		nIHH and obesity, ACTH deficiency, hypoglycemia, gastrointestinal symptoms	162150
LHβ		Isolated LH deficiency, delayed puberty	152780
FSHβ		Isolated FSH deficiency, primary amenorrhea, defective spermatogenesis	136530
Combined pituitary hormone deficiency			
PROP1		GH, TSH, LH, FSH, prolactin, and evolving ACTH deficiencies	601538

(continued)

Table 5.3 (continued)

Gene	Condition/phenotype	OMIM number
LHX3	Variable CPHD including HH, limited neck rotation	600577
LHX4	Variable CPHD	602146
Specific syndromes		
HESX1	SOD and other pituitary deficits including HH	601802
SOX3	Pituitary hormone deficits including HH, mental retardation	313430
SOX2	Anophthalmia/micro-ophthalmia, anterior pituitary hypoplasia, HH, esophageal atresia	184429
GLI2	Holoprosencephaly with MPHD including HH, multiple midline defects	165230
PNPLA6	Laurence-Moon syndrome with chorioretinopathy, ataxia, spastic paraplegia, and short stature	245800

ACTH adrenocorticotropic hormone, *AHC* adrenal hypoplasia congenita, *FSH* follicle-stimulating hormone, *GH* growth hormone, *HH* hypogonadotropic hypogonadism, *KS* Kallmann syndrome, *LH* luteinizing hormone, *MPHD* multiple pituitary hormone deficiency, *nIHH* normosmic isolated hypogonadotropic hypogonadism, *SOD* septo-optic dysplasia, *TSH* thyroid stimulating hormone
Adapted from Mehta and Dattani [79], Cangiano, et al. [80]

Transient Central Hypogonadism

Although many of the etiologies of central hypogonadism listed in Table 5.2 are permanent, some may prove to be transient in nature. This is the case for several of the known genetic abnormalities that lead to iHH, including mutations in *FGFR1*, *CHD7*, *GNRHR*, and *PROKR2* [82, 83]. Spontaneous gonadal function has been documented to begin years after it would have been expected and may occur during stable long-term testosterone treatment [82]. It is estimated that 10–20% of patients may have spontaneous reversal [84]. Common clinical findings in boys with congenital iHH include anosmia/hyposmia, undescended testes, small penis, and small testes. Puberty may start but then fail to progress in 40% of those with normosmic iHH [85].

Puberty is often delayed in the setting of chronic illnesses that impair nutritional status or lead to chronic inflammation, such as inflammatory bowel disease or cystic fibrosis. Improvement of nutrition, treatment of the inflammatory condition, or other appropriate therapies leads to onset or progression of puberty. In such cases, gonadotropin concentrations are in the prepubertal range, and testosterone levels are low. Stimulation of pituitary gonadotrophs with GnRH analogs does not result in increases of LH concentrations. Skeletal maturation is typically delayed and is often less than 12 years.

Constitutional Delay of Growth and Puberty

Constitutional delay of growth and puberty ("constitutional delay," CDGP) is a common variant of normal that results from a delayed onset of otherwise normal puberty, and it can be considered a form of transient hypogonadotropic

hypogonadism (Fig. 5.4). The typical history is of a full-term gestation with a normal birth weight and length. Growth is normal for the first year, but between 1 and 3 years of age, linear growth velocity slows and the child's height percentiles on the growth chart decline. Weight may follow a similar pattern but is often relatively preserved. The child is clinically well during this period, without constitutional symptoms suggesting illness. By 3 years of age, the linear growth velocity normalizes, with the child's height percentiles stabilizing and being maintained at the lower end of the normal range or slightly below normal. The rates of height and weight gain through mid-childhood are normal, allowing the child to maintain his height relative to his peers. However, the onset of puberty is delayed, and as other boys enter puberty and begin their pubertal growth spurts, the child with constitutional delay begins to lose height relative to peers. This is exacerbated by the decline in height velocity seen in normal boys with late puberty. Spontaneous pubertal maturation begins by age 15–16 years, and linear growth continues until adult height is attained, often several years after the boys' peers. Adult height may not be reached until the young man is 20–21 years old. Common features of constitutional delay include a positive family history, with one or more parents or second-degree relatives entering puberty later than average or continuing to grow into young adulthood. Dental development is often delayed, with the first primary tooth being lost at 7–8 years rather than at 5–6 years.

Boys with constitutional delay and their families are often worried about short stature, and this becomes particularly acute in the early teenage years, when the height discrepancy increases and delayed puberty becomes apparent. Because the presentation of constitutional delay and isolated hypogonadotropic hypogonadism have a great deal of overlap, it is difficult to distinguish the two conditions in the absence of anosmia or syndromic features (Table 5.3). Historically, the physician and the patient have engaged in watchful waiting to see if puberty begins spontaneously. As this approach is anxiety-inducing for the patient and family, other approaches have been explored. Administration of GnRHa causes a brisk rise in serum LH concentrations in normal boys in the early stages of puberty. Boys with constitutional delay may also have an increase in LH levels, but failure to increase LH after GnRHa does not exclude constitutional delay or confirm HH. Additionally, modest increases can be seen in those with partial HH. Other approaches have used hCG-stimulated testosterone levels and baseline levels of LH, inhibin B, anti-Mullerian hormone, kisspeptin, and insulin-like factor 3 in various combinations [86–89]. Reported sensitivities and specificities vary widely, due in part to different ages of the test subjects, different assays used, and variations in the composition of the subjects in terms of etiologies and the proportion of those with partial HH. However, none of the protocols have demonstrated sufficient reproducibility to be of practical use [90], and watchful waiting remains the gold standard.

The delay in skeletal maturation in boys with CDGP permits a longer phase of active growth than in unaffected boys. This allows affected boys to continue growing into the late teen years or even into the early 20s. Because the average boy attains near final adult height at 17 years, this extra time leads to an increase in height standard deviation scores in late adolescence. The adult height of boys with

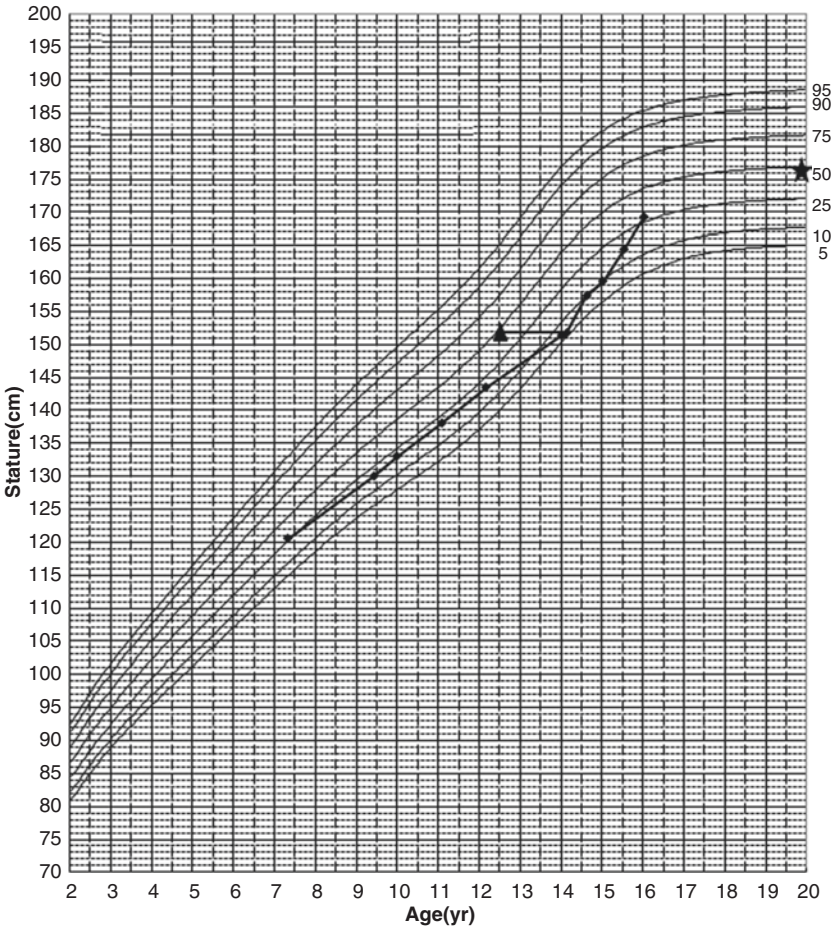


Fig. 5.4 This patient presented at 14.1 years of age for evaluation of delayed puberty. He was otherwise healthy. His sense of smell was normal. His family history was remarkable for delayed menarche in his mother, whose first menstrual period was at age 15. His father recalled that he continued to grow taller after completing secondary school. The patient's height percentiles had gradually declined since age 12. At the time of the exam, his height and weight *Z*-scores were −1.55 and −1.73, respectively. Midparental height *Z*-score was −0.05. His exam revealed a prepubertal boy with otherwise normal genitalia. Bone age was 12.6 years. The diagnosis of constitutional delay of growth and puberty was made. After discussion with the patient and family, the patient received intramuscular testosterone cypionate, 50 mg monthly for 4 months. Six months later, pubic hair was Tanner stage 2 and a small amount of phallic growth had occurred, but testes remained prepubertal. He was observed without further treatment until age 15.0. At that time, there had been no further pubertal maturation except that testicular volume had increased to 3–4 mL. Because the patient remained distressed at the discrepancy between his development and that of his peers, he received four additional monthly doses of testosterone cypionate at 75 mg each. When next seen at age 15.5, there was further pubertal maturation, with Tanner stage 3 pubic hair and testicular volumes of 5 mL bilaterally as well as a small amount of unilateral gynecomastia. He did not receive any further testosterone, and at age 16.0 his gynecomastia had resolved, his height velocity remained pubertal at 9.6 cm/year, he was Tanner stage 4 for pubic hair, and testicular volume was 10 mL bilaterally. He was discharged from the clinic and continued to grow normally. Subsequently, his two younger brothers had delayed puberty and followed a similar course (triangles represent bone age; star indicates midparental height)

CDGP is typically in the normal range, although it may be less than their midparental height [91, 92].

In boys with CDGP, bone mineral density is lower than in controls during the early and mid-teen years, related in part to the delayed exposure to sex steroids and the resulting discrepancy in bone growth and mineralization [93]. Although boys with CDGP and normal boys have comparable increases in bone mineral density as puberty progresses, the bone mineral density in adulthood may still be somewhat less than normal, even after testosterone treatment [94, 95].

Treatment of Delayed Puberty

The decision to treat boys with delayed puberty must consider the wishes of the individual as well as the likelihood of the delay in puberty actually being due to permanent hypogonadism. For boys thought to have CDGP, watchful waiting may be appropriate, as central puberty is likely to begin spontaneously by age 15–16 years. However, as they become increasingly different from their peers, many boys with delayed puberty are quite anxious and are ready to start treatment as soon as possible. In patients likely to have permanent hypogonadism, the practitioner should consider starting treatment soon after the patient reaches 14 years old. Boys known to have permanent hypogonadism may begin treatment at an age when their peers demonstrate signs of puberty, typically around age 12 years.

Treatment of Constitutional Delay of Growth and Puberty

Treatment of CDGP is indicated when the boy is at least 14 years old, prepubertal, expresses anxiety about the pubertal delay, and has a bone age of at least 12 years. When treatment for CDGP is needed, a long-acting injectable testosterone ester is the drug of choice, as these agents have been well studied and are able to generate serum testosterone concentrations appropriate for an early pubertal boy. The initial dose is 50–100 mg of testosterone cypionate or enanthate injected intramuscularly or subcutaneously once monthly [96]. This is usually continued for 4–6 months, with periodic clinical reassessment. After testicular enlargement is noted, testosterone may be discontinued, as the boy should continue to progress spontaneously. If testicular volume has not increased after 6 months, another 4–6 month treatment course may be given at the same or slightly higher dose. Failure of testicular enlargement by age 16–17 should call into question the diagnosis of CDGP, as the likelihood of permanent hypogonadism increases from this point.

More recently, oral testosterone undecanoate has been shown to be effective in the treatment of boys with CDGP [97, 98]. Using a starting dose of 40 mg daily that was then increased to 40 mg twice daily and then to as high as 80 mg twice daily, treatment for an average of 0.8 years led to progression of puberty and an increase in height SDS without excessive advancement of skeletal age. Subcutaneous injections of testosterone have been also become more mainstream, as the ease of injection is increased [96].

Pubertal Induction for Known HH or Primary Hypogonadism

In boys known to have permanent hypogonadism, treatment is initially similar to that of boys with CDGP. There are many possible approaches, but one regimen uses a starting dose of 50–100 mg of testosterone enanthate or cypionate monthly. The dose of testosterone is increased by 25–50 mg every 4–6 months until a dose of 200 mg monthly is reached. From that point, the interval between injections is decreased until a dose of 150–200 mg every 2 weeks is attained. After a stable adult dose is reached, serum testosterone concentrations should be measured midway between injections to ensure that levels are in the mid-portion of the normal adult male range. Weekly subcutaneous injection is approved for treating adults and has been used for gender affirming hormone therapy in transgender males [99] but has not been formally evaluated for pubertal induction [100].

Transdermal preparations of testosterone have the advantage of avoiding injections and providing more stable serum concentrations than injectable testosterone. However, the preparations are designed for adult replacement, and the dose cannot be readily titrated in small increments using currently available preparations. This makes it difficult to attain the low levels needed at the beginning of pubertal induction. Once boys are receiving full adult doses, transdermal preparations can be used. Given adolescent boys' frequent difficulty adhering to medication regimens, periodic testosterone injections may be a better option for some.

Side effects of testosterone preparations are usually minimal and are related to the dosage form. Intramuscular injection may be painful and result in minor tenderness or bruising at the injection site, but this may be improved with subcutaneous injection. Priapism is a rare complication. Transdermal patches are often associated with local skin reactions. A major concern for testosterone gels is the inadvertent passage of testosterone to others, particularly women and children, and the patient should be carefully instructed to wash his hands thoroughly and cover the exposed skin with clothing. There have been many case reports of children with virilization after exposure to transdermal gels [101, 102].

Conclusions

Puberty is a time of rapid physical and emotional change and is often challenging for teens. When pubertal maturation occurs abnormally early or late, it is a source of even more distress. When disordered puberty is recognized, it is critical to evaluate the patient to exclude serious pathology. Fortunately, the evaluation and diagnosis are often straightforward, allowing administration of any needed treatment and normalization of the maturational trajectory.

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Gynecomastia

6

Alexandre Hohl, Marcelo Fernando Ronsoni, and Simone van de Sande Lee

Pathophysiology

An imbalance in the action between androgens and estrogens in male breast tissue is considered the main mechanism leading to gynecomastia, since this tissue has receptors for both. Estrogens promote glandular growth stimulation, while androgens exert an inhibitory action on the gland [1, 2].

In men, most androgens are produced by the testes, while estradiol is derived from the aromatization of testosterone and androstenedione, primarily in Sertoli cells. A small portion of estrogens is converted from testosterone by the action of the aromatase enzyme at the peripheral level with adipose tissue being the largest site [3].

There are many causes for gynecomastia, which can be classified as physiological or associated with other conditions, as given in Table 6.1.

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Table 6.1 Etiologies of gynecomastia [3–5]

Physiological gynecomastia
• Neonatal gynecomastia
• Pubertal gynecomastia
• Senile gynecomastia
Pathological gynecomastia
• Increase in serum estrogens
– Exogenous estrogens
– Increased production of estrogens
Gonads: Leydig cell tumors, sertoli cell tumors, germ cell tumors, disorder/difference of sex development (DSD)
Adrenals: adrenocortical tumors, congenital adrenal hyperplasia
Peripheral tissues: obesity, liver cirrhosis, hepatocellular carcinoma, malnutrition, hyperthyroidism, familial aromatase excess syndrome
– Abnormal production of human chorionic gonadotropin (hCG)
Eutopic: germ cell tumors
Ectopic: lung, liver, stomach, and kidney tumors
• Decreased production/action of testosterone
– Primary testicular failure
Congenital/genetic: congenital anorchia, Klinefelter syndrome, defects in testosterone biosynthesis, disorder/difference of sex development (DSD)
Acquired: viral orchitis (such as mumps and SARS-CoV-2), trauma, castration, neurological and granulomatous diseases (such as leprosy), radiotherapy/chemotherapy
– Secondary testosterone deficiencies: including hypogonadotropic hypogonadism (Kalman syndrome and other genetic defects), pituitary adenomas, cranial irradiation, traumatic brain injury, obesity, opioid abuse, renal failure
• Other endocrinological causes: acromegaly, hyperprolactinemia, after anabolic androgenic steroid abuse, genetic causes (such as Kennedy syndrome), hyperthyroidism, hypothyroidism, Cushing's syndrome
Drugs
Anabolic steroid abuse
Alcohol and illicit drugs
Environmental polluting substances
Idiopathic

Physiological Gynecomastia

Neonatal

It occurs in the first few months after birth due to increased levels of circulating estrogens. These hormones produced by the mother during pregnancy are transferred to the fetus through the placenta, promoting the growth of the newborn's breast. In most cases this increase is bilateral [6].

Gynecomastia disappears within a few weeks, but it can persist for longer periods. In addition to breast enlargement, **Physiological** there may also be an opaque physiological secretion, popularly known as "witch's milk" [6].

Pubertal

At the beginning of puberty, there is a phase where the increase in serum estradiol levels precedes the increase in testosterone levels. This dysregulation of the estrogen/testosterone ratio is responsible for the basic pathophysiology of breast tissue growth at this stage [6].

Other factors may influence, such as the concomitant increase in serum concentrations of growth hormone (GH) and insulin-like growth factor-1 (IGF1). In addition to their role in linear growth, these hormones also act on receptors in the breast tissue, causing their proliferation [7]. Since most cases of gynecomastia occur during peak growth velocity, it is postulated that there may be a relationship between peak GH/IGF1 and gynecomastia [8–10].

As previously described, estrogens are in part derived from peripheral aromatization in adipose tissue. Therefore, gynecomastia is more frequent in obesity and may present as true gynecomastia or, in many cases, only as lipomastia (without glandular proliferation) [9].

In pubertal gynecomastia, mild degrees of breast enlargement usually occur starting 1 year after the onset of puberty, around 13 or 14 years of age. It presents asymmetrically and bilaterally, in some cases associated with local pain. The natural course of this form, which occurs in up to 95% of cases, is spontaneous regression between 6 months and 2 years [11, 12]. Some adolescents may present a voluminous enlargement of the breast (breast tissue greater than 4 cm) and persistent, called persistent pubertal macromastia [3].

Once the breast tissue fibrosis stage is reached, whether in macromastia or in the physiological form, the regression of breast volume is very small [13]. Interestingly, a family history was present in more than half of the patients with persistent pubertal gynecomastia [12]. Often, in these cases, pharmacological or surgical therapy is necessary to correct the glandular dysplasia [13].

Men over 65 years of age often present with relative hypogonadism, with decreased plasma levels of total testosterone (TT), increased sex hormone-binding globulin (SHBG), and decreased free testosterone (FT). Added to this, there is a progressive increase in adiposity, favoring the peripheral activity of aromatase [12, 14, 15].

Senile gynecomastia also presents bilaterally, it is painless and there is usually no spontaneous regression [6].

In this age group, several comorbidities can coexist, and several drugs can contribute to causing or worsening gynecomastia, ceasing to be just physiological gynecomastia [16].

Pathological Gynecomastia

Approximately, 15% of male estradiol and most testosterone are secreted directly by the testes. These bind to SHBG produced by the liver and are transported through the bloodstream to target organs. Sex hormones in their free form enter target tissues, where the aromatase enzyme complex converts testosterone into estradiol [2]. Several conditions can alter these pathways, resulting in pathological gynecomastia (Fig. 6.1).

Testicular estradiol secretion may be pathologically increased due to Leydig cell or Sertoli cell tumors [2, 12]. Pathological secretion of human chorionic gonadotropin (hCG) also indirectly stimulates testicular estradiol secretion. Examples of hCG-producing tumors include gonadal and extragonadal germ cell tumors, large cell lung, gastric and renal cell carcinomas [2, 11].

Another mechanism includes the deficient formation of testosterone by the testes, resulting from primary causes such as testicular trauma, orchitis, congenital anorchia and Klinefelter syndrome, or secondary causes resulting from hypothalamic or pituitary diseases [12, 17].

There are indications about the possible association between SARS-CoV-2 infection and gynecomastia, but more studies are needed [18].

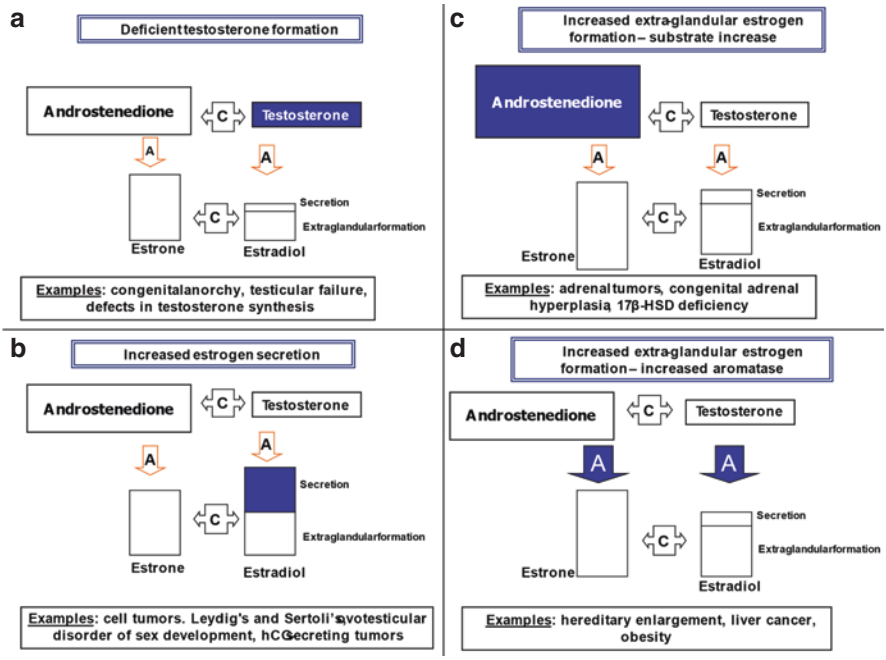

Fig. 6.1 (**a**–**d**) Abnormal patterns of the androgen–estrogen ratio [3]. The abnormal component of each pattern is shown in each frame in blue. *Enzyme A: aromatase; enzyme C: 17-β-HSD (hydroxysteroid dehydrogenase); *hCG* chorionic gonadotropic hormone

Adrenal tumors, congenital adrenal hyperplasia, and 17-β-hydroxysteroid dehydrogenase (17-β-HSD) deficiency generate an increase in androstenedione and other androgenic precursors, which are converted into estrogens in peripheral tissues [3, 11].

An increase in aromatase activity has been reported in several patients with gynecomastia associated with pathological processes that include thyrotoxicosis, Klinefelter syndrome, as well as obesity [2, 11].

Estradiol and estrone bind less avidly to SHBG than does testosterone. An increase in SHBG concentrations, which occurs in hyperthyroidism and some liver diseases, can lead to a greater binding of testosterone in relation to estrogens, leading to a decrease in the ratio between FT and free estrogen [11, 19].

Hyperprolactinemia causes gynecomastia through multiple mechanisms, including secondary hypogonadism, as well as hindering the androgen-mediated inhibition of breast tissue growth [2, 7].

A study in boys with pubertal gynecomastia showed increased serum levels of leptin [20]. Furthermore, it has been reported that the presence of certain polymorphisms in the gene encoding the leptin receptor may predispose to the development of pubertal gynecomastia [21]. Leptin may contribute to the development of gynecomastia by increasing aromatase expression in adipose and/or breast tissue. In addition, it may increase the sensitivity of breast epithelial cells to estrogens through greater expression of estrogen receptors or by direct stimulation of breast epithelial cells [22].

Abnormalities in androgen receptors due to a genetic defect, blockade of androgen receptors or stimulation of estrogen receptors by drugs or environmental endocrine disruptors can also result in gynecomastia [11].

Gynecomastia Secondary to Medication Use

Over the years, several drugs have been associated with the development of gynecomastia, the main ones are given in Table 6.2. Different mechanisms seem to be involved, such as estrogen-like activity, stimulation of testicular estrogen production, inhibition of testosterone synthesis, inhibition of androgenic action, inhibition of estrogen metabolism, increased peripheral aromatization, and hyperprolactinemia [23, 24].

Table 6.2 Main drugs associated with gynecomastia and respective level of evidence [4]

Antiandrogens		**Cardiovascular drugs**	
Flutamide, bicalutamide	A	Calcium channel blockers	C
Finasteride, dutasteride	A	Amiodarone	C
Spironolactone	A	ACE inhibitors	C
Eplerenone	B	Digoxin	C
Ketoconazole	B	**Drugs of abuse**	
Lavender oil	C	Alcohol	B
Antibiotics		Amphetamines	C
Isoniazide	C	Heroin	C
Metronidazole	C	Marihuana	C
Anti-ulcer drugs		Methadone	C
Cimetidine	B	**Hormones**	
Ranitidine	B	Estrogens, clomiphene citrate	A
Proton pump inhibitors	B	hCG	B
Chemotherapy		Anabolic steroids	C
Imatinib	C	GH	C
Methotrexate	C	**Others**	
Alkylating agents	C	Metoclopramide	A
Psychoactive drugs		Antiretrovirals	B
Haloperidol	B	Phenytoin	C
Phenothiazines	B	Penicillamine	C
Diazepam	C	Theophylline	C

Gynecomastia from Anabolic Steroid Abuse

The use of anabolic steroids is frequent among elite athletes, practitioners of recreational, and bodybuilding sports. When considering the effects of these drugs, we must take into account that very high and often unknown doses are administered, in addition to possible mixtures with other substances, such as GH and hCG [4].

Gynecomastia is a common adverse effect of anabolic steroid abuse, especially when using aromatizable androgens such as testosterone and androstenedione. Therapies used after cycles of anabolic steroids to reactivate the hypothalamic-pituitary-gonadal axis, such as clomiphene and hCG, can also cause or worsen gynecomastia [4].

Idiopathic Gynecomastia

In most of the published series, 50% or more of the evaluated gynecomastia do not have endocrine alteration, associated disease or use of identifiable medication, thus being classified as idiopathic gynecomastia [3].

Endocrine disruptors not yet identified may be involved in the pathophysiology in these cases. The deleterious effects of exposure to phthalic acid esters (phthalates) have attracted the attention of the medical community and regulatory agencies around the world. They are plasticizing additives for polyvinyl chloride (PVC) resins, polyurethane, and cellulosic resins used in the production of synthetic fibers for clothing or upholstery, toys, cosmetics, food additives, and packaging. High

molecular mass phthalates are considered teratogenic, hepatotoxic, nephrotoxic, and also have antiandrogenic effects and are associated with feminization of male fetuses, early thelarche and, possibly, gynecomastia [25–27].

Food products derived from soy contain phytoestrogens, a group of naturally occurring compounds that are structurally similar to estrogen and that can confer estrogen-like activity in tissues. Excessive consumption, such as milk and juices derived from soy, offered for a long period, may represent a risk of developing gynecomastia in certain populations [28].

The association of gynecomastia with exposure to products based on lavender, tea tree oil, and linseed oil, which seem to have estrogenic and antiandrogenic effects, has also been reported [29–32].

Gynecomastia and Obesity

Obesity is a condition associated with mainly secondary hypogonadism, while androgen aromatization occurs mainly in adipose tissue; consequently, men with obesity have a high estrogen/androgen ratio [4].

Leptin, a protein synthesized by adipose tissue and therefore more abundant in obese people, has receptors on epithelial cells of breast tissue, making it necessary for normal breast development and during lactation [21].

Previous studies have reported a correlation between leptin and sex hormones, as well as a possible increase in estrogen secretion, increased aromatase activity, and activation of alpha-estrogen receptors [33, 34]. Another study reported higher levels of leptin in cases of pubertal gynecomastia compared to the control group, which could cause an increase in estrogen receptors in breast tissue [20].

Excessive local deposition of fat in obese men can lead to the differential diagnosis of lipomastia or even worsen the clinical picture of true gynecomastia, requiring special attention during the physical examination, as shown in Fig. 6.2 [4].

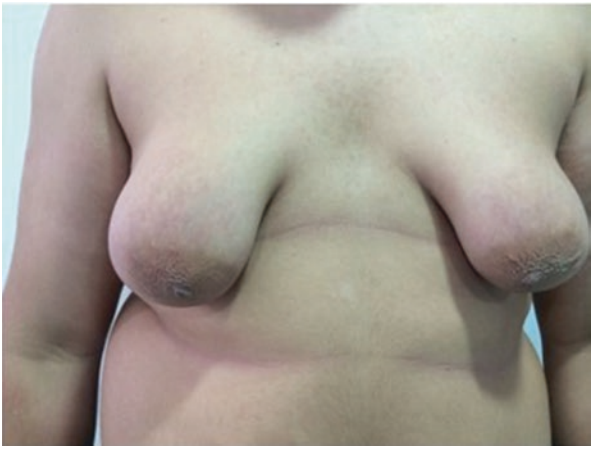

Fig. 6.2 Gynecomastia and bulky lipomastia

Features and Clinical Investigation

A careful anamnesis and physical examination, including the relevant elements presented in Table 6.3, is the first step to determine whether breast enlargement is true gynecomastia and guide its investigation [17]. Lipomastia is characterized by an increase in subareolar fat without an increase in the glandular component of the breast, and further investigation is not necessary in this case [11, 17].

The presence of breast pain (mastodynia) or its recent increase, outside the age ranges considered physiological, should lead to a more detailed etiological investigation [3].

Gynecomastia is usually bilateral, but in some patients, it may be asymmetrical or unilateral. Mastodynia occurs in 25% of cases, and increased local sensitivity is described by 40% of patients. Chronic gynecomastia is usually asymptomatic. On physical examination, it is normally palpable, mobile and firm, but not hardened as in breast cancer and centrally located under the nipple-areola complex [3, 17].

Table 6.3 Clinical roadmap for evaluating the patient with gynecomastia [3, 17]

Anamnesis
• Age of onset
• Progression speed
• Presence of pain
• History of mumps or other viral illnesses, liver or kidney disease
• Detailed history of use of medications, supplements, illicit drugs, anabolic steroids, or exposure to substances known to be associated with gynecomastia
• Occupation
• Family history
• Sexual function
• Psychosocial damages
Physical examination
• Weight, height, body mass index, waist circumference, arm span, and body segment measurements
• Pubertal stage
• *Habitus* (marfanoid, eunuchoid)
• Stigma of chronic liver disease
• Signs suggestive of hypogonadism
• Signs of hyperthyroidism
• Assessment of regional lymph nodes
• Assessment of the breasts: size of the mammary gland, asymmetry, contour, presence of masses, existence of papillary discharge—differentiate from lipomastia
• Gonadal assessment: testicular size, position in relation to the scrotum, asymmetry, consistency, presence of palpable masses

Physical Examination

The physical examination includes anthropometric measurements, such as weight, height, body mass index (BMI), and waist circumference, to quantify obesity. The assessment of body proportions to document eunuchoid and marfanoid habitus may be relevant among younger patients [4]. Remembering that the arm span measurement is the maximum distance between the ends of the middle fingers, of the right and left hands, when both arms are extended laterally at shoulder level.

The physical examination also includes the assessment of signs of hypogonadism, such as reduced hair and muscle mass; palpation of the thyroid gland and identification of signs of hypo or hyperthyroidism, liver or kidney failure and Cushing's syndrome [4].

The genital examination encompasses the pubertal stage according to the Tanner scale, which assesses the presence of pubic hair, penis size, and testicular volume [35]. This can be estimated by the Prader orchidometer or scrotal ultrasound. Testicular palpation may reveal the presence of abnormal areas, which must be confirmed by ultrasound [4].

In many cases, it is difficult to differentiate between gynecomastia and lipomastia on physical examination alone. After inspection, palpation should be performed with the patient in the supine position, with hands clasped under the head. Using the thumb and forefinger apart, the examiner slowly brings the fingers together on either side of the breast. In patients with gynecomastia, firm breast tissue concentric with the nipple-areola complex is palpated, whereas in lipomastia no tissue is felt with the fingertips. True gynecomastia can also be differentiated from lipomastia by means of mammography or breast ultrasound [11, 36].

"Drug treatment aims to correct the imbalance between estrogens and androgens. Currently, there is no specific medication for the treatment of gynecomastia, all of which are used off-label. We have androgenic and antiestrogenic substances as options, represented by estrogen receptor blockers (tamoxifen, raloxifene, clomiphene) and aromatase inhibitors (anastrazole, letrozole), as show in (Figs. 6.3 and 6.4) [4].

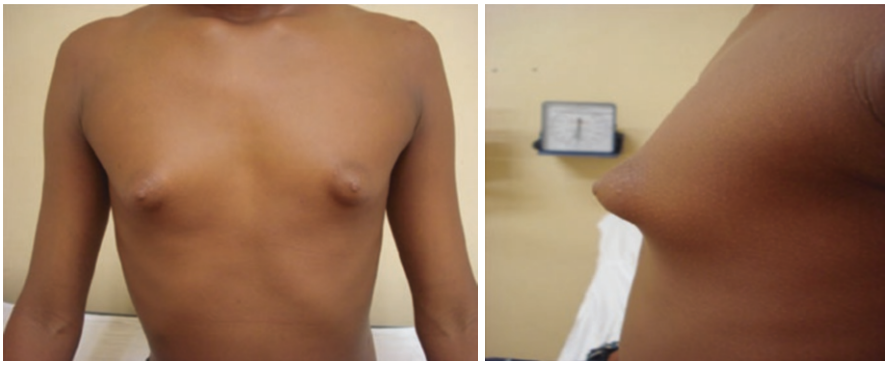

Fig. 6.3 Gynecomastia before tamoxifen treatment

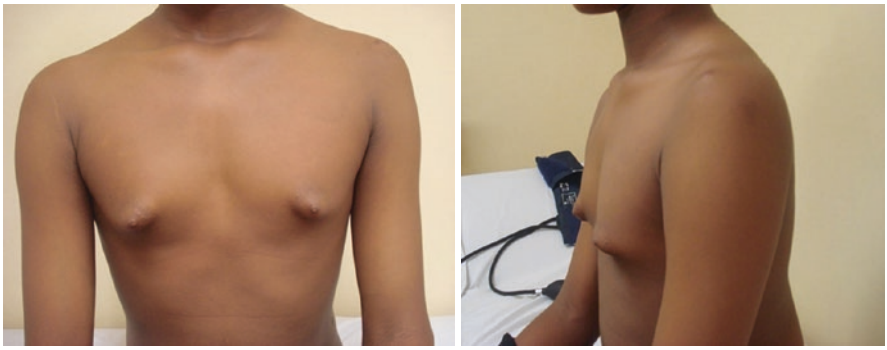

Fig. 6.4 Gynecomastia after tamoxifen treatment

Laboratory Investigation

Laboratory evaluation is performed in cases of true gynecomastia without a clear explanation. Liver, kidney, and thyroid function tests exclude the respective medical conditions.

Hormonal assessment should include the measurement of serum levels of estradiol, TT, LH, FSH, TSH, hCG, alpha-fetoprotein, and prolactin [11, 17].

In men whose initial TT concentrations are at the lower limit of normal, we should determine the FT concentration. As equilibrium dialysis, the reference method for its dosage is not widely used in practice due to cost, and immunoassay methods are imprecise, FT levels are estimated by the equation of Vermeulen et al., which includes the values of SHBG, albumin, and TT [37].

Imaging Exams

Although the vast majority of diagnoses are made through anamnesis and physical examination, an additional evaluation with breast ultrasound and/or mammography may be necessary to measure the glandular component and perform a differential diagnosis with other volumetric increases in the region, such as lipomastia [38].

It is recommended that the genital physical examination be complemented by testicular ultrasound due to the low sensitivity of tumor detection by gonadal palpation [4].

In addition, adrenal computed tomography (CT) and pituitary MRI may be useful in the diagnostic investigation of gynecomastia, as illustrated in Fig. 6.5 [17, 38].

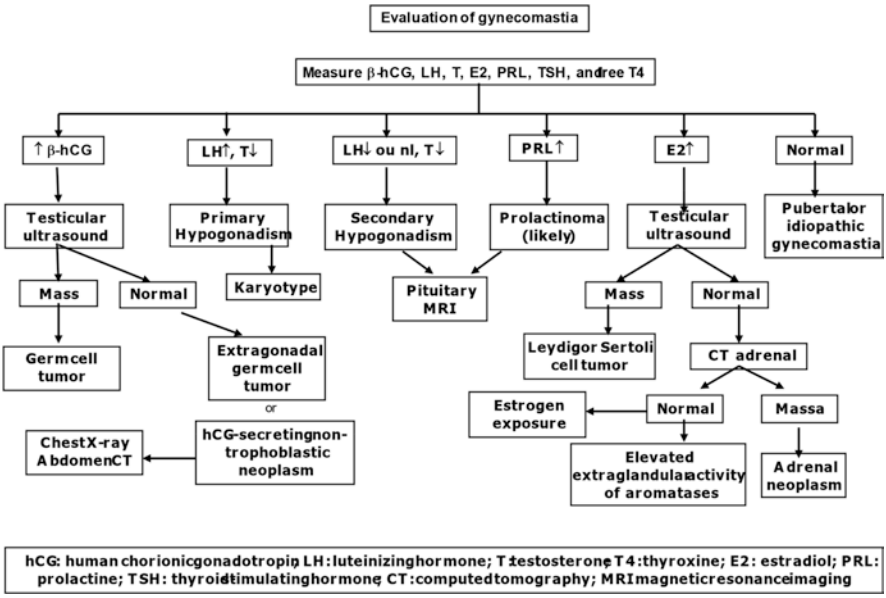

Fig. 6.5 Diagnostic investigation of gynecomastia [3]

Genetic Investigation

A study of 25 patients with gynecomastia revealed that 72% had no identifiable endocrine abnormalities. Among the others, the following were detected: Klinefelter syndrome, androgen insensitivity syndrome (androgen receptor gene mutation p.Ala646Asp and p.Ala45Gly), and 17α-hydroxylase/17,20-lyase deficiency (heterozygous mutation of the CYP17A1 gene) [39].

Kennedy syndrome is rare (1 in 40,000 men) and is caused by an increase in the number of CAG (polyglutamine) repeats in the androgen receptor gene, which results in lower receptor sensitivity (spinal and bulbar muscular atrophy). Although there is phenotypic variability, in the classic phenotype, clinical signs of androgen deficiency, such as gynecomastia, are combined with elevated levels of testosterone and LH, implying partial androgen resistance. Neuromuscular problems (weakness, atrophy, fasciculation) usually occur after androgen resistance at 40–50 years of age [4].

Overexpression of CYP19A1, which encodes the aromatase enzyme, results in a rare genetic disorder known as aromatase excess syndrome, also associated with gynecomastia [40]. Polymorphisms in the G protein-coupled estrogen receptor gene (GPR30) could also explain the presence of gynecomastia in certain adolescents, just as leptin receptor polymorphisms can affect the susceptibility of developing gynecomastia [21, 41].

The relationship between kisspeptin levels and breast enlargement has also been studied, and their high levels may be related to the onset of gynecomastia in neonates and prepubertal children [42]. More in-depth genetic studies are necessary; however, it is suggested to assess the need for investigation of patients previously defined as having physiological gynecomastia regarding the possibility of the existence of genetic mutations [39].

Differential Diagnosis

The differential diagnosis of a palpable breast mass in a male patient includes lipomastia, breast cancer, and other benign conditions such as lipomas, dermoid cysts, sebaceous cysts, neurofibromas, ductal ectasia, hematomas, and fat necrosis. Breast carcinoma and other benign conditions are usually unilateral and eccentric, while gynecomastia characteristically begins in the subareolar area and increases concentrically [36, 43].

Treatment

Due to its benign and self-limiting character, most cases of gynecomastia do not require treatment, only clinical observation. If a specific cause is identified and treated during the painful proliferative phase, there may be regression of breast augmentation [17, 44].

If possible, potentially gynecomastia-causing medications or anabolic agents should be discontinued immediately. If this is the cause, a decrease in breast tissue and sensitivity will generally be apparent within 1 month after drug discontinuation [11, 12].

However, if gynecomastia from any cause has been present for more than 2 years, it is unlikely to substantially regress, either spontaneously or with drug therapy, due to progression to fibrous tissue [11, 17, 44].

Drug treatment aims to correct the imbalance between estrogens and androgens. Currently, there is no specific medication for the treatment of gynecomastia, all of which are used off-label. We have androgenic and antiestrogenic substances as options, represented by estrogen receptor blockers (tamoxifen, raloxifene, and clomiphene) and aromatase inhibitors (anastrozole and letrozole), as show in Figs. 6.3 and 6.4 [44, 45].

The use of testosterone is indicated only in cases of proven hypogonadism and may have beneficial results when the hormone deficiency is recent, as well as in anorchia and viral orchitis. However, in many cases, as in Klinefelter syndrome, it has inconsistent effects and can even worsen gynecomastia, as it is aromatized to estradiol. Dihydrotestosterone can be used topically in the mammary gland with good results, since it is not aromatized [44, 46].

Estrogen receptor blockers are increasingly used, especially in severe and painful pubertal gynecomastia, with good results demonstrated in several studies. A systematic review by Lapid et al. to assess the efficacy of tamoxifen in the management of idiopathic pubertal gynecomastia showed to be potentially effective and safe, but controlled and randomized studies are needed to confirm this indication [47].

Another 10-year prospective study evaluated the use of tamoxifen in idiopathic gynecomastia, demonstrating complete resolution of the condition in 90.1% of cases [48]. Gastrointestinal and cardiovascular problems are the main adverse events associated with the use of tamoxifen. However, in addition to being rare, they are more prevalent when used in cases of prostate cancer and breast cancer [49].

Cases of gynecomastia and/or mastodynia resulting from antiandrogen therapy for prostate cancer occur with a cumulative prevalence of up to 70%, leading to discontinuation of therapy in up to 16% of patients [2]. As prophylaxis, both radiotherapy and the daily use of tamoxifen significantly reduce the incidence of gynecomastia and/or mastodynia [50–52]. Tamoxifen seems to be an effective alternative to radiotherapy in the treatment of gynecomastia in these cases, but its side effects, such as dizziness and hot flashes, must be considered [50]. Furthermore, it seems to be better than anastrozole, and the daily administration of tamoxifen seems to be more adequate than its weekly use [53–55].

A systematic review of treatments for gynecomastia identified several available options, all with a low level of evidence and discrepant diagnostic criteria [5].

The indication for surgical treatment is based on the patient's suffering, including psychosocial stress and pain, as well as esthetic distortion. Also, in cases of gynecomastia with progression to fibrosis or macromastia, surgery has been shown to be the best therapeutic option. The objective of every surgical procedure is to remove the fibrotic hypertrophic glandular tissue and reestablish the shape of the male breast [56].

Due to the wide variety of surgical techniques described for the treatment of gynecomastia, an individualized approach based on the degree of gynecomastia and the patient's preference can help the surgeon to provide the best results [57]. The combination of traditional surgical excision of glandular tissue and liposuction has shown the most consistent results and a low rate of complications [53].

Conclusion

Gynecomastia is a clinical sign defined by the enlargement of the breasts in males, caused by glandular proliferation. It is a very common change in certain periods of life, especially in the neonatal period, puberty, and the elderly. In these situations, gynecomastia is called physiological. Adult men with gynecomastia need to be investigated. Clinical treatment of gynecomastia can be done with medication in the first 2 years of its appearance. After breast tissue fibrosis occurs, the only possible treatment is surgery.

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Hypogonadotropic and Hypergonadotropic Hypogonadism

7

Prativa Rajbhandari, Jerry Sanghun Han, Christina Wang, and Ronald Swerdloff

The Hypothalamic-Pituitary-Testis Axis

The interrelationship between the hypothalamus, pituitary, and testes is a vital part of the successful initiation and maintenance of male reproductive function and is briefly described here (See Chap. 3 for details). The axis is dependent on the pulsatile hypothalamic secretion of gonadotropin-releasing hormone (GnRH) that will stimulate luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary that in turn initiates both intra-gonadal testosterone production and spermatogenesis. The testes, through secretion of testosterone and inhibin B from the Leydig and Sertoli cells, respectively, exert negative feedback on LH and FSH secretion (Fig. 7.1) [1].

Androgen Biosynthesis

LH binds to a G protein coupled receptor to activate cyclic AMP pathway in the Leydig cells. Activation of the LH receptor induces steroid acute regulatory protein (StAR) and translocator proteins that move cholesterol from the outer to the inner mitochondria membrane and converts cholesterol to pregnenolone. The major steps involved in testosterone synthesis are summarized in Fig. 7.2. The main testosterone biosynthesis pathway is from pregnenolone to dehydroepiandrosterone, to androstenediol, and to testosterone. There is a "backdoor" pathway that is important in androgen biosynthesis disorders [2] and details can be found in Chap. 3.

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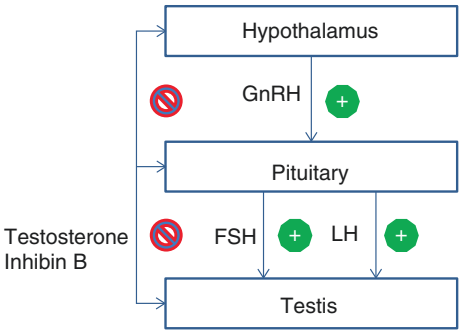

Fig. 7.1 The hypothalamic-pituitary-testis axis

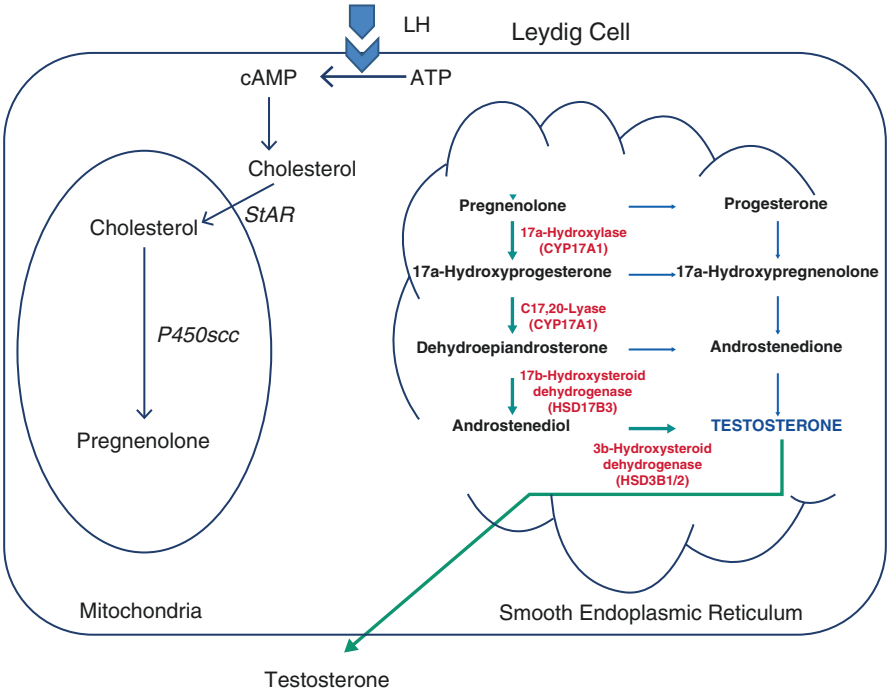

Fig. 7.2 Testosterone biosynthesis

Testosterone Metabolism

Testosterone is converted to DHT by α-reductase isozymes (SRD5A1 and 2) or to estradiol by the aromatase enzyme (CYP19) (Fig. 7.3). In males, majority of circulating testosterone is derived from testicular secretion and only a small amount is derived from the adrenal gland. About 44% of circulating testosterone is bound with high affinity to sex hormone binding globulin (SHBG) and 54% with low affinity to albumin. About 2–3% of testosterone is unbound and is referred to as free

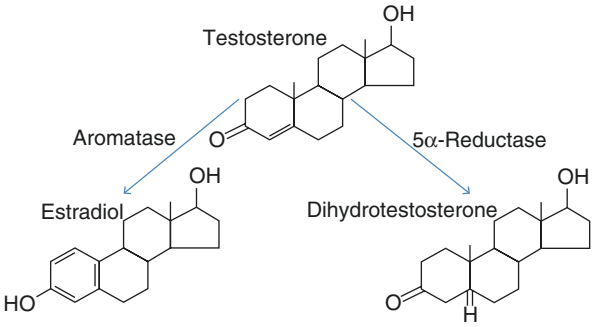

Fig. 7.3 Testosterone conversion to estradiol and dihydrotestosterone

testosterone. SHBG concentrations are decreased by androgens, obesity, hyperinsulinemia, and nephrotic syndrome and SHBG concentrations are elevated by estrogen administration, hyperthyroidism, chronic inflammatory illnesses, weight loss from excessive exercise, and aging.

Testosterone is metabolized mainly in the liver. A series of enzymatic steps leads to conversion to inactive metabolites and these compounds eventually undergo glucuronidation or sulfation before being excreted by the kidneys.

Clinical Diagnosis of Hypogonadism

History

The medical history for a man being evaluated for hypogonadism depends on age of onset. The clinical syndrome is different due to the age of onset in an adult versus fetal or pre-pubertal periods. A full history from childhood is important including information on testicular descent, onset of puberty and pubertal development, shaving frequency, any changes in quality or amount of body hair and prior or current systemic illnesses.

Post-pubertal men who developed hypogonadism may complain of low libido, hot flashes, and erectile dysfunction as well as less specific symptoms such as fatigue, loss of vigor, irritability or low mood, poor concentration, reduced physical performance, or sleep disturbance [3–5]. A complete sexual history includes frequency of sexual thought, masturbation, erectile function, sexual activity, and fertility. Information regarding previous orchitis, sinus or pulmonary problems, sexually transmitted infections, and HIV status should be obtained. Surgical history including prior trauma or irradiation to the testes is another important aspect in evaluating for hypogonadism that might affect the genital tract. A thorough drug history that includes recreational drugs, opioids, anabolic steroids, 5-alpha reductase inhibitors, cytotoxic drugs, and psychiatric medications should also be included.

Physical Examination

The general physical exam should include height, weight, and span assessments to determine whether there is eunuchoid proportions. Men whose hypogonadism is of prepubertal onset and who were not adequately treated will exhibit eunuchoid proportions, delayed development of secondary sex characteristics, and high-pitched voice. Characterization of facial, pubic, axillary and body hair distribution should be included. The presence of acne should be assessed as well as breast examination for the presence of gynecomastia. Assessment of muscle mass and adiposity should be evaluated. If hypogonadotropic (secondary) hypogonadism is suspected, patient should have a visual field exam performed. Genitourinary exam is needed for a complete examination that includes penile length and location of meatal opening. Testicular exam should include size and consistency, evaluation for hydroceles, hernias, and varicocele, and the presence or absence of vasa deferentia. Testis volume is usually over 15 mL, and small testes indicate decreased spermatogenesis because over 80% of the adult testis are occupied by seminiferous tubules. The prostate gland volume and nodularity can be assessed by a digital rectal examination and should be performed in men ages older 50 years.

Laboratory Studies

Blood levels of testosterone should be determined in the morning given that there is a diurnal rhythm in testosterone secretion. It is important to confirm low testosterone concentrations in men with an initial testosterone level in the mildly hypogonadal range because 30% of men may have a normal range testosterone level on repeat analysis [3]. Testosterone is usually quantitated by immunoassays, but low concentration should be measured by liquid chromatography and tandem mass spectrometry because this method is more specific and accurate than immunoassays [6]. Circulating testosterone is bound to sex hormone binding globulin (SHBG) and to albumin. Free or bioavailable testosterone concentrations should be measured when total testosterone concentrations are close to the lower limit of the normal range and when altered SHBG levels are suspected. Free testosterone can be calculated from total testosterone and SHBG but different formulae may give different values [7]. Direct measurement of free testosterone using equilibrium dialyses is the gold standard but suffers from the lack of standardization and reference ranges (see Chap. 4 for details).

Measurement of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) can help differentiate between hypergonadotropic vs. hypogonadotropic hypogonadism. Prolactin should be measured in all men who have a low serum LH and testosterone concentration, especially in patients with testosterone well below the reference range, an MRI of the sella would be the next step. Dihydrotestosterone (DHT) and androgen biosynthetic precursors should be measured in patients of abnormal genital differentiation for the diagnosis of 5-alpha reductase deficiency and congenital adrenal hyperplasia. Chromosome analysis is essential for the

diagnosis of Klinefelter's syndrome especially in those with testicular volume less than 5 mL, although men with mosaic Klinefelter's syndrome may have larger testicular volumes [1, 3, 5].

Diagnosis of Hypogonadism

The diagnosis is based on clinical symptoms and signs and a reduced serum testosterone level. The normal range of serum total testosterone in a young adult male population varies across different laboratories and 300–1000 ng/dL has been used as a convenient reference range. A recent report showed that the harmonized reference range for testosterone based of four cohort studies in men aged 19–39 years, from Europe and USA was reported to be between 264 and 916 ng/dL [8].

Men suspected to have hypogonadism should have their total testosterone measured in the early morning and if the level is below 250 ng/dL on at least two occasions with symptoms consistent with hypogonadism, the patient is probably hypogonadal and will need testosterone replacement therapy. If the serum testosterone is between 250 and 320 ng/dL with normal serum LH level or if there is a condition that alters sex hormone binding globulin, a free testosterone concentration should be obtained using equilibrium dialysis method or calculation from total testosterone, SHBG and albumin concentration if equilibrium dialysis is not available. With levels above 350 ng/dL, hypogonadism is unlikely and further investigation of other causes of the symptoms is warranted.

Evaluation of other pituitary hormones is essential if there is clinical evidence of hyperprolactinemia, hypopituitarism or sella abnormality on imaging. Pituitary imaging should be performed in patients with severe secondary hypogonadism (serum T <150 ng/dL) along with hyperprolactinemia and visual impairment to exclude pituitary tumor [3, 5].

Classification of Hypogonadism

When there is disruption of the hypothalamic-pituitary-testis feedback system, it can result in hypogonadism. It can present as hypergonadotropic hypogonadism, hypogonadotropic, compensated hypogonadism, or a combined etiology. Due to deficiency or absence of Leydig cell function. Laboratory evaluation will reveal low testosterone and elevated LH and FSH levels. The elevated gonadotropins reflect the lack of negative feedback on the hypothalamus/pituitary from the Leydig cells.

Failure of the episodic GnRH secretion or gonadotropin secretion can result in the clinical syndrome of hypogonadotropic hypogonadism. Laboratory evaluation will reveal low or normal levels of LH and/or FSH with low levels of testosterone. In some instances, men may present with nonspecific symptoms of hypogonadism and testosterone concentration in the lower reference range but with elevated LH concentrations, this may be named compensated hypogonadism and usually occurs in middle aged men predominantly with physical symptoms [9].

Combined dysfunction of the whole hypothalamic/pituitary and testis axis results in impairment of spermatogenesis, low testosterone concentration, and variable gonadotropin levels depending on the domination of the pathology in the testis or the hypothalamus/pituitary. It can occur in aging and systemic diseases including but not limited to type 2 diabetes mellitus, hemochromatosis, metabolic syndrome, obesity, and HIV.

Hypergonadotropic Hypogonadism

Hypergonadotropic hypogonadism, which is also known as primary hypogonadism, is associated with subnormal testosterone secretion and impaired spermatogenesis may be present. Many testicular diseases cause greater damage to the seminiferous tubule than the Leydig cells resulting in low sperm count, normal or high FSH (Sertoli cell abnormality), and normal range serum testosterone level; these patients present with infertility. Hypergonadotropic hypogonadism, is more likely to be associated with gynecomastia, presumably due to the stimulatory effect of the supranormal serum FSH and LH concentration on testicular aromatase activity. This results in increased conversion of testosterone to estradiol and enhanced testicular secretion of estradiol relative to testosterone. The common causes of hypergonadotropic hypogonadism are listed in Table 7.1.

Table 7.1 Causes of hypergonadotropic hypogonadism

Disorders	Examples
Congenital Disorders	Chromosome disorders—Klinefelter's syndrome
	Testosterone biosynthetic enzyme defects
	5α-reductase deficiency
	Androgen resistance syndromes
	Myotonic dystrophy
Developmental Disorders	Cryptorchidism
Acquired Defects	Orchitis—mumps and other viruses including HIV
	Granulomatous disease
	Torsion of testis, and other traumatic injuries
Toxins	Alcohol, fungicides, insecticides, heavy metals, phthalates, bisphenol A
Drugs/Irradiation	Irradiation
	Cytotoxic agents
	Alcohol
	Ketoconazole, spironolactone, flutamide, cimetidine
Autoimmune Testicular Failure	Isolated or associated with other autoimmune syndromes

Congenital Defects

Chromosome anomalies—Klinefelter's syndrome (KS) is the most common congenital abnormality causing primary hypogonadism, occurring in 1 in 500–1000 live male births [10]. The clinical manifestation of a male with an extra X chromosome in tall stature with eunuchoid proportion, decreased virilization, gynecomastia, low muscle mass, and increased visceral fat. The most common genotype is 47 XXY. Men with KS can have damage to the seminiferous tubules as well as damage to the Leydig cells. This results in small testes, azoospermia or very severe oligozoospermia, elevated FSH and LH concentrations, subnormal testosterone concentrations [11]. More recent studies in large numbers of men with Klinefelter's syndrome indicate that the changes in body composition may lead to metabolic syndrome and type 2 diabetes. They have learning ability and once diagnoses special education may help their development [12–14]. Adolescents and adults with Klinefelter's syndrome and low testosterone are treated with testosterone for the hypogonadism. Fertility may be possible as the seminiferous tubules may have few pockets of sperm which can be aspirated by micro-testicular extraction of sperm, intra-cytoplasmic sperm injection, and in vitro fertilization.

Cryptorchidism—Cryptorchidism refers to testes not descended into the scrotum. It can affect one or both testes. If only one testis is undescended, the sperm count can be subnormal and the FSH concentration can be slightly elevated. If both testes are undescended, the sperm count will usually be severely oligozoospermic, FSH elevated, and the serum testosterone may also be reduced. Cryptorchidism occurs in 2–9% of full-term births. The undescended testis has spermatogenesis defects and is associated with increased incidence of testicular cancer [15]. Surgical correction may improve fertility but does not reduce the risk of cancer [16].

Androgen Biosynthesis Defects—A congenital decrease in testosterone synthesis and secretion can result from mutations of the genes that encode the enzymes necessary for testosterone biosynthesis. These mutations are rare (Fig. 7.2). Children with 5-alpha-reductase 2 deficiency have ambiguous genitalia and are often raised as girls. At puberty because of the marked increase in testosterone allowing conversion to 5-alpha dihydrotestosterone, virilization occurs frequently with a gender role change [17]. The enzymes 3 beta-hydroxysteroid dehydrogenase, and 17 alpha-hydroxylase, are both present in the adrenal gland and the testes. Each of these mutations can result in decreased testosterone secretion which can result in incomplete virilization (congenital adrenal hyperplasia).

Androgen Insensitivity Syndromes—Androgen resistance syndromes present with a wide range of phenotypes from female to men with infertility. The mechanism may be related to gene mutations of the androgen receptors or post-receptor defects [18]. Gonadotropins are usually elevated.

Acquired Defects

Infections—After puberty, mumps is associated with clinical orchitis and over 50% of those affected after puberty can become infertile. During acute orchitis the testes are inflamed, painful and swollen. This can result in a decrease in size of the testes. The testes can return to normal size and function, or they become atrophic. Spermatogenic defects occur often due to destruction of seminiferous tubules which can result in normal LH with increased FSH levels. Over time low testosterone levels and lower LH levels can develop. Leprosy can also lead to orchitis and gonadal insufficiency.

Chemotherapy and irradiation—Chemotherapy and irradiation exposure of the testes in the treatment of malignant diseases can damage the seminiferous tubules to cause azoospermia and markedly elevated FSH concentrations. With chemotherapy, particularly alkylating agents such as cyclophosphamide, testosterone secretion is also impaired, leading to a decrease in the serum concentration of testosterone and increase in serum luteinizing hormone (LH).

Drugs—Medical or self-treatment with androgens and synthetic anabolic steroids can intratesticular testosterone and sperm count. Medications that interfere with testosterone biosynthesis (e.g., ketoconazole) and androgen action (spironolactone, flutamide) can cause hypogonadism. Environmental toxins such as fungicide and insecticides, and by-products of the plastic industry (phthalates and bisphenol A) may have anti-androgenic effects. These pollutants are called endocrine disruptors [19].

Trauma—The testes located in the scrotum are susceptible to injury. Surgical injury during hernia repair, varicocele and vasectomy can result in permanent testicular damage resulting in hypogonadism and infertility. Testicular torsion if not treated emergency may result in atrophic testes.

Autoimmune orchitis—Autoimmune testicular failure is a result from antibodies against the microsomal portion of the Leydig cells may occur either as an isolated disorder or as part of a multi-glandular disorder [20].

Hypogonadotropic Hypogonadism

Hypogonadotropic hypogonadism represents a deficiency in the secretion of gonadotropins (LH and FSH) because of an abnormality in the hypothalamus or pituitary gland. A summary of causes is listed in Table 7.2 (Congenital) and Table 7.3 (Acquired).

Table 7.2 Causes of idiopathic/congenital hypogonadotropic hypogonadism

Disease	Examples
Isolated hypogonadotropic hypogonadism	Kallmann syndrome—mutations of ANOS1, FGFR1, PROK2, PROK2R and others
With and without anosmia	Prader-Willi syndrome, Laurence-Moon Biedl syndrome, basal encephalocele
Associated with other syndromes	Fertile eunuch syndrome
Partial deficiency of gonadotropin-releasing hormone	PROP-1, POU1F1, TPI mutations
Multiple hypothalamic and/or pituitary deficiency	
Pituitary hypoplasia/aplasia	

Table 7.3 Causes of acquired hypogonadotropic hypogonadism

Disease	Examples
Pituitary and sella tumors	Prolactinoma and other pituitary adenomas
	Craniopharyngioma
	Meningioma
	Rathke's pouch cyst
Infiltrative and infectious diseases of hypothalamus/pituitary	Sarcoidosis, tuberculosis, coccidioidomycosis, syphilis, histiocytosis X
	Hemochromatosis, hemosiderosis
Autoimmune hypophysitis	Lymphocytic hypophysitis
	Cancer immunotherapy with checkpoint inhibitors
Traumatic brain injury	May be transient
Obesity, Malnutrition, and weight loss	
Exogenous hormones and drugs	Androgens, estrogens, progestins, glucocorticoids, anti-androgens
	Drugs inducing hyperprolactinemia,
	Opioids

Congenital Defects

Isolated hypogonadotropic hypogonadism—Congenital abnormalities from decreased secretion of GnRH and gonadotropins are called isolated gonadotropic hypogonadism are but easy to recognize due to the presentation of delayed sexual development. Isolated hypogonadotropic hypogonadism can be associated with anosmia due to impaired migration of the GnRH neurons with impaired olfactory axonal development. Patients with Kallmann syndrome can present with cryptorchidism, micropenis, absence or delayed puberty, eunuchoid proportions, small testes, and anosmia. Many genetic defects have been identified including ANOS1, FGFR1, Prokineticin 2 and its receptor (PROK2 and PROK2R). Other genetic causes include mutation of the kisspeptin-neurokinin B-dynorphin (KNDy) neurons are less common/rare [21]. It should be noted that patients with congenital hypogonadotropic hypogonadism may recover spontaneously although they may have been misclassified and were really extremes of delayed puberty [22].

Hypogonadotropic hypogonadism associated with syndromes—Patients with Prader-Willi syndrome have low testosterone, hyperphagia, and gross obesity. Laurence-Moon-Biedl syndrome patients have polydactyly, rod-cone dystrophy, learning disabilities, renal abnormalities, obesity, and hypogonadotropic hypogonadism. Mutations in leptin or the leptin receptor leads to morbid obesity and hypogonadotropic hypogonadism.

Multiple pituitary hormone deficiencies—Combined pituitary hormone deficiency may be caused by genes involved in the differentiation of the pituitary cells. Hypogonadotropic hypogonadism is associated with other hypothalamic-pituitary hormone deficiencies such as the PROP-1, POU1F1, and TPI gene mutation that lead to hypo-secretion of anterior pituitary hormones.

Acquired Defects

Pituitary tumors—Prolactinomas manifest differently in men depending on size of adenoma when diagnosed (i.e., microadenomas versus macroadenomas). Male patients with prolactinomas often present with hypogonadism, erectile dysfunction, and visual manifestations from suprasellar extension. In small tumors, hypogonadotropic hypogonadism may be due to suppressive effects of elevated prolactin levels on GnRH and thus decreased LH and testosterone secretion. Large non-prolactin-secreting pituitary tumors can also produce gonadotropin insufficiency from damage to the adjacent normal pituitary gland resulting in decreased serum LH and testosterone levels.

Infiltrative diseases—This may include sarcoidosis, tuberculosis, fungal infections, and histiocytosis X. Hemochromatosis is a genetic disorder of iron transport that results in iron deposits in many tissues including the pituitary gland [23]. Transfusion related hemosiderosis in hemoglobinopathies also present with hypogonadotropic hypogonadism [24].

Autoimmune hypophysitis and infundibulitis—Lymphocytic hypophysitis or infundibulitis is an autoimmune disorder of the pituitary and its stalk resulting in enlargement of the pituitary gland or the stalk. The autoimmunity may spontaneously involute or require pharmacological treatment of the inflammation and may cause hypogonadotropic hypogonadism [25]. Recent studies showed that cancer immunotherapy with checkpoint inhibitors may induce hypophysitis resulting in adrenal, thyroid, and gonadal insufficiency. Recovery from central hypogonadism from checkpoint inhibitors is common when compared to autoimmunity in the adrenals or thyroid gland [26].

Traumatic brain injury—Pituitary dysfunction occurs during the acute period but may persist depending on the severity of the injury. Surveillance is required to ensure the patient does not develop persistent hypogonadotropic hypogonadism [27] (See Chap. 10).

Anorexia nervosa and weight loss can result in functional defects resulting in low serum LH and testosterone levels. Stress and critical illness also lower gonadotropin and testosterone levels. Severe obesity is associated with suppression of the

hypothalamic-pituitary axis. Treatment of the underlying disease process may improve gonadotropin secretion.

Drugs—Exogenous administration of hormones including androgens, estrogens, progestins, and glucocorticoid suppresses the hypothalamic-pituitary axis and results in hypogonadism. Simultaneous use of a progestin together with supplemental androgens is used for experimental hormonal male contraception [28]. Opioid administration can disrupt the axis and can cause profound suppression of gonadotropin and testosterone secretion [29].

Compensated Hypogonadism

Middle aged and older men may present with the symptoms of hypogonadism and serum testosterone concentrations in the lower reference range, but serum LH are above the reference range. This has been reported in population-based studies and termed compensated hypogonadism and is associated with physical symptoms [30]. Others suggest that compensated hypogonadism may be a response of the hypothalamic-pituitary-testis axis to somatic illnesses [31] (See Chap. 8).

Hypogonadism Associated with Systemic Diseases

Abnormalities of the hypothalamic-pituitary-testis axis occur in systemic diseases including liver failure, renal failure including those on chronic hemodialysis, severe malnutrition, sickle cell anemia, advanced malignant disease, severe obesity, metabolic syndrome, type 2 diabetes, cystic fibrosis, and amyloidosis.

HIV infection is associated with both hypogonadotropic and hypergonadotropic hypogonadism [32]. In the prior era when successful treatment was not available, many AIDS patients had low serum testosterone levels [33]. The pathophysiology was complex with both hyper- and hypogonadotropic patterns seen. In addition, there were alterations in the SHBG levels resulting in low testosterone levels. Fortunately, HIV infections are successfully treated in many instances and the frequency of low testosterone states has reduced. In hemochromatosis and hemosiderosis, iron deposition can occur in the testis and the anterior pituitary, but hypogonadotropic hypogonadism is more common [23, 24].

In liver disease, the etiology of testicular dysfunction is complex and may be either independent of or associated with the direct toxic effects of excessive alcohol use. Gynecomastia, testicular atrophy, and sexual dysfunction are common signs in cirrhosis. Estradiol levels are usually elevated which results in an increased ratio estradiol to testosterone which can lead to gynecomastia. As with other systemic conditions mentioned, cirrhosis can cause injury of both testes and hypothalamus-pituitary [34].

In obesity, metabolic syndrome, and type 2 diabetes mellitus, there is a decrease in SHBG resulting in lower total testosterone levels and either normal range free testosterone or low free testosterone due to hypothalamic-pituitary dysfunction [35,

36]. Moreover, visceral obesity is associated with hypogonadism and sexual dysfunction [37, 38].

Hypogonadism also occurs with aging and is associated with co-morbidities. Testosterone level appears to fall more rapidly in the last decades of life. However, the prevalence of testosterone deficiency in older men is less than 5%, which is considerably lower than what was described in earlier studies [9]. Recent data indicate that testosterone replacement will enhance sexual drive and function, mood, bone mineral density, and anemia in older men [39, 40] (See Chap. 11).

Summary

Hypergonadotropic hypogonadism results from diseases of the testes while hypogonadotropic hypogonadism from the pituitary or the hypothalamus. Measurement of LH and FSH can distinguish these two conditions. There are disease processes that can result in hypogonadism by both etiologies. The diagnosis of hypogonadism is made after repeat lab assessment of testosterone concentration in conjunction with signs and symptoms. It is imperative that thorough history, physical and lab assessment be obtained such that the cause of hypogonadism is identified because hypogonadism may be reversed with appropriate treatment of the underlying disease process. Symptoms of hypogonadism improved with testosterone substitution and infertility in hypogonadotropic hypogonadism may treated with gonadotropin replacement.

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8 Functional Hypogonadism: Diabetes Mellitus, Obesity, Metabolic Syndrome, and Testosterone

Ricardo Martins da Rocha Meirelles

Introduction

Male hypogonadism can be primarily caused by diseases or lesions of the testes or secondary due to pituitary or hypothalamic disorders. In both cases, hypogonadism can result from genetic, neoplastic, infectious, granulomatous, vascular, actinic, traumatic, or drug-induced injury. High blood gonadotropins are found in primary hypogonadism, while these hormones are low or in the normal range in secondary hypogonadism. Late-onset hypogonadism (LOH), occurring in some aging males, has features of primary and secondary disorder simultaneously [1]; in some instances, it is not possible to determine an organic origin, and hypogonadism is said to be functional or idiopathic [2]. In some of these cases, reversibility of the androgen efficiency can be achieved by lifestyle modifications [3]. This is considered the first-line therapy [4]. Bariatric surgery is the solution for many patients [5]. Testosterone replacement therapy (TRT) is left for those cases where fertility desire is not an issue and the lifestyle modifications are not feasible. Another treatment proposed for functional hypogonadism was the clomiphene citrate as an alternative for TRT in those men seeking fertility [6].

TRT results in normalized testosterone levels in 2 years of treatment and improves glycemia, endothelial function, lipids, and insulin sensitivity, reducing the cardiovascular risk [7].

Functional hypogonadism is prevalent in metabolic disturbs, remarkably in type 2 diabetes mellitus, obesity, and metabolic syndrome [8]. This chapter will study the prevalence, pathophysiology, clinical aspects, and treatment of the association between low testosterone levels and these diseases.

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Epidemiology

Diabetes

Many authors report an association between hypoandrogenemia and diabetes mellitus [9–15], and this disease is one of the conditions in which the Endocrine Society suggests measuring serum testosterone levels [16]. Some questionnaires have been developed to screen for male hypogonadism: The St. Louis University Androgen Deficiency in Aging Male (ADAM) [17], the Aging Male Survey (AMS) [18], and the Massachusetts Male Aging Study (MMAS) [19]. The comparison of the three questionnaires showed that ADAM has more sensitivity (97%) although less specificity (30%) than the others [20]. We found that in men with diabetes, the ADAM questionnaire shows less sensitivity (85%) and almost no specificity (2%) [21], being not useful for screening and reinforcing the Endocrine Society suggestion of measuring testosterone levels in type 2 diabetic patients.

A cross-sectional study of 1292 healthy men, 20–60 years old, reported a stepwise reduction of testosterone and increase of insulin, a physiological marker of insulin resistance, along the decades of life, independent of age, obesity, body fat distribution, plasma glucose, tobacco, and alcohol consumption [22]. Cross-sectional research is not suitable for a cause-consequence relationship, but the higher risk of type 2 diabetes in Klinefelter syndrome hypogonadism [23] suggests that the age-associated lowering of testosterone is the cause of increasing insulin resistance.

Dhindsa et al. [24] found a prevalence of 33% of hypogonadism in 103 men with type 2 diabetes. Although hypogonadotropic hypogonadism is frequent in middle-aged male patients with type 2 diabetes mellitus, in men with type 1

diabetes, we find normal total testosterone (TT) concentrations, and only 6% have low calculated free testosterone (cFT) [25]. However, Grossman [26] found a 20.3% prevalence of low levels of cFT in 69 men with type 1 diabetes, stating that hypogonadism was associated with insulin resistance in both types of diabetes. Even young type 2 diabetic patients have lower levels of total free testosterone when compared with type 1 diabetic patients matched for age [27]. Notwithstanding, a trial with 181 male type 1 diabetic patients showed a prevalence of 8.3% of hypogonadotropic hypogonadism in this sample, and they found an association with age, waist circumference, and insulin requirements [28]. Previously known and newly diagnosed type 2 diabetic patients showed the same prevalence and risk factors of testosterone deficiency in a cross-sectional study comprising 186 men [29]. In the group of previously known type 2 diabetic men, those with better control (HbA1c < 7%) had higher levels of total testosterone lower risk of low levels of this hormone [29].

Glucose metabolism is affected by serum testosterone levels even when glycated hemoglobin (HbA1c) is within the reference range. In a cross-sectional study of 1292 men from the Norfolk population of the European Prospective Investigation

into Cancer, the levels of HbA1c were higher in the lowest quartile of testosterone levels (Total Testosterone [TT] ≤ 12.6 nmol/L and Calculated Free Testosterone [cFT] ≤ 234.1 pmol/L) [30]. According to the progressive reduction of serum testosterone levels in men, there is a continuum in the prevalence of normal glucose metabolism to diabetes mellitus. In 775 men ≥55 years, those with impaired glucose tolerance diagnosed by 75 g oral glucose tolerance test (OGTT) showed significantly lower TT levels in age and body mass index (BMI) adjusted analysis [13]. TRT prevents glucose intolerance (prediabetes) evolution to diabetes in hypogonadal patients [31].

The number of cytosine-adenine-guanine (CAG) repeats in the androgen receptor (AR) gene is associated with diminished androgenic activity [32]. A higher number of these repeats in men with type 2 diabetes mellitus relates to a higher HbA1c, and the authors determined a U-shaped relation with mortality [33].

Some studies showed that low testosterone levels predict the development of type 2 diabetes in men [34–39]. The association of diabetes mellitus with low testosterone levels is so frequent that Corona and cols. wrote a paper on whether testosterone could be regarded as a new therapy for diabetes [40].

Obesity

The association between obesity and hypogonadotropic hypogonadism has been known for more than four decades [41–44]. Although some authors postulated that despite the total testosterone being low, the calculated free testosterone concentrations were average in obese men [43], Zumof et al. [45] showed that also free and bioavailable testosterone presented an identical percent decrease as body mass index (BMI) increases.

In Tromsø Study, comprising 1548 men aged 25–84 years, after adjustment for age and BMI, men with waist circumference (WC) ≥ 102 cm had significantly lower levels of total testosterone than those with WC < 94 cm [46]. Considering BMI instead of WC, in the European Male Aging Study (EMAS), 5.2% of 3219 men, 40–79 years, with BMI ≥ 30 kg/m^2 had late-onset hypogonadism and only 0.4% of men with BMI < 25 kg/m^2 had total testosterone <320 ng/dL and/or free testosterone <6.4 ng/dL [47].

The association between low testosterone levels and obesity is true among middle-aged male adults and younger men. Young obese pubertal and post-pubertal males, aged 14–20 years, with Tanner stage ≥4, present testosterone and calculated free testosterone concentrations 40–50% lower than non-obese men matched for age and pubertal status [48]. Once both testosterone and free testosterone are reduced in these obese young men, it is not reasonable to attribute to lower sex hormone-binding globulin (SHBG) the difference found compared to lean counterparts.

Metabolic Syndrome

Metabolic syndrome (MetS) components are abdominal obesity (measured by the waist circumference), dyslipidemia, high blood pressure, and elevated fasting glucose. The National Cholesterol Education Program—Adult Treatment Panel III (NCEP-ATP III) [49] defines metabolic syndrome as the presence of at least any three of these risk factors, and the International Diabetes Federation [50] requires central obesity associated with two or more other risk factors. Table 8.1 illustrates both criteria.

Many studies demonstrate the association between low testosterone levels in men and metabolic syndrome. For example, in a small sample of 130 nonsmoking men, from the Quebec Family Study, the frequency of men presenting three or more features of the MetS increased with decreasing testosterone levels, from 8.9%, in the third tertile, to 44.2%, in the first tertile ($P < 0.0005$) [51]. In addition, in a case-control study of 832 hypogonadal patients with Klinefelter Syndrome (KS) compared to 4033 randomly selected age-matched men, KS subjects significantly increased the risk of type 2 diabetes and obesity [52].

In the Third National Health and Nutrition Examination Survey, comprising 1226 men aged ≥20 years, those in the lowest quartile of total testosterone were more likely to have metabolic syndrome than men in the highest quartile (prevalence ratio 2.16, 95% CI 1.53–3.06) [53]. However, the European Male Aging Study (EMAS), performed 2 years later on 2966 community-dwelling men aged 40–79 years, found a significant association between low testosterone levels and the metabolic syndrome (odds ratio 9.94; CI 2.73–36.22) only in men with severe late-onset hypogonadism [54].

Meta-analysis of 52 observational studies, comprising 22,043 men, concluded that total testosterone (TT) and free testosterone (FT) are lower in men with MetS [TT mean difference = −2.64 nmol/L, 95% confidence interval (CI) −2.95 to −2.32; FT standardized mean difference = −0.26 pmol/L, 95% CI -0.39 to −0.13], and men

Table 8.1 Metabolic syndrome in men: comparison of the NCEP-ATP III and IDF diagnostic criteria

	NCEP-ATPIII	IDF
	3 or more risk factors	1st + 2 or more risk factors
Waist Circumference[a]	>102 cm	According to ethnicity[b]
Triglycerides	≥150 mg/dL (≥ 1.7 mmol/L)	
Blood pressure	≥130/85 mmHg	
Fasting glucose	≥110 mg/dL (≥6.1 mmol/L)	≥100 mg/dL (≥5.6 mmol/L)
HDL cholesterol	<40 mg/dL (<1.03 mmol/L)	

[a]If body mass index is >30 kg/m^2, central obesity can be assumed, and waist circumference does not need to be measured

[b] Male Waist Circumference according to ethnicity: Europids, Sub-Saharan Africans, Eastern Mediterranean, and Middle East (Arab) ≥ 94 cm; South Asians, South and Central Americans ≥90 cm; Chinese ≥90 cm; Japanese ≥85 cm

Adapted from references [3, 4]

in the highest tertile of blood TT levels had lower MetS risk (relative risk [RR estimate = 0.38, 95% CI 0.28–0.50) [55]. Erectile dysfunction, obesity, peripheral vascular disease, and alcohol intake significantly increase the probability of metabolic syndrome in men with testosterone deficiency [56]. Sexual symptoms, even when not associated with hypogonadism, can be accompanied by a higher prevalence of metabolic syndrome. Almost half of 280 men with erectile dysfunction (ED) had metabolic syndrome, and the ED was more severe among those with metabolic syndrome [57]. ED is quite common even in apparently healthy men, and we should also think of depressive symptoms in these men [58].

Not only metabolic syndrome is associated with low testosterone levels, but also the intima-media thickness (IMT). A trial in 935 men, the median age of 57 years, participating in a health examination showed a significant negative linear correlation of IMT with testosterone, and more hypogonadal subjects were in the two lower tertiles of mean IMT [59].

A few long-term prospective trials can be found in the medical literature. For example, Laaksonen et al. [35] followed 702 middle-aged Finnish men participating in a population-based cohort study for 11 years without diabetes or metabolic syndrome at the time they entered the study. More men in the lowest quartile of total testosterone developed metabolic syndrome (odds ratio [OR] 2.3, 95% CI 1.5–3.4) and diabetes (OR 2.3, 95% CI 1.3-4.1). The authors hypothesize that hypoandrogenism is an early marker for insulin and glucose metabolism disturbances that may progress to metabolic syndrome or frank diabetes [35].

The European Male Aging Study (EMAS), with 3369 community-dwelling men aged 40–79 years, established a link between the greater risk of metabolic syndrome in men with low levels of testosterone, independent of SHBG, BMI or insulin resistance [60]. A lower estradiol/testosterone ratio was associated with protection against the risk of developing metabolic syndrome [60]. A Korean study with 2172 men aged 21–79 years showed a negative association between total testosterone and the prevalence of metabolic syndrome independent of Age and BMI [61]. However, one study of 203 men with type 2 diabetes showed no difference in the proportion of patients with metabolic syndrome in the group with and without hypogonadism, using the International Diabetes Federation criteria [62]. Some authors state that the metabolic syndrome associated with hypogonadism needs clarification once it is not defined if the low testosterone levels are the cause or consequence of metabolic syndrome [63].

Pathophysiology

Hypogonadism diagnosis comprises low testosterone levels associated with clinically compatible symptoms and signs [64]. Although the most standard symptoms are sexual (loss of libido, erectile dysfunction, and absence of morning erections), it is usual to observe an increase in fat mass, a decrease of muscle mass, osteopenia or osteoporosis, anemia, loss of body hair, vegetative symptoms, and mood alterations. From these, fat mass increase and muscle mass decrease, and their consequences

may be the basis for the disturbance leading to type 2 diabetes mellitus, obesity, and metabolic syndrome. Insulin resistance is present in all these conditions. However, the inverse relationship between low testosterone levels and high insulin is independent of age or obesity [22].

For centuries, it has been known that animal castration favors weight and fat gain. There are many pathways through which testosterone can interfere with fatty tissue. The differentiation of stem cells into myocytes or adipocytes depends on circulating testosterone. Testosterone induces the differentiation of mesenchymal stem cells into muscle cells and inhibits adipocyte formation (Fig. 8.1) [65, 66]. Because this is a dose-dependent phenomenon, some athletes take supraphysiological doses of androgens as anabolic agents, with well-documented adverse effects [67].

Higher relative muscle mass is inversely associated with insulin resistance and lower levels of glycated hemoglobin (HbA1c), and a 10% elevation of SMI corresponds to an 11% reduction in the homeostatic model assessment of insulin resistance (HOMA-IR) [68]. The authors also found that the prevalence of glucose intolerance was reduced by 12% [68].

A study in male Sprague-Dawley rats indicated that testosterone replacement treatment decreases visceral fat cell size characteristic of central obesity and metabolic syndrome [69]. In men, testosterone, but not dihydrotestosterone, inhibits lipoprotein lipase (LPL) activity and incorporation of triglycerides to adipocytes in the abdominal but not in the femoral fat [70]. Testosterone also influences the catecholamine-induced lipolysis by increasing the number of β-adrenoceptors and adenylate cyclase activity in adipose precursors cells in male rats [71].

The relationship between obesity and hypogonadism is bidirectional. Obesity is associated with high levels of inflammatory cytokines produced by adipocytes. Tumor necrosis factor (TNF)-α and interleukin (IL)-6 reduce GnRH and LH secretion by the hypothalamus and pituitary, respectively, both in animals and in vitro [72, 73], and can contribute to low testosterone levels seen in the obese.

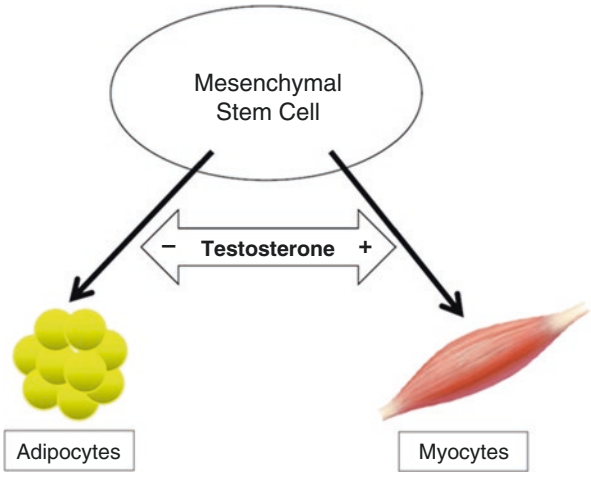

Fig. 8.1 The action of testosterone on mesenchymal stem cell differentiation

Higher content of aromatase in abdominal fat of obese men could contribute to the low levels of testosterone through its conversion to estradiol, and this hormone could lead to inhibition of gonadotropin production by the pituitary, explaining why there is no elevation of LH in such cases [74]. However, this was not confirmed by Dhindsa et al. [75]. Notwithstanding, the LH pulse amplitude decreases in obese men, pointing to a hypothalamo-pituitary abnormality in these subjects [76].

Leptin levels elevate proportionally to the degree of obesity and seem to have a role in testosterone production. There are leptin receptors in the testes, and the stimulation of testosterone secretion by human chorionic gonadotropin (HCG) is inhibited by leptin, which can contribute to the low levels of testosterone found in obese men [77–79]. It is noteworthy that serum leptin concentration tends to be higher in hypogonadal men than in those with normal testosterone levels of equivalent BMI, and testosterone replacement therapy promotes the reduction of leptin concentration, as illustrated in Fig. 8.2 [80].

Compromised mitochondrial function induces metabolic alterations characteristic of insulin resistance [81–84]. Pitteloud et al. [85], using a hyperinsulinemic-euglycemic clamp in 60 men, demonstrated an association of impaired mitochondrial function with low testosterone levels promoting insulin resistance in men.

Bellastella et al. [86] suggested that the anti-pituitary antibodies (APA) targeting gonadotropin-secreting cells present at high titers in type 2 diabetes could account for an autoimmune pathogenesis of hypogonadotropic hypogonadism in these patients, but prospective studies are needed to confirm this hypothesis.

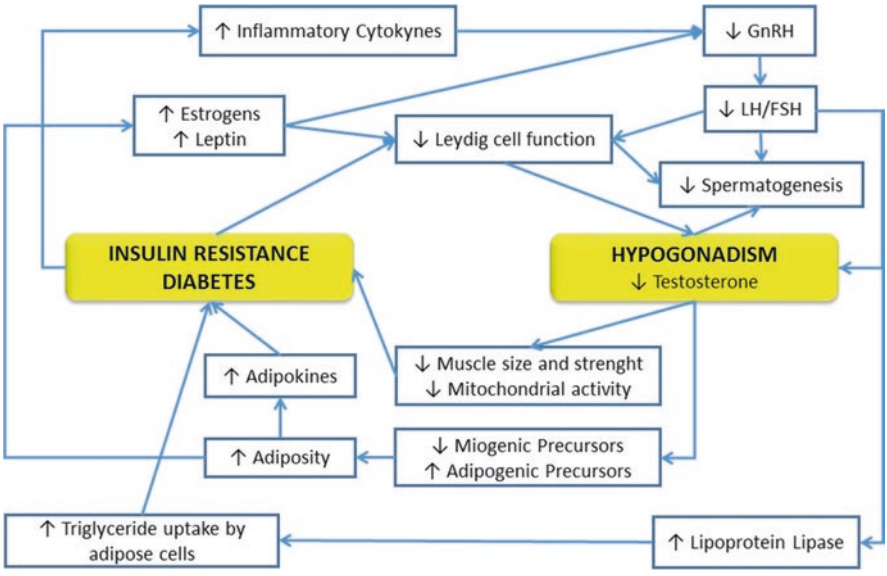

Fig. 8.2 Bidirectional relationship between hypogonadism and insulin resistance. (Adapted from references [3, 74])

Testosterone Replacement Therapy

Although some authors consider that the risk-to-benefit ratio of testosterone therapy in patients with type 2 diabetes requires further elucidation [87–91], most of the published trials point out benefits overcoming risks. However, three papers that concluded for higher cardiovascular risk with testosterone replacement therapy (TRT) [92–94] were criticized for methodological issues or misinterpretation of data, and many world endocrine societies and individual researchers asked for a retraction of one of them [95, 96]. An extensive systematic review and meta-analysis of placebo-controlled randomized clinical trials did not support a causal role between TRT and adverse cardiovascular events [97].

It is important to remember that the interval between the beginning of TRT and its effect is different for each focused parameter. Although the first measured change can be apparent in 3–6 months, the maximum effect will be obtained after a period of 12 months for increasing muscle mass and strength, 24 months for fat mass and waist circumference, and up to 36 months for growing bone mineral density [98]. The effects on carbohydrate metabolism can be detected in a few days (improvement of insulin sensitivity), but significant improvement in glycemic control can last 3–12 months (insulin level, HOMA-IR, HbA1c), and fasting glucose can reach a better status in 24 months [98].

Reducing excessive fat mass and increasing lean mass is one of the main goals of treating obesity, type 2 diabetes, and metabolic syndrome to lessen cardiovascular risk factors and outcomes. Interestingly, in a long-term study of 255 hypogonadal men for 5 years, those overweight or obese lost weight, and those within the normal BMI range gained weight, probably by increasing muscle mass [99]. It is noteworthy that the study was not designed to investigate weight change in hypogonadal men treated with testosterone. Another study of 261 hypogonadal older men with erectile dysfunction for 5.5 years concluded that TRT reduces obesity and improves metabolic syndrome parameters: total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, fasting glucose, HbA1c, and systolic and diastolic blood pressure [100].

Mortality is higher in men with low testosterone levels. A study of 794 older community-dwelling men aged 50–91 years, followed up for an average of 11.8 years, showed 40% higher mortality in men with serum testosterone in the lowest quartile than those in the higher quartile, mainly by cardiovascular and respiratory disease [101]. Another study confirmed these data in 3637 older community-dwelling men aged 70–88 years, with a mean follow-up of 5.1 years [102]. The Health In Men Study (HIMS) from Australia found that men with serum testosterone in the 50–75% quartile (measured by liquid chromatography-tandem mass spectrometry) had the lowest death rates [103].

Men with prostate cancer treated with luteinizing hormone-releasing hormone agonist and antiandrogen (chemical castration) with previous coronary artery disease-induced congestive heart failure or myocardial infarction were at increased risk of all-cause mortality in a study of 5077 patients with a median age of 69.5 years [104].

Low serum testosterone levels are associated with higher mortality [105], and treatment of hypogonadism improves risk factors in patients with obesity, diabetes, and metabolic syndrome. However, the first evidence that TRT could reduce mortality came when Shores et al. [106] compared death rates of hypogonadal men treated and not treated with testosterone replacement. This observational cohort study of 1031 male veterans older than 40 years with low testosterone levels depicted that those treated with testosterone had half the mortality rate of those not treated (10.3% and 20.7%, respectively) [106]. Similar results were found in patients with type 2 diabetes. In a 6-year follow-up study, including 581 subjects with type 2 diabetes and serum levels of testosterone measured, mortality rates of those receiving TRT (9.1%) were not different from eugonadal diabetic men but were much higher in the group that did not receive testosterone replacement (20.1%) [107].

Conclusion

Diagnosis and treatment of hypogonadism in men with type 2 diabetes mellitus, obesity, and metabolic syndrome can help control these diseases and decrease mortality. Inversely, weight loss and lowering glycated hemoglobin levels can ameliorate hypogonadism in men. It should be kept in mind seeking hypogonadism in patients with insulin-resistant disorders.

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Male Hypogonadism and Aging: An Update

9

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Abbreviations

Bio	Bioavailable
BMD	Bone mineral density
CRP	C-reactive protein
CVD	Cardiovascular disease
CVRF	Cardiovascular risk factors
DHT	Dihydrotestosterone
E1	Estrone
E2	Estradiol
FSH	Follicle-stimulating hormone
FT	Free testosterone
hs-CRP	High-sensitivity C-reactive protein
IL-6	Interleukin 6
LH	Luteinizing hormone
LOH	Late-onset hypogonadism
MS	metabolic syndrome
SHBG	Sex hormone-binding globulin
T	Testosterone
TNF-α	Tumor necrosis factor α
TRT	Testosterone replacement therapy

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Introduction

Serum testosterone (T) levels usually decline after the fifth decade of life due to defects in testicular and hypothalamo-pituitary function, and a high percentage of men over 60 years of age have T concentrations below the lower limit of normal for a young adult male [1–3]. This clinical situation is known as androgen deficiency in the elderly male, andropause, late-onset hypogonadism (LOH) or functional hypogonadism [4]. On the other hand, as in young- and middle-aged men, the elderly male can develop classic hypogonadism, both primary (hypergonadotropic hypogonadism due to testicular failure) and secondary (normo- or hypogonadotropic hypogonadism due to hypothalamic or pituitary insufficiency). There are also different clinical situations that may be accompanied by reversible hypogonadism, and multiple risk factors for developing hypogonadism in older men. The clinical benefits of hormone replacement therapy with T in older men with classic hypogonadism are well established; however, the long-term benefits and risks of T therapy in older men with LOH are under study. In 2017, we conducted a descriptive review on the main aspects related to the definition, clinical manifestations, diagnostic criteria, and therapeutic options of androgen deficiency in the elderly male [5]. In the present review, we update each of the sections by providing the main novelties reported in the last 5 years.

Aging and Gonadal Function

T is the main androgen in humans and, in addition to its main primary function on male secondary sexual characteristics, fertility and sexual behavior, it plays an important regulatory role in metabolism, skeletal musculature, and CV function [6, 7].

In men, serum T progressively decreases with aging in accordance with the decline in testicular function, a condition that is common in older men [8]. On average there is a decrease in total serum T about 3 ng/dL/year in the aging man [9]. This decline is quite evident, especially after the age of 50 years. For example, T levels have been reported to decrease by as much as 49% in males aged 50–80 years [10]. However, more recent studies have shown that, in the absence of serious disease, serum T in older men may be comparable to those found in younger men [11, 12]. The main changes in hypothalamic-pituitary-gonadal (HPG) axis function associated with aging are summarized in Table 9.1.

Table 9.1 Changes in hypothalamo-pituitary-gonadal axis function associated with aging[a]

Decreased amplitude of GnRH pulses
Increased number of GnRH pulses
Decrease negative T feedback
Elevated serum concentrations of SHBG
Altered LH pulses in the T synthesis
Decreased and altered androgen receptors
Increased or low FSH
Increased or low LH
Decreased T production
Decreased sperm production
Alterations in the androgen receptor

GnRH gonadotropin releasing hormone, *T* testosterone, *SHBG* sex hormone-binding globulin, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone
[a] Allan and McLachlan [13], Veldhuis [14]

Definition of Hypogonadism in the Elderly

Male hypogonadism can be defined as a functional testicular deficiency involving insufficient T synthesis and impaired spermatogenesis [15]. This disorder can arise at any time in life and is accompanied by different clinical manifestations. In all cases, the biochemical marker is a low concentration of circulating T. Primary hypogonadism is characterized by elevated serum gonadotropins in the presence of low T levels due to insufficient production in the Leydig cells. The insufficient T production may be accompanied by impaired spermatogenesis. Causes may be congenital, such as Klinefelter syndrome, cryptorchidism, congenital adrenal hyperplasia, or acquired, such as gonadectomy, trauma, orchitis, testicular damage associated with radiotherapy or chemotherapy, or chronic diseases. Central hypogonadism results from insufficient gonadotropin production and is biochemically characterized by low or inappropriately normal gonadotropin levels in the presence of low T. Causes of hypogonadotropic hypogonadism also include congenital (Kallman syndrome) and acquired disorders (sellar or parasellar lesions, infiltrative or inflammatory diseases, trauma, radiation, hemorrhage, or drugs) [16–18].

Maintaining normal T levels is important in sustaining male secondary sexual characteristics, bone mass, muscle mass and strength, erythropoiesis, sexual and cognitive function, and well-being. Low levels of T may adversely affect multiple organ functions and quality of life. The significant decrease in androgen action is associated with a syndrome consisting of osteoporosis, weakness, redistribution of body fat, hypoproliferative anemia, decreased libido and sexual function, malaise, and cognitive abnormalities [19, 20].

It is convenient to differentiate true hypogonadism from suppression of the pituitary-gonadal axis caused by systemic disease [21]. This form of non-gonadal illness may occur in a variety of conditions, such as stress from acute illness, surgery, burns, myocardial infarction, stroke, sepsis, hyperprolactinemia, depression, chronic obstructive lung disease, rheumatoid arthritis, renal disease, HIV-related disease, and severe vitamin D deficiency [20, 22, 23]. Several drugs, such as

glucocorticoids, antipsychotic, opioids, and statins, may induce T deficiency [20]. Furthermore, a progressive decline in serum T with age has been reported in several epidemiological surveys [2, 24]. The Baltimore Longitudinal Study showed that the average annual decrease in total T was 3.2 ng/dL in men older than 53 years, representing approximately 1%/year for a normal lower limit of 325 ng/dL [10]. The rate of fall in serum T with age varies among individuals and is affected by chronic diseases and medications [10]. Aging is also accompanied by an increase in the concentration of sex hormone-binding globulin (SHBG), whereby the concentration of free T (FT) is further reduced.

Age-related androgen deficiency may be exacerbated in the presence of abdominal obesity that results in elevated estrogen levels and SHBG [25]. A recent study conducted in China found a decrease in T levels similar to that found in European subjects, whereas older men who had maintained traditional non-Western diet and largely avoided weight gain did not exhibit lower T levels than younger Chinese men [26]. Interestingly, low serum T levels combined with potentially attributable sexual symptoms only occur in a small minority of aging men (2–6%) and can be largely attributed to comorbidities causing gonadotrophin suppression, and in particular obesity [20, 26].

In some older men, this fall in T can lead to clinical signs and symptoms such as decreased libido, impotence, decreased growth of body hair, reduced muscle mass, fatigue, and decreased bone mass [27, 28]. This situation has been described as androgen deficiency in the elderly male, andropause, LOH or functional hypogonadism [21, 29–31]. The International Society of Andrology and the International Society for the Study of the Aging Male define the LOH as a clinical and biochemical syndrome associated with advanced age and characterized by typical symptoms and a deficiency in serum T levels. It may result in significant detriment in the quality of life and adversely affect the function of multiple organ systems [32–34]. The symptoms most associated with hypogonadism are loss of libido, erectile dysfunction, decreased muscle mass and strength, increased body fat, decreased bone mineral density (BMD) and osteoporosis, decreased vitality, and depressed mood. None of these symptoms are specific of the low androgen state [35]. In the European Male Aging Study (EMAS) [8], the authors surveyed a population sample of 3369 men aged 40–79 years at eight European centers. They found that increased probabilities of sexual symptoms and limited physical vigor were discernible with decreased T levels. In this study, LOH was defined as the presence of at least three sexual symptoms (decreased sexual interest, morning erections, and erectile dysfunction) associated with a total T level of less than 320 ng/dL and a FT level of less than 64 pg/mL.

Epidemiology and Risk Factors for Hypogonadism in the Elderly

Currently, the exact prevalence of hypogonadism in the elderly is not really known. This is because the prevalence varies according to the definition used, the population studied, the method of analysis, and the T cut-off points used. On the other

Table 9.2 Prevalence of hypogonadism according to different criteria for diagnosis and age of the study subjects

Study (Author, year)	Criteria for diagnosis (serum testosterone)	Age (years)	Prevalence (%)
Mulligan et al. (2006) [37]	<300 ng/dL (10.4 nmol/L)	≥45	39
Araujo et al. (2007) [38]	<200 ng/dL (6.9 nmol/L)	40–70	6–12
Hall et al. (2008) [39]	<300 ng/dL (10.4 nmol/L)	30–79	5.6
Wu et al. (2010) [8]	<320 ng/dL (11.1 nmol/L)	70–90	2.1–5
Liu et al. (2015) [40]	<374 ng/dL (13.0 nmol/L)	40–79	9.1

hand, due to increased life expectancy it is expected that the prevalence of hypogonadism in the elderly will increase in the coming decades. Epidemiological studies have shown that its prevalence would be around 6–9.5% of healthy males aged 40–70 years living in the community, increasing to 15–30% in diabetic and obese males [36]. Other studies have shown a different prevalence ranging from 5.6% to 39% depending on the diagnostic criteria for hypogonadism, the geographic area, and the age of the subjects studied (Table 9.2).

The diagnosis of hypogonadism in the elderly is further complicated by the lack of a consensus threshold for defining T deficiency in the elderly. Serum T levels below 288 ng/dL (10 nmol/L) have generally been considered pathological, whereas T values above 350 ng/dL (12 nmol/L) make hypogonadism unlikely [8, 41].

Several risk factors for classical hypogonadism in the elderly can be considered, including all those processes that may affect the integrity of the HPG axis. Moreover, comorbidity is highly prevalent in the elderly, and it is known that several diseases such as those related to metabolic syndrome (obesity, hyperglycemia, insulin resistance, hypertension, and dyslipidemia), acute illnesses, and several drugs, including corticosteroids and opioids, can negatively impact serum T concentrations in the elderly [42, 43].

Hypogonadism and Morbidity

Low T concentrations in the elderly male can negatively influence different clinical aspects such as sexual function, body composition, muscle function, mood, cognition, health-related quality of life, nutritional status, BMD, blood pressure, lipid profile, carbohydrate metabolism, and red blood cell count [8, 29, 44–55]. Additionally, an inverse relationship between T deficiency and systemic inflammation and cardiovascular (CV) comorbidity has been also reported in elderly men [44, 56–65] (Table 9.3). However, it is often difficult to discern whether many of these clinical manifestations are symptoms related to hypogonadism per se or to the aging process and associated comorbidity. Therefore, exclusion of other pathological entities associated with subnormal serum T levels should always be considered for the diagnosis and possible treatment of LOH.

Table 9.3 Clinical and analytical alterations associated with hypogonadism in older men

Mood, cognition, physical function, and health-related quality of life
Depressive symptoms
Cognitive dysfunction
Impaired memory
Altered visuospatial performance
Derangements in executive functions
Decline in visual memory
Poorer self-rated health
Nutritional status and body composition
Poor nutritional status
Diminution of lean body mass
Increased fat mass
Sexual function
Poor morning erection
Low sexual desire
Erectile dysfunction
Low sexual thoughts
Bone
Decreased BMD
Increased bone resorption
Impaired static and dynamic balance
Higher risk of falls
Laboratory abnormalities
Anemia
Hyperglycemia
Hyperlipidemia
Hyperinsulinemia
Increased hs-CRP, TNF-α and IL-6
Elevated fibrinogen
High plasminogen activator inhibitor activity
Decreased IL-10
Cardiovascular
CV risk factors
Larger waist circumference
Visceral obesity
Insulin resistance
Hypertension
Metabolic syndrome
Type 2 diabetes
CV disease
Carotid and aortic atherosclerosis
Stroke
Transient ischemic attack
Lower extremity peripheral arterial disease
Intermittent claudication
Atrial fibrillation
Angina pectoris
Acute myocardial infarction
Congestive heart failure
Mortality
All-cause mortality
CV mortality

BMD bone mineral density, *hs-CRP* high-sensitivity C-reactive protein, *TNF-α* tumor necrosis factor α, *IL-6* interleukin 6, *IL-10* interleukin 10, *CV* cardiovascular

Sexual Function

As occurs in middle-aged men, low T levels contribute to sexual dysfunction in the elderly [29, 47, 66]. In fact, the symptoms more significantly related to low serum T concentration in this population seem to be sexual symptoms [45]. In this setting, the European Male Aging Study, performed in 3369 men aged 40–79 years, showed that three sexual symptoms (poor morning erection, low sexual desire, and erectile dysfunction) were the only symptoms that were significantly associated with a serum T concentration less than 320 ng/dL (11 nmol/L) and a FT level of less than 64 pg/mL (220 pmol/L), suggesting these values as necessary to establish the diagnosis of LOH [8]. These results were similar to those obtained in previous studies [38]. Different questionnaires have been developed to detect the presence of hypogonadism in the elderly, including LOH, and to differentiate them from other disorders, among them are the Aging Male's Symptoms Scale (AMS) [67], the Androgen Deficiency in Aging Males (ADAM) [31], the Massachusetts Male Ageing Study questionnaire or Smith's screener questionnaire (MMAS) [68], and the Androtest [69]. However, despite having a high sensitivity (>80%), the specificity is low (<50%), therefore they are not recommended for universal screening of hypogonadism [29]. Lastly, primary hypogonadism seems to be more likely associated to low sexual thoughts, irrespective of age, than those with secondary or compensated hypogonadism [52]. T therapy is associated with an improvement in sexual symptoms associated to LOH [70]; however, T treatment must be individualized for each particular patient in order to provide the safest and most beneficial results [71].

Nutritional Status and Body Composition

Elderly is usually associated with an increased risk of sarcopenia (low muscle mass), dynapenia (declining muscle strength), and subsequent disability [72]. Moreover, aging subjects show a decline in skeletal muscle metabolism which may be due to different predisposing factors, such as telomere attrition, epigenetic changes, mitochondrial dysfunction, age-related low-grade systemic inflammation, sedentary lifestyle, inadequate nutrition (reduction of appetite and low-protein diet), and hormonal alterations [72, 73]. Among the main hormonal changes associated with aging are a reduction in circulating levels of sex hormones (testosterone and estradiol, E2), growth hormone (GH), insulin-like growth factor type 1 (IGF-1), ghrelin and dehydroepiandrosterone (DHEA), and an increase in serum cortisol levels [72]. A cross-sectional study performed in patients older than 70 years showed that geriatric men with compensated hypogonadism (normal T and increased luteinizing hormone, LH) had worse nutritional status compared with healthy controls [53]. Furthermore, T is a well-known anabolic hormone that acts increasing fat-free mass and decreasing fat mass. A relationship between body composition and testicular function in older men has also been reported [74, 75]. Changes in body composition related to age, such as decrease of lean body mass and increased fat mass, are similar to those occurring in hypogonadism [50, 76, 77]. On the other

hand, body weight and lifestyle factors influence HPG axis function in aging. In fact, weight loss is associated with a rise, and weight gain with a fall, in T, FT, and SHBG in community-dwelling aging men [1]. T therapy is also associated with multiple benefits on muscle function and body composition in LOH patients. However, clinicians must be aware of and mindful of the clinical characteristics of each patient to make the necessary therapeutic adjustments in the management of LOH, with the aim of achieving the most beneficial and safe outcomes in each patient [71].

Bone Mineral Density

In males, both age and hypogonadism, separately, are well-known risk factors for BMD reduction [78–81]. Therefore, male patients diagnosed with LOH are at risk for osteoporosis and osteoporotic fractures [80]. Hypogonadal elderly men show increased bone resorption, greater static and dynamic imbalance, increased risk of falls, and slightly lower BMD [82]. In addition, male aging is associated with variations in reproductive hormones that appear to be related to longitudinal changes in BMD [49]. Different studies suggest that serum E2 levels are more strongly associated with BMD, bone turnover, and bone loss than T levels in middle-aged and older men [54, 83, 84]. A prospective (4.6 years of follow-up) study of 1238 men aged at least 65 years old showed that low bioavailable (Bio) E2 and high SHBG levels were associated with lower BMD and faster hip BMD loss. The combination of low Bio E2, low Bio T, and high SHBG was associated with significantly faster rates of BMD loss [85]. These same changes have also been associated to a higher risk of nonvertebral fractures in a prospective cohort study of men over age 65 years [83]. More recently, an observational (6 years of follow-up) study performed in 1705 men aged 70 years and older from the Concord Health and Ageing in Men Project (CHAMP) study showed that lower SHBG, follicle-stimulating hormone (FSH), LH, and higher estrone (E1) levels protected against loss of BMD. On the other hand, in this study a relationship between incident fractures and serum concentrations of T, dihydroT (DHT), and E2 was not found [49]. Most studies have confirmed that T replacement therapy (TRT) increases BMD and prevents further bone loss in hypogonadal males [86–89]. However, data on the effect of TRT on fracture prevention are not yet available [80]. To date, there are no data on the possible therapeutic use of estrogens on BMD in hypogonadal males. In men with LOH, the presence of osteoporosis should be ruled out in order to establish a specific bone treatment, bearing in mind that TRT should always be initiated following the international guidelines for the treatment of male hypogonadism and that it can be accompanied by a favorable effect on BMD [80].

Mood, Cognition, Physical Function and Health-Related Quality of Life

An association between low circulating T levels and depressive symptoms in elderly men has been reported [90]. The Rancho Bernardo Study [91], a cross-sectional population-based study performed in 856 community-dwelling older men, aged 50–89 years, showed that depressed men had significantly lower levels of Bio T than those without depression. More recently, a 3-year longitudinal population-based study, based on the data of the Longitudinal Aging Study Amsterdam (LASA) including 608 men, aged ≥65 years (median age 75.6 years) revealed that FT levels below 64 pg/mL (lowest quintile) and 50 pg/mL, predicted the onset of depressive symptoms and were associated with depressive symptoms, respectively [92]. So far, there is not enough evidence linking low T levels with major depressive disorder in older men [90]. There is evidence that treatment with T in hypogonadal men improves depressive mood. This effect may appear within 3–6 weeks, with a maximum after 18–30 weeks [71, 93]. Although T therapy improves the positive and reduces the negative aspects of mood, the significance of its effect on mood in the elderly is small [16]. There is some clinical evidence showing that T improves depressive symptoms in elderly males with low T concentrations and persistent, low-grade, late-onset depressive disorder [94]. Therefore, due to the risks of T treatment and its long-term efficacy are not yet well defined in the elderly, is necessary to assess the risks and benefits on an individualized basis before continuing treatment.

Neuropsychological function decreases substantially with age in men [95]. On the other hand, as reported in hypogonadal young men [96] and in men receiving androgen deprivation therapy for prostate cancer [97], low serum T levels in older men may negatively influence some aspects of cognitive function, memory, executive functions, and spatial performance [48, 95, 98]. A prospective, longitudinal (10 years) study performed in 407 men, aged 50–91 years, investigated the relationships between age-associated decreases in endogenous serum T and free T concentrations and declines in neuropsychological performance showing that hypogonadal older men had significantly lower scores on measures of memory and visuospatial performance, and a faster rate of decline in visual memory than eugonadal subjects [99]. Given the increase in life expectancy, it is more than likely that the coming decades will see a considerable increase in both the prevalence of cognitive impairment in older men and LOH. Although two clinical trials conducted in community-dwelling elders found no significant improvements in memory or other cognitive functions with T therapy [100, 101], the results of the effect of T treatment on cognitive function in elderly males with hypogonadism have not been clearly conclusive to date [102–104]. Therefore, further observational studies and clinical trials are needed to clarify the role of TRT on cognition, especially in older men with hypogonadism [95, 104, 105].

Aging is known to be accompanied by a worsening of general health in males. The age-associated reduction in T appears to be related to the deterioration of general health status in these individuals. A cross-sectional, longitudinal study

examined the relationships between reproductive hormones and self-assessed health and quality of life in community-dwelling older men at baseline, as well as changes during a 2-year follow-up. Lower serum T and E1 levels were associated with worse self-rated health, whereas lower serum E1 levels predicted subsequent deterioration in self-rated health over time. No association was found between T levels and longitudinal changes in overall health status [106]. A more recent cross-sectional study performed in 788 participants older than 65 years with evidence of sexual dysfunction, diminished vitality, and/or mobility disability, and an average of two T <275 ng/dL from T trials showed that FT and T levels were consistently, independently, and positively associated, albeit to a small degree, with measures of sexual desire, erectile function, and sexual activity, but not with measures of vitality or physical function in symptomatic older men with low T [45]. A prospective, observational and longitudinal analysis performed in 261 patients (mean age 58 years) diagnosed with LOH and treated with long-acting intramuscular T undecanoate for up to 5 years showed a clear improvement in both psychological and physical characteristics as physiological T levels were reached and maintained contributing to an improvement in the health-related quality of life in these LOH men [107].

Erythrocyte Count

Anemia is common (~11%) in elderly men. It is usually primarily due to iron deficiency, chronic disease/inflammation, and chronic kidney disease, and it has been negatively associated with morbidity and mortality [108, 109]. T has a stimulatory effect on erythropoiesis [110]. This effect might explain, at least in part, the association of anemia in severe hypogonadism in males. In 2006, Ferrucci et al. [46] reported for the first time that serum T level is a risk factor for anemia in older persons. Older men with low T levels tend to have lower hemoglobin levels, are more likely to have anemia, and have a higher risk of developing anemia over a 3-year follow-up period. On the other hand, T therapy in hypogonadal males is associated with a dose-dependent increase in hemoglobin. This hemoglobin response to T is greater in the elderly than in young males [111]. In this regard, the Endocrine Society suggests that T therapy be offered on an individualized basis after an explicit discussion of the potential risks and benefits to males over the age of 65 who have symptoms or conditions suggestive of T deficiency (such as low libido or unexplained anemia) and consistently and unequivocally low morning T concentrations [16].

Inflammation, Metabolic Derangements, and Cardiovascular Disease

Several observational studies have shown an inverse association between circulating T levels with CV disease in elderly males. A lower risk of CV events has been described in older men with higher serum T levels [112–114]. On the contrary, T

deficiency in older men has been associated with both CV risk factors (CVRFs) and CV morbidity [59, 61, 62, 64, 65, 115, 116]. Other studies found a positive association between low serum T and high serum E2 with lower extremity peripheral arterial disease [61] and lower free T and higher LH levels with abdominal aortic aneurysm in this population [65]. In one study performed in 2703 men aged 70–89 years, lower levels of T or DHT, but not E2, were associated with the presence of intermittent claudication independently of age, smoking, obesity, and other CVRF [62]. An association between hypogonadism and personal history of heart disease (heart failure, angina pectoris, and/or acute myocardial infarction) in aged men hospitalized for acute disease has also been reported [42]. In this study congestive heart failure and acute cerebrovascular disease were the second and the third causes of hospitalization, respectively. Lastly, in very old men (≥80 years) T deficiency was strongly associated with atrial fibrillation risk [59]. However, these observations have not been demonstrated in younger males. For example, in a study of community-dwelling men aged 40–69 years, lower total T concentrations were not associated with an increased risk of myocardial infarction (MI), stroke, heart failure (HF), or major adverse cardiovascular events (MACE) [117].

Chronic low-grade inflammation is a risk factor for atherosclerosis [118]. Elevated serum levels of C-reactive protein (CRP) and certain cytokines such as tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) have been shown to be harmful to the CV system, increasing the propensity for cardiovascular disease (CVD) [115]. It is now known that T plays an immunomodulatory role. Low T levels have been related to an inflammatory condition that is associated with (1) increased high sensitivity (hs-CRP) [119], proinflammatory cytokines (TNF-α and IL-6) [120], as well as fibrinogen and plasminogen activator inhibitor activity promoting a hypercoagulable state [121], and (2) decreased IL-10, an anti-inflammatory cytokine, promoting a pro-atherosclerotic condition [122]. On the other hand, other more recent studies have shown that higher levels of T are inversely associated with TNF-α and IL-6 [123].

Results from the EMAS have shown that severe LOH in community-dwelling men aged 40–79 years is associated with larger waist circumference, insulin resistance, and the metabolic syndrome (MS) [58, 60]. Other studies have reported that low serum T concentration is independently associated with insulin resistance in non-diabetic older men [63] and has a predictive value for the development of not only MS but also visceral obesity [44] and type 2 diabetes (T2D) [56]. The relationship between low T and diabetes could be via a bidirectional relationship with visceral fat, muscle, and possibly bone [56]. Hypertension has also been associated with increased prevalence of low T [124]. Furthermore, T levels in men are inversely associated with the degree of carotid and aortic atherosclerosis suggesting that loss of androgens in older men might adversely affect CV risk [57, 58, 125, 126]. On the other hand, a number of studies [112, 127], but not all [128], have reported a relationship between gonadotropins, mainly LH, and an adverse CVD risk profile in older men. In this setting, high LH levels have been positively associated with increased incidence of MACE in older men with sexual dysfunction [112, 127]. There are numerous studies on the effect of T treatment on the CV system with

mixed results, which do not make clear the potential beneficial effects of hormone replacement therapy in men with late-onset hypogonadism. On the other hand, questions related to safety and its possible limitation of use in routine clinical practice should always be taken into account [129].

Hypogonadism and Mortality

An inverse relationship between LOH and mortality in the elderly male population has been reported [130–133] (Fig. 9.1). A case-control prospective study performed in 11,606 men, aged 40–79, showed an inverse relationship between serum T levels and mortality from all causes [134]. Furthermore, independent of multiple risk factors and various pre-existing health conditions, low serum T levels in older men were predictors of long-term (20-year) mortality [131, 132]. A cross-sectional study showed that low T at admission in aged hospitalized male patients admitted for acute disease was associated not only with prevalent morbidity (personal history of heart disease, cancer, respiratory disease, and renal insufficiency) but also with in-hospital mortality [42]. In fact, the likelihood of death during hospitalization in this population increased at lower serum T concentrations (Figs. 9.2 and 9.3). In spite of the probability of recovery of gonadal function is high after discharge [135], the presence of low levels of reduced serum T concentration at hospital admission also behaves as a powerful predictor, even more than age, not only for hospital mortality but also for long-term (5 years) all-cause mortality [136] (Fig. 9.4).

Similarly, some studies have shown an association between low serum T concentrations and CV mortality in community-dwelling aged men [134, 137]. This fact might be in relation to the association of hypogonadism with risk factors for CVD, mainly insulin resistance, MS, and T2D as previously indicated. Low T levels (<200 ng/dL) discovered during hospitalization for acute disease in older men was accompanied by a median survival time for CV mortality significantly lower than eugonadal patients after hospital discharge. In these patients hypogonadal status

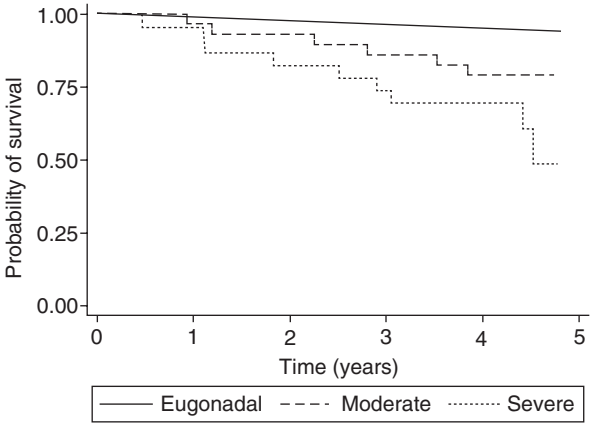

Fig. 9.1 Unadjusted Kaplan–Meier survival curves for late onset hypogonadism (LOH) status in community-dwelling aging men showing a strong association between LOH and all-cause mortality with a progressive decline in the probability of survival over time. (Data from [133]. Reproduced with permission of The Endocrine Society)

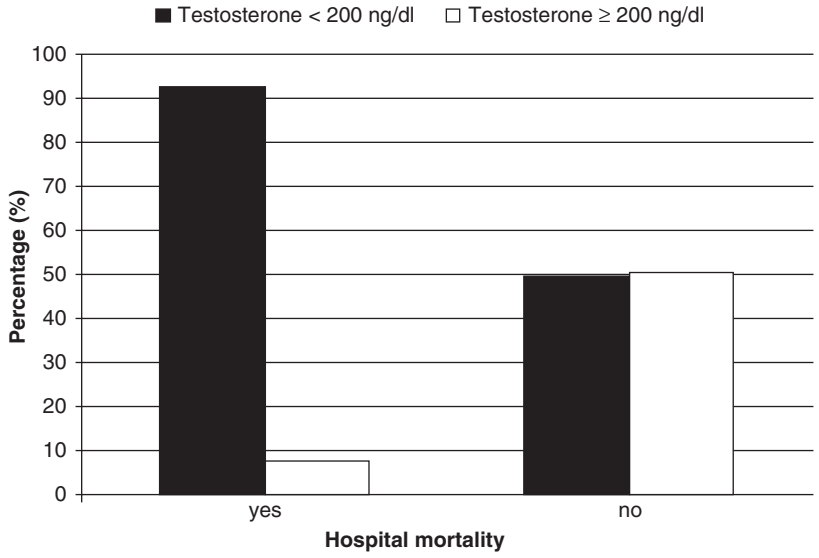

Fig. 9.2 Percent distribution of 150 aged male patients hospitalized for acute disease according to gonadal status at entry and hospital mortality [13 patients (8.7%) died during hospitalization]. (Data from [42]. Reproduced with permission of Springer Science + Business Media)

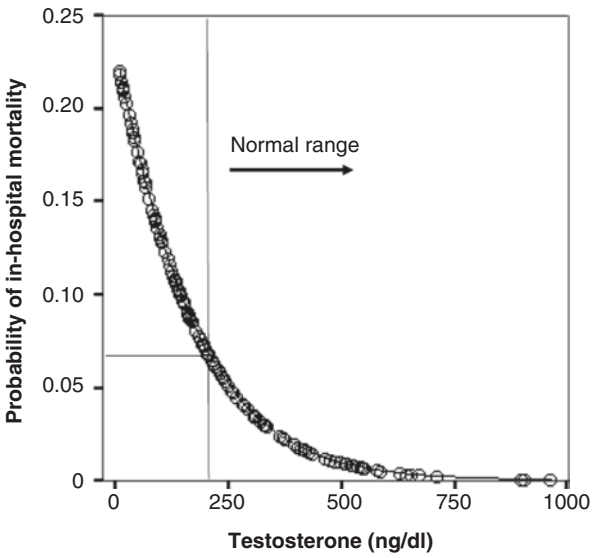

Fig. 9.3 Probability of death during hospitalization in 150 aged hospitalized male patients as a function of serum concentrations of total T at the time of hospital admission, according to a model of logistic regression analysis. (Data from [42]. Reproduced with permission of Springer Science + Business Media.)

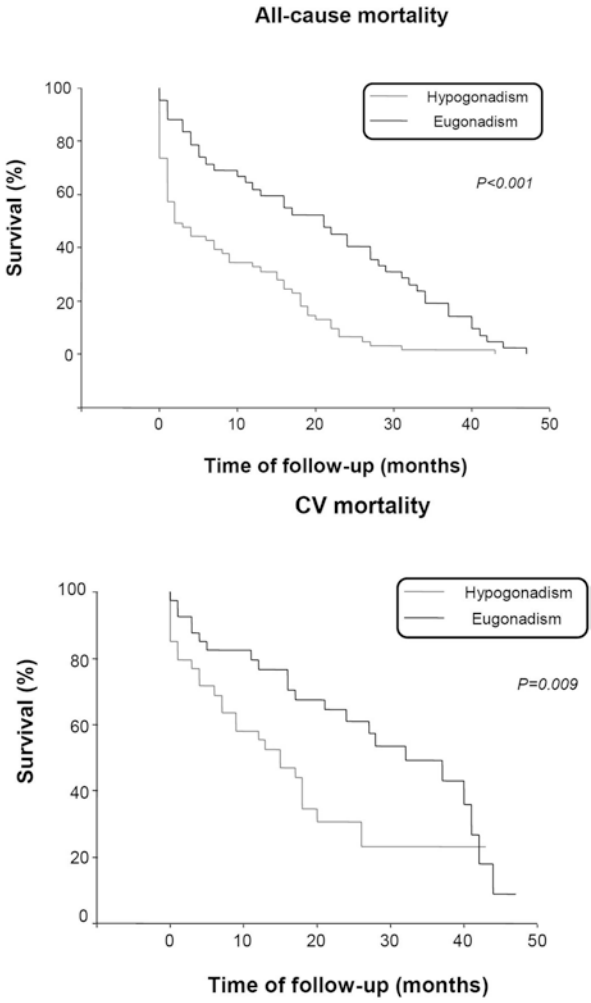

Fig. 9.4 Kaplan–Meier survival analysis for all-cause and CV mortality in 150 elderly male patients admitted for acute illness stratified according to gonadal status at hospital admission. (Data from [136]. Reproduced with permission of Thieme)

was also independently associated to CV mortality, even more than personal history of CVD and diabetes. The coexistence of two predictors of CV mortality such as LH and hypogonadism suggests that the primary gonadal failure might be an important marker of CV mortality risk in this population [136] (Fig. 9.4).

Lastly, male sex and older age are known to be risk factors for increased SARS-CoV-2 infection severity and fatality [138–140]. On the other hand, hospitalized men with coronavirus disease-19 (COVID-19) present with rather low T levels. It is known that serum T levels are known to correlate negatively with multiple inflammatory biomarkers that are associated with worse clinical outcome and increased risk of intensive care unit admission or death in SARS-CoV-2 infected patients [141]. Therefore, it is not surprising that elderly males hospitalized for COVID-19 have a worse prognosis and higher mortality rate than younger males, as has been demonstrated in different observational and retrospective studies [141–143].

Replacement Hormonal Therapy

Objectives of Therapy

The objective of treatment with T in the aging man is to reverse or prevent the symptoms and long-term effects of hypogonadism and to maintain a good general condition, including maintenance of virility, restoration of sexual function and libido, restoration of a sense of well-being, optimization of bone density and prevention of osteoporosis, and improvement of CV risk [144]. It is suggested that the treatment goal in elderly patients is to achieve T levels in the mid-lower part of the normal range of young men [16, 17, 33]. Improvement in signs and symptoms of T deficiency should be sought and failure to benefit clinical manifestations within a reasonable time interval should result in discontinuation of treatment [33].

T trials in older men have drawbacks such as small sample size, inclusion of healthy older men, variable dosing regimens, and the use of surrogate outcomes. Overall, these trials have shown that in comparison with placebo, T induces moderate effect on lumbar BMD, an increase in lean body mass, a reduction in fat mass, an improvement in grip strength. Imprecise or inconsistent results have been reported in relation to T effects on bone fractures, lower extremity muscle strength, sexual satisfaction, depression, cognition, or quality of life [16]. Although data on the effects of T treatment on bone fractures are not available, it is assumed that T treatment has the potential to contribute to fracture reduction, and assessment of bone density at 2-year intervals has been recommended in hypogonadal men with osteopenia [35].

Indications in the Aging Man

Androgen treatment is only indicated in older men with symptoms and signs of hypogonadism and a clear-cut decrease in serum T concentration on more than one occasion [16, 17]. However, routinely prescribing T therapy to all men 65 years of age or older with low T concentration is not recommended [16].

A recent study reported that in symptomatic men 65 years of age or older, raising T concentrations for 1 year from moderately low to the mid-normal range for men 19–40 years of age had a moderate benefit with respect to sexual function and some benefit with respect to mood and depressive symptoms but no benefit with respect to vitality or walking distance [145]. Other studies have shown increased hemoglobin and improvement in anemia [146], increased volumetric and areal bone mineral density and the estimated strength of trabecular and peripheral bone in the spine as well as hip [147].

In cases of older men with symptoms suggestive of hypogonadism, but whose androgen levels are normal, the elevation of serum T by exogenous administration of this hormone is not accompanied by a relief of symptoms. Before beginning therapy in older men, clinicians should consider the severity of androgen deficiency, the contribution of comorbidities and medications to clinical manifestations, and

the treatment of potentially reversible functional causes of hypogonadism [148]. LOH is often comorbid to obesity and several chronic diseases [21]. For this reason, lifestyle modifications should be encouraged in patients with obesity or type 2 diabetes [149]. The potential clinical benefits, risks and contraindications should also be discussed with the patient. T should not be prescribed for the treatment of symptoms that are not specific of LOH, and that can be caused by other conditions, such as obesity, depression, diabetes, or chronic diseases even though a low T level is associated with these conditions [4, 21, 150].

There is no evidence-based consensus on the need for T treatment in aging men, therefore, current recommendations are based on expert opinion [151]. Most assays for total T display 250–300 ng/dL as the lower limit of normality for young men, while the lower limit for FT measured by equilibrium dialysis is 5.0–6.5 ng/dL. However, a single threshold value may not be valid for all men. A population-based study showed that total T thresholds below which a man is considered to have low levels of this androgen were 251 ng/dL, 216 ng/dL, 196 ng/dL and 156 ng/dL for men of 40 years, 50 years, 60 years, and 70 years, respectively [152]. The American Association of Clinical Endocrinologists considers that men with symptomatic hypogonadism and total T levels below 200 ng/dL are potential candidates for treatment [144]. Morley [153] has proposed that the proper diagnosis of LOH, and therefore an indication of substitution treatment, is made only in elderly men who meet three conditions: (1) symptoms of hypoandrogenism (with special reference to sarcopenia and osteopenia), excluding the most common causes of these symptoms; (2) low total T levels (below 268 ng/dL) or Bio or FT (measured or calculated) if total T is between 268 and 380 ng/dL; and (3) an adequate response to a 3-month treatment trial with symptom improvement [153]. The Endocrine Society does not clearly define serum T levels below which T therapy should be offered to older men. The updated edition of clinical practice guidelines for T therapy suggest that, in hypogonadal men 55–69 years old, who are being considered for T therapy and have a life expectancy >10 years, the potential benefits and risks of evaluating prostate cancer risk and prostate monitoring should be discussed with the patient [16].

Contraindications

Androgen therapy is contraindicated in men with breast cancer or prostate cancer [16, 32, 33]. Androgen therapy should not be initiated until a complete urologic evaluation has been performed in men with palpable prostate nodule or induration, with prostate-specific antigen (PSA) >4 ng/mL or PSA >3 ng/mL in men at high risk of prostate cancer, such as African American men or men with first-degree relatives with prostate cancer [16, 17].

The Endocrine Society also suggest that clinicians offer evaluation of prostate cancer risk beginning at age 40 in hypogonadal men who are being considered for T therapy and are at high risk of prostate cancer. In patients who agree to prostate cancer monitoring, clinicians should evaluate PSA levels and perform a digital prostate examination at baseline and at 3–12 months after starting testosterone

treatment. After 1 year of T therapy, it is recommended to follow the guidelines for prostate cancer screening based on the age and race of the patient [16]. Men successfully treated for prostate cancer and suffering from confirmed symptomatic hypogonadism are potential candidates for T substitution after a prudent interval if there is no clinical or laboratory evidence of residual cancer [35].

Androgen treatment is not recommended in men with hematocrit >50%, blood viscosity, and untreated severe obstructive sleep apnea syndrome [16, 17]. Conditions in which fluid retention can be harmful, such as congestive heart failure, and severe lower urinary tract symptoms are also contraindications. Mood disorders can be exacerbated by androgen preparations that cause supraphysiological increased plasma T concentrations. Age per se is not a contraindication to initiate T treatment, although individual assessment of comorbidities and potential risks is necessary in the elderly [35].

Testosterone Preparations

There are different T preparations with diverse advantages and disadvantages (Table 9.4). Clinicians should choose the most appropriate formulation for each patient according to his needs [154]. The choice depends on the availability, cost, convenience, dose needed, and preference of the patient.

Table 9.4 Advantages and disadvantages of the main T formulations for the treatment of male hypogonadism

Formulation	Dose	Advantages	Disadvantages
Non scrotal patch	1–2 patches per day (5–10 mg of T per day)	Avoids IM injection	Low serum T levels in some men
		No alterations in T:DHT ratio	Skin irritation
		Mimics the circadian rhythm of T	
		Lower increase in hemoglobin than with IM T esters	
Scrotal patch	A daily 6-mg patch	Avoids IM injection	Elevated DHT and reduced T:DHT ratio
			Shaved scrotal skin
T transdermal gels	5–10 g of gel per day (50–100 mg of T per day)	Avoids IM injection	Variability in T concentrations
		Dose flexibility	Moderate increase in DHT
		Easy application	Skin irritation in few patients
		Good skin tolerance	Potential transmission to another person by skin contact
		Less erythrocytosis than injectable T	High cost

(continued)

Table 9.4 (continued)

Formulation	Dose	Advantages	Disadvantages
T axillary solution	60 mg (2 pump or 2 twist actuations) once daily	Good skin tolerability	Potential transmission to another person
			Variability in T concentrations
			Skin irritation in few patients
			Moderate increase in DHT
T enanthate and cypionate	100–200 mg every 1–2 weeks, IM	Great experience and effectiveness	IM injection needed
		Dose flexibility	T levels and symptoms fluctuations
		Low cost	
IM T undecanoate	1000 mg every 10–14 weeks, IM	Low frequency of injections	High cost
			Large injection volume (4 mL)
Alkylated androgens	Not recommended	Active PO	Variable clinical response
			Hepatic adverse effects
			Altered lipid profile
Oral T undecanoate	40–80 mg, bid or tid	Active PO	Erratic serum T levels
			High frequency of administration
			Gastrointestinal side effects
			Elevated DHT
T pellets	4–6 200-mg pellets every 4–6 months, SC	Unchanged T:DHT ratio	Surgical incision required
		Normal values of T for 4–6 months	Infection
			Extrusion
Bioadhesive bucal tablets	30 mg bid	Absorption at buccal mucosa	Gingival adverse effects
		Unchanged T:DHT ratio	Twice daily application
Nasal T gel	11 mg bid or tid	Rapid absorption	Multiple daily intranasal doses
			Local nasal side effects
			Not appropriate for men with nasal disorders

T testosterone, *PO* orally, *DHT* dihydroT, *SC* subcutaneous, *IM* intramuscular

The most widely used preparations in clinical practice are transdermal formulations and intramuscular injections [154]. Use of transdermal patches and gels avoids injections, and their effect is less sustained than that of intramuscular esters, therefore being more attractive to elderly patients because the possible development of a contraindications requires rapid discontinuation of T [33, 35]. Transdermal preparations provide the closest approach to the normal circadian rhythm of T [155]. The main advantage of these preparations is the maintenance of relatively stable concentrations of T, which prevents fluctuations in mood, energy, and libido that may occur with intramuscular esters.

Scrotal patches require prior shaving of the scrotal skin and adherence may be poor (Table 9.4). Furthermore, because scrotal skin is rich in 5α-reductase, patients can have high levels of DHT [156]. Non-scrotal patches are used on non-genital

skin, usually in the back, thigh, or upper arm. Each patch releases 5 mg of T for 24 h [157, 158], and application at night is recommended with DHT levels remaining within the normal range [159]. The advantages of these preparations compared with intramuscular injections are its ease of use, lack of need for injection, and maintaining T levels without fluctuation. The disadvantages include higher cost and skin irritation requiring discontinuation in some patients. Several preparations containing 2.5 g, 5 g, and 10 g of a hydroalcoholic gel with 25 mg, 50 mg, and 100 mg of T, respectively, intended to provide 2.5 mg, 5 mg, and 10 mg of active principle, respectively, are available. Another formulation is supplied inside a vial with a pump mechanism that supplies 0.5 g of gel (10 mg of T) each time the piston is depressed. Thus, the dose can be adjusted in fractions of 10 mg per pulse [34, 160]. Serum DHT levels are moderately higher, and the T:DHT ratio is lower in hypogonadal men treated with T in comparison with healthy men (Table 9.4).

Gels are generally well tolerated, although can cause skin irritation that usually does not require treatment discontinuation [161]. The gel dries quickly after application, albeit it is possible to transfer to another person if there is direct skin contact.

T preparations for intramuscular injection are oily preparations that allow slow release over a long period after been injected. We currently have short-acting and long-acting formulations. The short-acting preparations, T enanthate and cypionate, have been used for many years [162]. Administering a weekly dose of 100 mg of T enanthate results in slightly supraphysiologic levels of T for 1 or 2 days after injection, followed by normal concentrations until the next injection. Administration of 200 mg every 2 weeks produces a higher peak during the first days followed by concentrations within the normal range that may fall below the lower limit of normality within the days before the next injection. Treatment schedules with higher doses, i.e., 300 mg every 3 weeks or 400 mg every 4 weeks, may lead to higher peaks and valleys. Advantages of these preparations are their low cost and high effectiveness (Table 9.4). Disadvantages include the need for intramuscular injection and fluctuations that occur in serum T after each injection. Some men have episodes of fatigue or depression during periods of low T levels and stages of breast tenderness and hyperactivity during periods of high levels of T. Mood and sense of energy can also fluctuate according to androgen levels [163]. Recently, administration of T cypionate and enanthate by subcutaneous injection at weekly dose of 50–100 mg has comparable pharmacokinetics and safety to intramuscular administration and a steady-state concentration of serum testosterone between dose intervals [164, 165]. These new preparations have the advantage over intramuscular preparations that they allow self-administration and, therefore, favor patient autonomy [166, 167].

The long-acting T undecanoate, administered by intramuscular injection at doses of 1000 mg every 10–14 weeks, has shown to maintain T levels within the normal range [168]. The injection can be painful and should be administered very slowly deep into the gluteal muscle. T levels should be measured just before the third injection in order to determine the frequency of future injection intervals.

There are other less commonly used preparations. Alkylated androgen therapy has been accompanied by hepatic adverse effects such as cholestatic jaundice, peliosis, and hematoma [169, 170]. Non-aromatizable oral preparations cause a rise in low-density lipoprotein cholesterol and a reduction in high-density lipoprotein cholesterol, which can increase the CV risk [171]. Therefore, alkylated androgens such as methylT, fluoxymesterone, and oxandronole should not be used in androgen replacement therapy for hypogonadal males.

T undecanoate, currently used intramuscularly, is also available in oral preparations [172]. An open-label, single-arm, multicenter study in 95 hypogonadal men receiving 225 mg of oral T undecanoate twice a day, without dose adjustment, showed that this preparation restored T levels to the normal range in most subjects. This new oral T replacement therapy can provide an option for no-titration oral T replacement therapy and has the potential to improve patient compliance.

T pellets are implanted subcutaneously and provide a prolonged release of T for 3–6 months [173]. The recommended dose is 200-mg pellets every 4–6 months. Infection and extrusion of the pellets have been described as adverse effects, although with current preparations the rate of these complications is low [174]. Because pellets release T over several months and are not removable, they are less desirable for treatment in older men [148].

Bioadhesive buccal tablets contain 30 mg T and are applied to the gum twice a day. The tablet should be pressed firmly for 30 s to cause adhesion and remain in the mouth for 12 h. The tablets can cause gingival irritation and alterations in taste [175].

Monitoring of Therapy

The efficacy of T treatment is monitored by self-reported improvement of symptoms and measurement of serum T levels, initially at 3–6 months after starting therapy and then on a yearly basis. If no benefit is reported by the patient, discontinuation of treatment should be considered and other causes of symptoms should be investigated [160].

Older men with hypogonadism should be monitored with regular checking of hematocrit, hemoglobin, and PSA levels and by initially doing digital rectal examinations at 3–6 months and then yearly while undergoing T therapy [16]. In men with osteoporosis and without high risk for fracture, bone mineral density measurements 1–2 years after initiating T therapy is recommended [16]. Metabolic parameters such as blood glucose and lipid profile can also be measured [149]. Other safety parameters that should be monitored during therapy include lower urinary tract symptoms, gynecomastia or self-reported breast tenderness, and induction or worsening of obstructive sleep apnea [148]. Formulation-related adverse reactions should also be checked at each visit. T therapy in men with classic forms of hypogonadism is usually life-long, whereas the optimal duration of treatment for late-onset hypogonadism is uncertain because this condition may spontaneously normalize [176].

Adverse Effects of Testosterone

Elevation of PSA Levels and Benign Prostatic Hyperplasia

The average increase in PSA after initiation of treatment with T is about 0.3 ng/mL in young males and 0.4 ng/mL in older men. Increases over 1.4 ng/mL are uncommon [177]. A meta-analysis of 15 studies including 739 patients that received T replacement and 385 controls concluded that T therapy does not increase PSA levels in men being treated for hypogonadism, except when it is given intramuscularly, and even the increase with intramuscular administration is minimal [178].

Several studies have failed to show an exacerbation of voiding symptoms attributable to benign prostatic hyperplasia (BPH) during T replacement therapy or a higher rate of urinary retention in patients receiving T compared with patients receiving placebo [158, 179–183]. Two recent studies [184, 185] and a meta-analysis [186] have confirmed that T therapy does not worsen lower urinary tract symptoms in men who do not have severe symptoms prior to treatment. However, some men may have an exacerbation of symptoms of BPH (Table 9.5), and in those cases an appropriate urologic evaluation should be performed before proceeding with the androgen treatment.

Prostate Cancer

A meta-analysis [187] showed that the rate of prostate events was significantly higher in men treated with T (n = 651) compared with those receiving placebo (n = 433) with a relative risk of 1.78 (95% confidence interval, 1.82–7.51). The rates of prostate cancer and PSA >4 ng/mL were not significantly different between the two groups. In a meta-analysis of 51 randomized and nonrandomized studies published between 2003 and 2008 [188], there was no significant effect of T therapy on the incidence of prostate cancer, the need for prostate biopsy, or the risk of other prostatic or urologic outcomes such as a significant increase of PSA, when

Table 9.5 Risks associated to therapy with different testosterone preparations

Associated risk	Comment
Acne and oiliness of skin	Uncommon
Benign prostatic hyperplasia	Requires surveillance
	Rarely clinically relevant
Prostate cancer	Essential surveillance
	An increased risk has not been conclusively demonstrated
Cardiovascular adverse events	An increase in cardiovascular risk has not been demonstrated
	Current data suggest beneficial effects
Lipid profile	Neutral effect. No data of overt worsening
Sleep apnea	Uncommon
Erythrocytosis	Essential surveillance
	3–18% with transdermal preparations
	Up to 44% with intramuscular injections
Gynecomastia	Uncommon, usually reversible
Skin irritation	Common with patches, uncommon with gels, rare with injections
Hepatotoxicity	Alkylated oral agents

compared with the placebo group. A registry-based study analyzed a cohort of 12,779 men who were newly diagnosed with hypogonadism [189]. During 58,224 person-years of follow-up, the use of T replacement therapy was not associated with an overall increased risk of prostate cancer compared with nonuse. In men with prostate cancer, a recent systematic review, including 2459 testosterone-treated patients found that, compared to nontreated patients, testosterone-treated patients may not have increased risks for disease progression. However, the quality of currently available evidence is extremely poor. The authors concluded that T replacement therapy may be harmful in men with advanced disease burden, in those with untreated prostate cancer undergoing active surveillance, and in those with successfully treated prostate cancer but having high-risk disease [190].

A recent systematic review [191] suggested that until more definitive data becomes available, clinicians wishing to treat their hypogonadal patients with localized prostate cancer with T replacement therapy should inform them of the lack of evidence regarding the safety of long-term treatment for the risk of cancer progression. Although some authors consider patients with a history of organ-confined prostate cancer for T replacement on an individualized basis (after radical prostatectomy, with undetectable PSA, and no detectable residual disease 2 or more years after surgery) [192]. The Endocrine Society precludes a general recommendation, because of the lack of data from randomized clinical trials [16]. Nevertheless, in patients without known prostate cancer, the evidence seems sufficient to think that androgen therapy does not increase the risk of subsequent discovery of prostate cancer.

In men older than 50 years, symptoms of BPH should be evaluated and treated before initiating T replacement. A rectal exam and PSA quantification are needed before beginning androgen treatment. Treatment should not be initiated without urologic evaluation in patients with suspicious findings on rectal examination (i.e., palpable prostate nodule or induration, asymmetry, or areas of increased consistency), or with PSA >4 ng/mL or >3 ng/mL in men at high risk of prostate cancer. During follow-up, patients should be monitored for prostate disease as previously stated [35]. The patient should be referred for full urologic evaluation if a prostate nodule is palpable, if PSA level is >4 ng/mL, or if the PSA increment is >1.4 ng/mL over a period of 1 year. In patients in whom a PSA level is used after 6 months of treatment with T and in whom data on PSA are available for more than 2 years, an increase in PSA >0.4 ng/mL/year also requires a urologic evaluation [17, 177]. Urologic consultation should also be performed in patients with severe lower urinary tract symptoms.

Cardiovascular Effects

Epidemiological studies have shown that low T levels are more predictive of CVD than high levels [57]. Older men with untreated hypogonadism have increased mortality compared with eugonadal men, even after adjusting for age, body mass index, current smoking, and poor general health [133, 193]. Other studies suggest that high levels of T may even have a favorable effect on CV risk [194–196]. Overall mortality and CV event rates did not differ among T- and placebo-treated men in a

meta-analysis [188]. T treatment did not differ from placebo in the incidence of diabetes or in the changes from baseline in cardiometabolic risk factors or systolic and diastolic blood pressure levels [188]. Nevertheless, a randomized, placebo-controlled trial of T treatment in older men with impaired mobility and low T levels who also had a high baseline prevalence of CV disease was stopped because of an increased number of CV events in T treated men [197], particularly in men who achieved high serum T levels [198]. Other studies have also reported increased CV risks in men who received T therapy [199, 200].

An extensive systematic literature review from articles published between 1940 and 2014 concluded that there is no convincing evidence of increased CV risk with T therapy. On the contrary, the authors of this review highlight that mortality and incident coronary artery disease are inversely associated with serum T concentrations, and that there appears to be a strong beneficial relationship between normal T and CV health [201]. A randomized placebo-controlled clinical trial has shown that the rate of subclinical atherosclerosis progression in older men with low or low-to-normal T levels did not differ significantly between men treated with T or placebo [202]. The rates of intima-media thickness progression or change in coronary artery calcium scores were not modified by T treatment. However, this study was not powered to evaluate CV events.

The NIH T trials studied a group of 790 men, 65 years of age or older, with low serum T concentration, and symptoms suggesting hypogonadism. Participants were randomly assigned to receive either T gel or placebo gel for 1 year [145]. The authors found that, in these patients, raising T concentrations for 1 year from moderately low to the mid-normal range for men 19–40 years of age had a moderate benefit with respect to sexual function and some benefit with respect to mood and depressive symptoms but no benefit with respect to vitality or walking distance. Although not powered to investigate the safety of T treatment, the NIH T trials reported that a total of 14 men (50% of them received placebo) had a myocardial infarction, stroke or death from cardiovascular causes.

More recently, a systematic review showed that T therapy in patients with heart failure was not associated with an increase in mortality or heart failure hospitalization rate [203]. In a large observational cohort of men with previous myocardial infarction, normalization of T levels with T therapy was not associated with an increased risk of recurrent myocardial infarction, and was associated with decreased all-cause mortality compared with those with non-normalized T levels [204].

Several epidemiological studies and meta-analysis have shown no association between T therapy and the risk of venous thromboembolism in men [205–208]. However, another study showed that T therapy was associated with an increase in short-term risk for venous thromboembolism among men with and without hypogonadism [209]. Some evidence in this study indicated that the association was more pronounced among younger men. These findings suggest that caution should be used when prescribing T therapy.

Our knowledge about the CV safety of T will be greatly expanded in the coming years thanks to two ongoing projects. The TRAVERSE trial is an ongoing study to evaluate the effect of T replacement therapy on the incidence of MACE and efficacy

measures in hypogonadal men [210]. To date, more than 5000 men, aged 45–80 years with low serum T levels and high cardiovascular risk, have been included and randomly allocated to T gel or placebo for 5 years. The final data collection date for primary outcome measure is June, 2022 [211].

The Testosterone Efficacy & Safety (TestES) Consortium was funded by the National Institute of Healthcare Research Health Technology Assessment (NIHR HTA) in the UK. TestES uses a rigorous approach called Individual Patient Data (IPD) to impartially select and combine clinical trials data from around the world. It will answer three specific questions related to the benefits and risks to men with hypogonadism taking androgen replacement therapy, the experience and acceptability of this therapy in men with hypogonadism, and the their cost-effectiveness [212].

Lipid Profile and Blood Glucose

T replacement produces a neutral effect on plasma lipids, sometimes accompanied by a minimal reduction in high-density lipoprotein (HDL)-cholesterol and a reduction in total cholesterol [158, 182, 213–216]. A meta-analysis on the effects of intramuscular T esters on serum lipids in hypogonadal men concluded that HDL-cholesterol levels were reduced in three studies and remained unchanged in 15 studies. Total cholesterol levels were reduced in five studies, increased in two studies, and remained unchanged in 12 studies. Finally, levels of low-density lipoprotein (LDL)-cholesterol decreased or remained unchanged in 14 of the 15 studies analyzed [216]. The decrease in HDL-cholesterol has been shown to be more marked in studies enrolling older patients using intramuscular T preparations [188].

In hypogonadal men with metabolic syndrome T replacement therapy has been associated with a significant reduction in fasting glucose, homeostatic model assessment (HOMA) index, and waist circumference in patients with MS [217]. An improvement of fasting glucose, hemoglobin A1c, and triglyceride levels has also been observed in subjects with T2D, thus suggesting a possible role of T replacement in improving the metabolic outcome in patients with metabolic disorders [149]. Other authors have confirmed an improvement in metabolic syndrome in older men with LOH [55].

Sleep Apnea

T treatment may worsen sleep apnea syndrome [32]. This usually occurs in men treated with high doses of T who have other risk factors for the development of sleep apnea. The mechanism of this effect is unknown, but it is thought to be related to a central effect [218].

Erythrocytosis

T therapy can cause erythrocytosis (hematocrit >54%) in some men with hypogonadism. This is more common in older men than in young men [213]. Its incidence has been estimated at 3–18% of patients treated with transdermal formulations and up to 44% of patients treated with injectable preparations [177]. In patients treated with transdermal preparations there is a direct relationship between the dose of T

and the incidence of erythrocytosis [34]. Two previous meta-analyses showed that the relative risk of having hematocrit >50% in men who were treated with T was 3.69 [187] and 3.15 [188], respectively, compared with men treated with placebo or without intervention. Furthermore, a recent meta-analysis, including four randomized clinical trials with 1779 hypogonadalmen, showed that T therapy was associated with an 8.5-fold higher relative risk of developing erythrocytosis than that found in placebo-treated subjects [186].

In men who develop erythrocytosis during T administration, treatment should be discontinued until hematocrit has returned to the normal range, and evaluation for hypoxia and sleep apnea is indicated [16]. Using therapeutic phlebotomy to lower hematocrit is also effective in managing T-induced erythrocytosis [16].

Liver Toxicity

Liver toxicity effects, including the development of benign and malignant tumors, have been associated with oral preparations of T, particularly alkylated T [170]. Intramuscular and transdermal preparations have not been associated with hepatic injury [171].

Other Adverse Effects

Other potential side effects of T treatment include increased skin fat, acne, headache, weight gain, prolonged painful or frequent erections, and male pattern baldness [16]. Gynecomastia and infertility have been described in patients receiving T replacement [171]. T levels above the physiological range may cause irritability, impulsive aggression, and signs of major depression [32]. T therapy can cause fluid retention and edema and potentially worsen edema associated with heart failure or other edematous states [16]. Skin lesions, mainly erythema and pruritus, are relatively common with T patches and require discontinuation of therapy in some patients. Less commonly, treatment with gels can also cause local irritation. Another possible adverse effect of the gels is the interpersonal T transfer after topical application [219].

Conclusion

Older males often have low circulating serum T levels. This hormone deficiency may be permanent or reversible and manifest as the classic form of hypogonadism or refer to LOH. Often, the symptoms associated with aging are similar to those of hypogonadism and are therefore difficult to distinguish. This decrease in serum T concentrations has been associated with several CVRFs, CVD, and all-cause and CV mortality in this population. LOH should be considered as a clinical and biochemical syndrome associated with advanced age and is characterized by typical symptoms and a deficiency in serum levels of T. It has been proposed that the proper diagnosis of LOH, and therefore the indication for replacement therapy, should be made only in elderly men who present with symptoms of hypoandrogenism associated with low T levels and an adequate response to a 3-month treatment trial with

symptom improvement. The goal of therapy is to achieve T levels in the middle or lower part of the normal range for young men and to alleviate symptoms caused by androgen deficiency. Androgen therapy would be contraindicated in men with breast or prostate cancer. Age alone would not be a contraindication to initiating T therapy, although an individual assessment of comorbidities and potential risks would be necessary.Conflict of InterestThe authors have no conflict of interest and financial support in relation to the present manuscript

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Male Hypogonadism and Traumatic Brain Injury

10

Alexandre Hohl and Roger Walz

Traumatic Brain Injury: A Silent Epidemic

Traumatic brain injury is very common and a serious social and public health problem [1, 2]. Among the different types of traumatic injuries, the traumatic brain injury (TBI) is a major cause of morbidity, mortality, and neurological disability among young adult men [3–5], being a global concern regardless of the economic development of countries. In Brazil, United States of America (USA), Germany, and Australia, TBI is the leading cause of death in people under 45 years old [3, 6] and, in severe cases, survivors usually have clinical, physical, cognitive, psychological, and psychiatric sequelae [7–10]. Fifty percent of deaths from TBI occur at the accident site, during transport in the ambulance or during the period of medical treatment in emergency rooms [11].

Several studies show a trimodal incidence of higher occurrence of TBI: children under 1 year, late youth/early adulthood, and elderly people (> 64 years old) [3, 11]. As to gender, the incidence is higher in men, especially in teenagers and young adults [3]. The main causes of TBI are: traffic accidents [5], work accidents and sports accidents [12], and violence [13].

A deeper knowledge of TBI may be useful to develop new strategies for diagnosis and treatment, including rehabilitation. The lack of knowledge on TBI led to the use of the term "silent epidemic" because the number of patients with TBI is still underestimated. Many patients with TBI do not go to the hospital in minor trauma causes—about 80%. The hospital care ends up being directed for victims of severe

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and moderate TBI, representing 20% of cases [14], and most patients with severe TBI dies before being hospitalized.

In the 90s, the mortality rate due to TBI in the US was three times higher for men than for women. Half of hospitalizations for TBI in the US in 1994 were caused by traffic accidents and 25% were for falls. Only 10% of the cases of hospital admissions result from injuries from firearms, given that these weapons cause a high rate of pre-hospitalization mortality. In 1996, the *Center for Disease Control and Prevention* (CDC) in the US estimated that 5.3 million people were living with a disability caused by TBI (2% of the US population) [15].

According to a systematic review published in 2006, the incidence of TBI in Europe ranged from a minimum of 91/100,000 (Sweden) to 546/100,000 (Spain) [16], with the main cause being traffic accidents followed by falls. In northern Europe, falls were more prevalent, while car accidents were predominant in southern Europe. The average incidence of hospitalization for TBI in Europe is higher than in the US (235/100,000 and 103/100,000, respectively). The mortality rate for TBI in the US and Europe are similar, around 15–20/100,000. TBI-related deaths in the USA account for approximately one-third of deaths caused by trauma [3].

In other continents, the incidence of TBI has also increased in the past decades. The rates of average incidence of hospitalization were above 300/100,000 in South Africa [17] and Australia [18]. Variations must be taken into account in making comparisons between these studies, including study design and differences in the populations studied.

Epidemiological studies on TBI conducted in Brazil in the last two decades have shown that 12% of injuries resulted in death. Traffic accidents were a major cause, and among these, the motorcycle accidents are rapidly increasing. Despite hospitalizations for TBI not being the rule, as most cases are mild, the mortality rates in cases requiring hospitalization are very high. In Brazil, TBI occurs mostly among men, accounting for 80% of the cases. The number of hospital admissions (50–60/100,000) is lower when compared to other countries like the US or the European countries, which may be explained by management problems and the difficulty to obtain pre-hospital care, in addition to the difficulty of the victims in moving from the site of the accident [19–22].

Some measures such as the use of seat belts and helmet and the improvement of primary and secondary care of patients managed to reduce the number of TBI in developed and developing countries [23]. How patients with TBI are rescued is also crucial to reduce the mortality rates and the care costs of these patients. Patients with other injuries in addition to severe TBI have the best cost-effective ratio for emergency care by helicopter when compared to ground assistance [24]. The major issue is how this resource is made available in most countries.

TBI Classification and Concepts

TBI may be defined as lesions of the brain tissue caused by external mechanical forces, evidenced by the loss of consciousness due to head trauma, amnesia, other neurological or neuropsychological abnormalities, diagnosed skull fracture, and intracranial lesions or death [25].

TBI may also be defined as a change of brain function, manifested as confusion, altered level of consciousness, convulsions, coma or sensory or motor neurological deficit resulting from the application of a force, penetrating or not, on the skull [11, 26].

TBI is classified according to the injury mechanisms, clinical severity or morphological changes (Chart 10.1) [27].

In closed trauma, the forces of acceleration and deceleration, such as those that commonly occur in traffic accidents, cause diffuse lesions and local contusions due to the force of the impact. In penetrating injuries, the penetrating object causes local destruction and, depending on the kinetic energy transmitted to the tissue, diffuse devastating injuries [28, 29].

Although modern approaches to disease classification use anatomical, physiological, metabolic, immunological, and genetic attributes, the TBI still is broadly classified based on clinical signs [26].

The Glasgow Coma Scale (GCS) is the main clinical method used to classify the severity of TBI (Chart 10.2) [30]. The GCS assesses and scores three items of the physical examination: eye opening, verbal responses, and motor responses. Severe TBI is classified as a trauma that causes coma as long as it is not related to extracranial conditions (such as severe intoxication) and as long as it remains at least beyond the initial resuscitation period [26].

Chart 10.1 Classification of Traumatic Brain Injury

Mechanism	Closed	High speed (collision of vehicles)	
		Low speed (falls, aggressions)	
	Penetrating	Injuries caused by firearms	
		Other penetrating injuries	
Severity	Mild	Score 14–15 in Glasgow coma scale	
	Moderate	Score 9–13 in Glasgow coma scale	
	Severe	Score 3–8 in Glasgow coma scale	
Morphology	Skull fractures	Skull cap	Linear versus starred
			With or without depression
			Exposed or closed
		Basilar	With or without liquor loss
			With or without paralysis of the VII pair of cranial nerve
	Intracranial injuries	Focal	Epidural
			Subdural
			Intracerebral
		Diffuse	Mild concussion
			Classic concussion
			Diffuse axonal injury

Chart 10.2 Glasgow coma scale (GCS)

Eye opening		Motor response	Verbal response	Eye opening	
Spontaneous	4	Obeying	6	Oriented	5
To commands	3	Locates	5	Confuse	4
To pain	2	Nonspecific withdrawal to pain	4	Inappropriate sounds	3
None	1	Abnormal inflection	3	Unintelligible sounds	2
		Extensor response	2	None	1
		None	1		

Chart 10.3 Classification of TBI based on CT

Category	Definition
Type I diffuse injury	No visible alterations in CT
Type II diffuse injury	Cisterns with midline deviation of 0–5 mm and/or density injury, no injury of high density or mixed density greater than 25 ml in volume may include bone fragments and foreign bodies
Type III diffuse injury (*swelling*)	Compressed or absent cisterns with deviation from the midline of 0–5 mm; no injury of high density greater than 25 mL in volume
Type IV diffuse injury	Deviation from the midline greater than 5 mm; no injury of high density greater than 25 mL in volume
Mass lesion surgically treated	Every lesion surgically treated
Mass lesion not surgically treated	Injury of high density greater than 25 mL in volume not surgically treated
Lesion of brainstem	Lesion of the brainstem

Patients with TBI will be diagnosed in coma when they do not open their eyes even in response to painful stimuli, do not pronounce words, and do not follow simple commands, which corresponds to a GCS score of eight or less [31]. The neurological assessment of a patient in a coma for TBI should always include an assessment of the GCS and an assessment of pupillary response to light stimuli [32].

Computed tomography (CT) and magnetic resonance imaging (MRI) are imaging exams used to evaluate the morphology of lesions [28, 33]. CT scan is the exam of choice in emergencies in patients with TBI and assesses the presence and location of hematomas, contusions, cerebral edema, and herniation across the midline and the tentorium [34]. In 1991, Marshall and colleagues proposed a scale for rating TBI according to the findings at CT (Chart 10.3) [35]. This scale differentiates patients into six categories according to the presence or absence of abnormalities, obliteration of basal cisterns, presence of midline deviation, and mass lesions [36].

Injury Mechanisms in TBI

Traumatic brain injury and neurological damages from TBI are consequences of primary injuries caused on impact and of secondary lesions that occur after trauma [31]. Secondary injuries include the effects of hypoxia, hypotension, hyperglycemia, sepsis, anemia, hyperthermia, and high intracranial pressure (ICP) secondary to mass effect [37].

The primary lesions may cause diffuse axonal injury, petechial hemorrhages to cerebral hematoma, cerebral edema, and alterations in the permeability of the blood–brain barrier due to lesions in small venules [38, 39]. Intracranial hematomas can be classified as epidural, subdural (the most common, present in 20–25% of patients with severe TBI), or intraparenchymal [28].

Secondary injuries may be prevented and treated. They occur within hours to days after the trauma and may be considered determinant in the neurological outcomes of the patients, directly influencing their recovery. They are the main cause of in-hospital mortality and morbidity after TBI [40]. Often the secondary brain injury is caused by cerebral edema, which causes an increase in ICP and subsequent decrease in cerebral perfusion, leading to ischemia [41]. The cerebral edema is caused hours after TBI by the accumulation of vasogenic substances, such as prostaglandins and nitric oxide. If the edema is not effectively prevented or treated, it may exacerbate morbidity and mortality [42].

Hypotension and hypoxemia commonly occur before the patient reaches the hospital, significantly increasing the risk of secondary brain injury and may worsen the prognosis [43].

Hypophysis and Hypothalamus: Anatomy and Physiology

Hypophysis, also known as pituitary gland, weighs 0.5–1 g and is located in a cavity of the sphenoid bone in the skull base, called sella turcica, and does not have contact with the brain due to the diaphragm, a reflection of the dura mater that covers it. However, it communicates with the hypothalamus through a neural stem, which passes through the diaphragm, establishing communication between both structures. Anatomically, the pituitary gland is divided into the posterior hypophysis, also known as neurohypophysis, and the anterior hypophysis or adenohypophysis, which represents 80% of the total volume of the gland [44].

The blood system of the hypothalamus and the hypophysis is formed between the eighth and fourteenth week of pregnancy. The posterior lobe receives blood supply mainly from the superior hypophyseal artery and from the inferior hypophyseal artery. The super hypophyseal artery emanates a capillary plexus formed by portal veins that irrigate the anterior hypophysis. The anterior lobe also receives blood from another set of shorter portal veins coming from the inferior hypophyseal artery. Therefore, little blood reaches the cells of the anterior pituitary, which makes it very fragile both in mechanical and vascular aspects [45].

The neurohypophysis only stores and secretes hormones synthesized by the hypothalamus, i.e., the vasopressin and oxytocin, while the anterior pituitary has specific endocrine cells that synthesize, store, and secrete a group of hormones. This group consists of adrenocorticotropic peptide hormones (ACTH), thyroid stimulating hormone (TSH), growth hormone (GH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and prolactin [46–48].

The gonadotropic hormones (LH and FSH) are glycoproteins and regulate growth, development, pubertal maturation, reproduction processes, and the secretion of sex steroid hormones. Gonadotropins usually secrete both hormones and represent 10–15% of the anterior lobe cells, which are spread throughout the gland. LH and FSH act on Leydig and Sertoli cells localized in the testicles and stimulate the production of testosterone and sperm cells [9].

Hypophysis, Critical Illness, and TBI

Before addressing the issue of hypophyseal dysfunction in patients with TBI, it is important to be aware of abnormalities in the endocrine function during critical illness. Most patients with severe or moderate TBI will be treated in the acute phase in an intensive care unit, and important abnormalities about the secretion of hypophyseal hormones in patients in intensive care have been documented.

The testosterone levels decline significantly in the acute phase (first days) of critical illness in male patients [49], despite the maintenance of circulating concentrations of gonadotropins. The high levels of cytokines seen in critical illness are postulated to be responsible for this alteration, and the sharp reduction in testosterone levels has been considered to reduce unnecessary anabolism, possibly facilitating survival [50].

Although hyperprolactinemia may occur during critical illness as a response to stress and this hormone increase may cause hypogonadotropic hypogonadism, the high prolactin in these situations does not contribute significantly to reduce gonadotropins in these men [51].

All these issues must be considered in the interpretation of hormonal changes of the pituitary gland in critically ill patients due to severe TBI.

There are two main mechanisms that explain the types of lesions caused by head trauma. The first would be the mechanism by contact, causing lesions in the scalp, skull fractures with or without depression, and localized hematomas. The second mechanism would be for acceleration–deceleration, resulting in the displacement of the head and forming traumatic waves that reach throughout the brain. Both may affect the hypophysis and the hypothalamus [29, 48].

Hypophyseal dysfunction post-TBI was first reported in 1918 by Cyran, in the context of a patient with hypophyseal failure caused by a skull base fracture [52]. After this case report, a number of autopsies in the second half of the twentieth century have shown high rates of pituitary damage after fatal TBI [53]. The two first systematic studies of hypophyseal dysfunction in TBI focused on long-term

hypopituitarism have reported high rates of hormone deficiencies of the anterior hypophysis, in particular deficiency of gonadotropins [54, 55].

Hypopituitarism is the partial or complete failure of the secretion of anterior hypophysis hormones, and it may result from hypophyseal or hypothalamic diseases [56]. In TBI, these hormonal changes mainly occur due to weak hypothalamic-hypophyseal infundibular structure and insufficient vascularization. The blood vessels that irrigate the hypophysis pass along the neural stem, and this region is very vulnerable to mechanical compression, which may be caused by cerebral edema or edema of the hypophysis itself after trauma [57].

Cases of post-TBI hypopituitarism may occur only by a temporary change during the acute phase after trauma or may occur at any time in the evolution after trauma, becoming permanent due to the lesion of the hypophysis or the hypothalamus [48]. The diagnosis may be established within a few weeks to months after TBI, and it may take years to be recognized, so it is not possible to establish a precise period to the onset of hypopituitarism [58]. As so, it is necessary to be alert to a possible change in the hypophysis, even if it is a late alteration, in order to reduce the incidence of undiagnosed cases, since the hormonal dysfunction may cause or exacerbate symptoms that may be attributed to TBI [59]. There are also reports of patients who developed hormone deficit after TBI, which was been reversed spontaneously after a few years [60].

Regarding the severity of the trauma and the hypophyseal dysfunction as a consequence, there are some controversies. Benvenga and colleagues have reported that 93% of patients with post-TBI hypopituitarism were in a coma or unconscious soon after trauma [55]. Kelly and colleagues have identified a score on the GCS under 10 and the presence of cerebral edema on CT as risk factors for the development of hypopituitarism after trauma [61]. Water and colleagues found no relationship between trauma severity and consequent hormonal dysfunction [62].

In general, one of the first hormonal deficiencies that arise in cases of post-TBI hypopituitarism is the alteration in gonadotropins, probably due to the anatomical position of the gonadotropic cells in the hypophysis and its relation to the vascularization, which can be easily affected during or after trauma [55, 61, 63].

Male hypogonadism may cause several signs and symptoms in adult men [64]. The patient may experience loss of libido, worsening of the erection quality, increased body fat, abdominal obesity, dyslipidemia, insulin resistance, hypertension, increased thrombotic factors and cardiovascular mortality and, decreased bone mass and muscle strength. Also, the patient may experience a decrease in physical energy, memory, attention span, and some patients may even experience emotional disorders, social isolation and decrease in psychic and physical well-being [9, 64]. In patients with a history of TBI, these signs and symptoms may be confused with changes arising solely by the trauma, such as neurological or motor deficits.

In principle, the combination of low blood levels of peripheral hormones and inadequately low levels of pituitary hormones (below the upper limit of the reference range) indicates hypopituitarism [9, 64]. Table 10.1 provides a summary of the attempts to evaluate the gonadotropic function.

Table 10.1 Criteria for hormone deficiency of the male gonadotropic axis

Testosterone: Low (<10–12 nmol/L or < 288–346 ng/dL)	LH and FSH: Normal or inadequately low

Table 10.2 Inclusion criteria for investigation of hypopituitarism in patients with mild, moderate, or severe TBI

Inclusion criteria:
1 Patients requiring hospitalization for at least 24 h or who were in the ICU should be investigated in the acute phase and prospectively
2 Patients with the history of complicated mild TBI, moderate TBI or severe TBI at any time after the event, especially those with signs and symptoms suggestive of hypopituitarism
Definition of complicated mild TBI: Presence of at least one of the following conditions:
• Any anatomical change in the initial CT or MRI (diffuse axonal lesion, skull fracture, skull base fracture, diffuse cerebral edema, intracerebral hematoma, multiple contusions)
• Presence of acute pituitary hormonal changes
• Need for hospitalization for more than 24 h
• Need for monitoring in the ICU and/or need for any neurosurgical intervention
• Presence of anti-hypophyseal antibodies and anti-hypothalamus antibodies

There is still no consensus about which patients who have had TBI need to be investigated. Table 10.2 describes a recent investigation proposal.

The testosterone replacement is indicated and well established when the deficit of androgens in male patients is diagnosed. Once the hormone deficiency is recognized, the treatment should be held with the hormone deficiency alone or together in case of a panhypopituitarism [9, 65]. With hormone replacement therapy, you can see several beneficial effects, such as improving sexual function, increased muscle mass and capacity for physical exertion, mood enhancement, improved quality of life, and all of these items also contribute to the recovery from TBI [57, 66].

The testosterone replacement in post-TBI hypogonadism is not different from other forms of treatment of male hypogonadism. It can be made with injectable testosterone formulations, transdermal (gel or adhesive) or subcutaneous implants. Oral testosterone is little used in the treatment of male hypogonadism [64].

Biomarkers, Hormones, and Post-TBI Prognosis

In 2006, Perel and colleagues analyzed 31 studies published since 1990 involving prognostic models for patients with TBI using multiple logistic regression [67]. A study was conducted by Mushkudiani and colleagues in 2008 [68]. Critically, they suggested that studies of prognostic models in TBI need a better description of the extent and validity of the variables included in the model, demonstration of interactions in the multivariate analysis, increased sample size, proper management of continuous variables and losses, clear description of the calculation of the prognostic score, appropriate presentation of model performance measures, and external validation [67, 68]. The authors also highlighted the need for studies in developing countries, where most cases of TBI occur [67].

Until a few years ago there was no study about severe TBI prognostic in Brazil. Recently a prognostic model of mortality of patients with severe TBI at the time of hospital discharge has been developed using multiple logistic regression analysis applied to 748 patients with severe TBI admitted in a hospital in southern Brazil during a decade [22].

In this model, the overall correct prediction was 77%, with 88% to predict survival and 56% to predict mortality. The inclusion of other clinical and laboratory variables, such as the treatment and the presence of hemodynamic instability, hypoxia, anemia, fever, seizures, infections, increase in ICP, renal, hepatic or respiratory failure, may contribute to improving the prediction capacity of the model. In this context, considering the anatomical location of the hypophysis and the hypothalamus in the CNS, the evaluation of the hormonal response resulting from direct injuries and from a phenomenon adaptive to trauma may be associated with the prognosis of TBI.

The identification of biomarkers and their association with clinical, laboratory, radiological, and neurosurgical variables are a major scientific challenge to identify possible therapeutic targets in TBI [69].

A biomarker is indicative of a disease or a specific biological condition that can be measured using samples taken from any affected tissue or peripheral body fluids. These markers may be an altered enzyme activity, changes in protein expression or post-translational modification, alteration in gene expression or in the composition of reserve lipids, or a combination of these changes. As a result, several strategies have been used to discover biomarkers, including transcription profiling, proteomic, and metabolomic approaches [70].

Recently, the association between mortality of patients with severe TBI and the plasma levels of lipid peroxidation (thiobarbituric acid-reactive species; TBARS) and protein oxidation (carbonyl) have been investigated, both being used as markers of oxidative stress. Plasma levels of TBARS and carbonyl increased significantly in the first 70 h after severe TBI but they were not independently associated with mortality in hospital [71].

Several cytokines are involved in TBI and high serum levels of interleukin 10 (IL-10) in the first 3 days after TBI have been shown to be significantly correlated to the severity of the GCS and have been proven to be independently associated with in-hospital mortality in patients with severe TBI. The multiple logistic regression analysis showed that increased IL-10 levels (190 pg/mL) at 10 and 30 h after TBI was 5–6 times more associated with the in-hospital mortality rate than the lower levels (<50 pg/mL), regardless of age, GCS score, pupils at admission, and associated trauma. Based on these data, serum IL-10 has demonstrated that it can be a useful marker for prognosis in severe TBI [72].

Pentraxin 3 (PTX3) is a humoral component of the innate immune system that has been studied as a marker of inflammation, infection and cardiovascular disease. The association between serum levels of PTX3 and in-hospital mortality of patients with severe TBI has been investigated. Like IL-10, serum PTX3 after severe TBI is an independent factor associated with mortality and is a potential biomarker for the prognosis of these patients [73].

Several evidences suggest that androgens may influence the mechanisms of cell death. In men, low testosterone levels have been associated with a worse prognosis after acute ischemic events [74, 75]. Androgen levels are inversely associated with the severity of cerebral ischemia, with the infarction size and with mortality in 6 months, and the total and free testosterone levels tend to normalize within 6 months after the ischemic injury. The beneficial effects of androgens after brain trauma have also been reported in animals [76]. These seemingly conflicting findings can be reconciled by the hypothesis that androgens are deleterious during the acute lesion, but beneficial during the recovery phase. Potential mechanisms by which androgens could improve recovery after ischemia and post-TBI include normalization of reperfusion, the promotion of axonal regeneration, neurogenesis, and synaptogenesis [77].

Recently, Wagner and colleagues evaluated 117 adults with severe TBI [78]. In addition to increased age and increased severity of the injury, the increase of estradiol and testosterone levels over time was associated with increased mortality and worse overall results for both men and women. These results represent a potential shift on the role of sex steroids in neuroprotection after TBI, justifying further studies in this area.

In this regard, the inclusion of hormones such as biomarkers would help to improve the predictive efficiency thereof. The independent association among plasma levels of TSH, LH, FSH, GH, free T, cortisol, IGF-1, and total testosterone was measured 10 h and 30 h after severe TBI and the hospital mortality of 60 consecutive male patients was evaluated. The multiple logistic regressions showed a trend for an independent association among hospital mortality and normal or elevated LH levels measured at 10 h and 30 h. In conclusion, the hormones plasma levels, excepting the LH, are not highly consistent with the hospital mortality of male patients [79]. Current data suggest that LH levels during the acute phase of TBI might contribute to accurate prognostication and further prospective multicentric studies are required to develop more sophisticated predictive models incorporating biomarkers such as LH in the quest for accurate outcome prediction following TBI. Moreover, the potential therapeutic benefits of modulating LH during the acute phase of TBI warrant investigation [80, 81]. Mitochondrial dysfunction may be important in preventing neuronal cell death. TBI deregulates the neuroendocrine system, suppressing the action of androgens. Some authors suggest as an early therapeutic target in the acute stages of TBI the role of androgen signaling in the regulation of gliosis and in the protection of mitochondrial function under stress [82].

Conclusions

TBI is a public health problem in most countries and it is associated with high morbidity and mortality, affecting mainly young adult men. As this is an economically active population, the economic and social impact is significant. Several studies have shown that post-TBI hypopituitarism is more common than previously thought 20 years ago and that the gonadotropic axis is highly impacted by it. Testosterone

replacement is indicated in cases of post-TBI hypogonadism. Thus, it is necessary to identify these patients and treat them properly in order to reduce morbidity, optimize rehabilitation, and improve the quality of life of these patients. The role of gonadotropins and testosterone in the acute phase as prognosis biomarkers of TBI in men needs to be further investigated.

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Male Hypogonadism and Fertility

11

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Introduction

Hypogonadism is the presence of low serum testosterone levels with symptoms and signs that correspond to the state of low testosterone [1]. It is a common condition that affects men's sexual and reproductive health. Hypogonadism is classified according to the level of dysfunction into primary hypogonadism which correlates to testicular failure of testosterone production and secondary hypogonadism which correlates to hypothalamic and/or pituitary failure of gonadotropin production that is responsible for the body's testosterone production [2, 3]. The Baltimore Longitudinal Study of Aging reported an 8% prevalence in men below 45 years of age, while the Massachusetts Male Aging Study estimated the crude incidence of androgen deficiency was 12.3 per 1000 person-years and that it increases significantly with age [2].

Infertility is described as the inability to conceive after 1 year of regular unprotected intercourse. It is believed that men contribute to 50% of the etiology of infertility with 30% acting as a sole male factor [4]. Sigman et al. reported a 9.6%

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cross-prevalence of hypogonadism in infertile couples presenting for assessment, whereas later reports by Bobjer et al. found that one-third of infertile men under the age of 50 were hypogonadal [5, 6]. When investigating the prevalence of hypogonadism within cohorts defined by semen parameters, Sussman et al. identified 16.7% of men with obstructive azoospermia, 45% of men with azoospermia due to spermatogenic dysfunction, 42.9% of men with oligospermia, and 35.3% of men with normal semen parameters were hypogonadal [7].

Assessment of the gonadal state is a guidelines-based diagnostic measure in the evaluation of the infertile male with "impaired libido, erectile dysfunction, oligozoospermia or azoospermia, atrophic testes, or evidence of hormonal abnormality on physical evaluation" [8].

Physiology of Hypogonadism and Its Relation to Spermatogenesis

Spermatogenesis is the process of germ cell meiotic division and the formation of spermatozoa that occurs in the seminiferous tubules [9]. The process is spermatogenesis. It should have a period after many factors and before hormones, especially testosterone, are amongst the most important. Testosterone is produced by interstitial Leydig cells, and it has been reported that the intratesticular testosterone levels are 50–200 times the circulating blood levels, annotating its significance in regulating and maintaining spermatogenesis in men. Intratesticular testosterone exerts its paracrine role through the diffusion into the seminiferous tubules and binding to the androgen receptors initiating the functional responses required to support spermatogenesis [10].

This can be summarized into four main roles, starting with remodeling of the blood–testis barrier through various critical proteins expression. Secondly, testosterone is involved in the process of germ cell meiosis by regulating oxidative metabolism, DNA repair, and RNA processing. Third, testosterone modulates protein expression needed for the Sertoli spermatid adhesion, which in its absence causes premature germ cell release and absence of elongated spermatids [11]. Lastly, testosterone aids in the release of mature sperm in the seminiferous tubules and prevents its retention and phagocytosis by Sertoli cells.

An understanding of the hypothalamic pituitary testicular (HPT) axis is critical to diagnose the etiology of hypogonadism as well as understand the therapeutic options and their potential implications on spermatogenesis. The hypothalamus and pituitary glands modulate testosterone synthesis through the initial secretion of gonadotropin-releasing hormone (GnRH) in a pulsatile fashion that reaches the anterior pituitary gland via the hypothalamo-hypophyseal portal circulation. GnRH stimulates luteinizing hormone (LH) and follicle stimulating hormone (FSH) production. Both FSH and LH synchronize the process of spermatozoa production via endocrine, autocrine, and paracrine stimuli [12]. Sertoli cells through FSH stimulation regulate spermatogenesis and produce inhibin. Leydig cells through LH stimulation form testosterone and other steroid hormones such as androstenedione and dehydroepiandrosterone (DHEA).

The HPT axis has a negative feedback pathway that depends on the presence of estradiol and inhibin hormones. Testosterone is converted to estradiol by the enzyme

aromatase and inhibin is influenced by FSH levels. An excess of either estradiol or inhibin will inhibit the production of GnRH, LH, and FSH on the hypothalamic and pituitary levels [13].

Classification of Hypogonadism

Primary hypogonadism is characterized by low testosterone levels and elevated gonadotropins (FSH and LH), termed hypergonadotropic hypogonadism, due to testicular Leydig cell failure. Secondary hypogonadism is characterized by low levels of gonadotropins and low testosterone levels due to dysfunction at the hypothalamus and pituitary gland level. Causes for this imbalance could be further classified into congenital and acquired etiologies. Potential etiologies of primary and secondary hypogonadism are outlined in Tables 11.1 and 11.2, respectively [14].

Table 11.1 Primary hypogonadism

Congenital	• Klinefelter Syndrome • Rare chromosomal abnormalities: XX male syndrome 47 XYY syndrome, 48 XXYY syndrome, 21 Trisomy (Down syndrome), Noonan syndrome, Autosomal translocations1, Defects of testosterone biosynthesis, CAH (testicular adrenal rest tumors), Disorders of sex development (gonadal dysgenesis), LHR gene mutations, Myotonic dystrophy (including type I and II), Uncorrected cryptorchidism (including INSL3 and LGR8 mutations), Bilateral congenital anorchia, Sickle cell disease, Adreno-leukodystrophy
Acquired	• Drug induced: Chemotherapy agents, alkylating agents, methotrexate, testosterone synthesis inhibitors, ketoconazole, aminoglutethimide, mitotane, metyrapone • Bilateral surgical castration or trauma • Testicular irradiation • Orchitis (including mumps orchitis) • Autoimmune testicular failure • Testicular torsion • Alcohol/cirrhosis • Environmental toxins • Chronic systemic diseases[a] • Chronic organ failure[a] • Glucocorticoid excess (Cushing syndrome)[a] • Aging[a] • HIV • Malignancies • Lymphoma • Testis cancer • Spinal cord injury • Vasculitis • Infiltrative diseases (amyloidosis; leukemia)

[a]Conditions acting at central and peripheral levels resulting in either primary or secondary hypogonadism

Table 11.2 Secondary hypogonadism

Congenital	• Hemochromatosis • Combined hormone pituitary deficiency • Idiopathic hypogonadotropic hypogonadism • (IHH) with variants: Normosmic IHH, Kallmann syndrome, Isolated LH β-gene mutations, Prader-Willi Syndrome
Acquired	• Drug induced: Estrogens, testosterone or androgenic anabolic steroids, progestogens (including cyproterone acetate), hyperprolactinemia-induced drugs, opiates, GnRH agonist or antagonist, glucocorticoids • Traumatic brain injury • Pituitary neoplasm (micro/macro-adenomas) • Hypothalamus tumors • Pituitary stalk diseases • Iatrogenic • Surgical hypophysectomy • Pituitary or cranial irradiation • Inflammatory and infectious diseases • Lymphocytic hypophysitis • Pituitary infections • granulomatous lesions, sarcoidosis, Wegener's granulomatosis • encephalitis • Langerhans' histiocytosis • Hyperprolactinemia as a consequence of localized problems (hypothalamus-pituitary mass) • Chronic systemic diseases[a]: Type 2 diabetes mellitus/metabolic syndrome/metabolic diseases • HIV infection • Chronic organ failure • Chronic inflammatory arthritis • Glucocorticoid excess (Cushing syndrome)[a] • Eating disorders[a] • endurance exercise • acute and critical illness • Aging[a] • Spinal cord injury • Transfusion-related iron overload (β-thalassemia)

[a]Conditions acting at central and peripheral levels resulting in either primary or secondary hypogonadism

Congenital Hypogonadism

Klinefelter syndrome (KS) is considered one of the most common chromosomal abnormalities in men who present with infertility and hypogonadism. It has an incidence of 1–2 per 1000 live male birth, and it is the most common form of hypergonadotropic hypogonadism [15]. The syndrome was named in 1940 after Harry Klinefelter who initially described features observed in 9 men that included tall stature, gynecomastia, small testicular volume, and infertility in the form of azoospermia due to spermatogenic dysfunction [16]. Jacobs and Strong, unmasked the genetic component of this syndrome in 1959, demonstrating an extra X

chromosome (47, XXY) [17], caused by nondisjunction during paternal and maternal meiosis in 80% of patients [18]. The remaining 15–20% are believed to have multiple mosaic types of KS. This latter subset is distinguished by varying degrees of spermatogenic failure, with milder phenotypic aberrations and greater chance for sperm in the ejaculate, albeit severely diminished [19]. The hormonal imbalance greatly impacts spermatogenesis and treating the patients hypogonadal status may further increase the chance of sperm retrieval or production [20].

Kallmann Syndrome was first described by Franz Kallmann in 1944 [21]. It is a congenital disorder characterized by low levels of sex hormones (testosterone, estradiol, and progesterone) due to the production or function failure of hypothalamic gonadotropin-releasing hormone. It has an incidence of 1 in every 48,000 of the population and 1 in 30,000 of males [22]. It is considered the most common cause of hypogonadotropic hypogonadism (HH) and distinguished from other forms of HH by the presence of disruption in the GnRH neuron migration from the nasal region to the hypothalamus during embryo development, causing anosmia (total loss of smell) or hyposmia (reduced smell sensation), and hypogonadism [23, 24]. Males present with delayed puberty, detained secondary sexual characters, and abnormal sense of smell. Moreover, it can be accompanied with undescended testes, renal abnormalities, and midline facial defects [23]. Treatment goals focus on establishing normal secondary sexual characters, preserving fertility, and maintaining appropriate sex hormone levels that are essential for general and bone health. Therapeutic options attempt to re-establish the hormonal axis if spermatogenic function is desired, otherwise focusing on testosterone replacement for symptomatic improvement [25].

Prader-Willi syndrome is another genetic form of HH, caused by certain paternal gene deletions or mutations (q11 or q13) on chromosome 15. It was first described by Andrea Prader and Heinrich Willi in 1956. Among many features this syndrome can present with obesity due to increased appetite, intellectual delay, cryptorchidism, hypogonadism, and infertility are the most related to the scope of this chapter. It has an incidence of 1 in every 10,000 to 30,000. Treatment aims only as supportive and rarely patients seek fertility [26].

Congenital Adrenal hyperplasia (CAH) is a defect in cortisol synthesis and one of the most common autosomal recessive disorders. It is typically identified at birth due to manifestation of adrenal shock but may present later in life with milder forms of adrenal hormone imbalances [27]. 21-Hydroxylase enzyme is the most common type of enzymatic deficiency that impacts the gland final out products—mineralocorticoids, glucocorticoids, and sex hormones [28]. The pituitary gland compensates for this deficiency by increasing the production of adrenocorticotropic hormone (ACTH), which acts on the adrenal gland resulting into hyperplasia [29]. Adrenal hyperplasia releases excess androgens which suppress gonadotropin secretion from the HPT axis, causing a state of low testosterone which in turns affect spermatogenesis and fertility [30, 31]. It is believed that an element of gonadal damage by the intratesticular adrenal remnants might contribute to the etiology of infertility [32].

There are different forms of CAH depending on the defective enzyme that prompts peculiar presentations based on age and sex of the patient, enzyme insufficiency, and degree of excess androgens [33].

Acquired Causes of Hypogonadism

Hyperprolactinemia is the atypical production of the polypeptide hormone prolactin from the anterior pituitary gland [34]. It is a well-known cause for secondary hypogonadism and low testosterone due to the inhibition of GnRH secretion which in turn decreases the LH production and function on the Leydig cells [35]. Men often present with decreased libido, sexual dysfunction, gynecomastia, and infertility [36]. The AUA guidelines for the evaluation and management of testosterone deficiency have stated that "serum prolactin levels should be measured in patients with low testosterone levels combined with low or low/normal luteinizing hormone levels (Strong Recommendation; Evidence Level: Grade A)" and "patients with persistently high prolactin levels of unknown etiology should undergo evaluation for endocrine disorders (Strong Recommendation; Evidence Level: Grade A)" [37].

Apart from the hormonal effect of hyperprolactinemia on male infertility, multiple experimental studies have identified the presence of prolactin receptors in different testicular cells and male accessory glands, suggesting a possible direct role of prolactin in spermatogenesis and indirectly affect in gonadal steroid secretion [38].

Prolactin is highly regulated by dopamine. Increased levels of dopamine result in decreased prolactin through production pathway inhibition. Differential diagnosis for hyperprolactinemia are prolactinomas, whether macro (>1 cm) or micro (<1 cm) tumors; other pituitary and hypothalamic tumors that impede dopamine production and delivery; and medication induced by affecting dopamine synthesis [39]. Treatment aims on stabilizing the prolactin hormones to reverse its effect on sex hormones, usually by dopamine agonists (cabergoline and bromocriptine). If a macroadenoma is present, initial treatment is often medical with surgical resection if there is a lack of appropriate therapeutic response [40]. Treatment commonly restores suppressed libido and erectile function, as well as improving bulk semen parameters [41].

Hyperestrogenemia: Estrogen is one of the sex hormones present in both men and women. The active component in men is named estradiol. It is mainly produced from testosterone conversion by the aromatase enzyme [42]. Estradiol exhibits its vital role in male reproductive functions through distinctive receptors (ER$\boldsymbol{\alpha}$ and ER$\boldsymbol{\beta}$) expressed on various reproductive cell types (Leydig cells, immature germ cells, and spermatozoa). Though its presence and significance are established, the full understanding of its role in spermatogenesis remains unexplained. It has been reported that at increased levels, it directly impacts cell communication through functional impairment of the Sertoli cell tight junctions and causes germ cell apoptosis [43]. Hyperestrogenemia disrupts the HPT axis by affecting the secretion of GnRH that impacts FSH and LH levels which subsequently causes down regulation

of the testicular function in the form of low testosterone and defective germ cell meiosis division [44, 45].

There are multiple contributing factors for hyperestrogenemia, including obesity, hepatic dysfunction, tumors producing estrogen, hyperthyroidism, medications (e.g., anti-androgens), and adrenal diseases. Excess adipose tissue in obese individuals, as an example, harbors enhanced aromatase activity leading to increased rates of peripheral conversion of testosterone to estradiol. The feedback inhibition further decreases gonadotropin release and gonadal function [46, 47].

Management of Hypogonadism

The first step in managing infertile men with hypogonadism is proper assessment for any identifiable etiology. Understanding the patient's reproductive goals is crucial in determining the appropriate treatment. Management of hypogonadism while preserving reproductive potential can be a challenge, yet many options exist.

Selective Estrogen Receptor Modulators (SERMs)

A class of medications that exert their action on estrogen receptors. They are also termed Estrogen agonist/antagonist drugs depending on the acting tissue and the intrinsic activity of each medication [48]. SERMs are off-label, non - FDA approved therapies for the treatment of hypogonadal men [49]. Their use was first described in 1964 and they serve as a well-established means of indirect testicular stimulation [50]. The most common agents used in male hypogonadism and infertility are clomiphene citrate and tamoxifen. They competitively inhibit the action of estrogen on its receptors, which in turns inhibits the negative feedback mechanism on the HPA axis leading to increased secretion of GnRH, LH, FSH and finally endogenous testosterone [51, 52]. Clomiphene citrate is composed of the cis-isomer zuclomiphene and trans-isomer enclomiphene with a half-life of 5 days. The combination of cis-isomers and trans-isomers contribute to both agonistic and antagonistic effects on estrogen receptors [53–55].

Side effects of these medications include changes in energy levels, mood, sexual desire, and aggression. Such changes are commonly mild and related to increased testosterone. Rarely, pituitary gland enlargement could occur, causing headaches and visual disturbance [56]. Although data has demonstrated limited polycythemia effects due to increased testosterone levels, monitoring of hemoglobin is advised [57, 58]. PSA levels were reported minimally elevated while on testosterone treatment, which could mask early detection of prostate cancer, therefore PSA surveillance should also be considered [59]. Moreover, clomiphene citrate may induce hyperestrogenism with resultant breast tenderness and gynecomastia, thus estradiol surveillance, particularly should the patient note symptoms, is advisable [60]. Lastly, a paradoxical hormonal response of SERMs was described in long-term usage. This was attributed to the imbalance of enclomiphene to zuclomiphene ratio,

having a more agonist estrogen effect than usual [61]. Furthermore, other reports for paradoxical spermatogenic response in the form of deranged sperm parameters up to azoospermia have been mentioned, therefore mandating proper patient counseling before prescribing such medications and regular surveillance of their hormonal and seminal parameters during treatment [62].

Helo et al. performed a prospective randomized, double blinded trial that compared the effect of clomiphene citrate 25 mg daily or anastrozole 1 mg daily on testosterone levels, semen parameters, and patient questionnaires (International Index of Erectile Function, Erection Hardness Scale, and the Androgen Deficiency in the Aging Male questionnaires) in 26 men with hypogonadism and infertility. The authors found that the clomiphene arm had increased testosterone levels at 6 and 12 weeks compared with anastrozole. No significant changes were noticed in semen parameters and patient questionnaire scores between both groups at 12 weeks follow-up compared to baseline data [63]. In 2016, Chandrapal et al. evaluated the safety of clomiphene citrate treatments (50 mg daily or every other day) in 77 hypogonadal men over 12 months follow-up. The authors noted a significant increase in the mean of total and bioavailable testosterone (200 ng/dL and 126 ng/dL, respectively), but no significant change in the PSA or hematocrit [64]. Two years later, Habous et al. investigated the testosterone response and symptom improvement in 282 hypogonadal men receiving either clomiphene citrate 50 mg, HCG injections 5000 IU twice weekly or a combination of both. Despite documented elevation of testosterone levels in all groups, no statistical difference increases across the groups were reported. The authors stated that clomiphene citrate was an effective and feasible treatment option to increase testosterone and preserve fertility compared to other treatment arms [65]. In the same year, Soares et al. conducted a randomized, placebo controlled, double blinded study on 78 obese men with secondary hypogonadism taking clomiphene citrate 50 mg daily for 3 months. They identified a significant increase ($P < 0.001$) in the total testosterone compared to baseline at 12 week follow-up in the clomiphene citrate group (225.54 ± 72.49 vs 687.94 ± 276.66) with no change noted in the placebo arm (220.28 ± 69.30 vs 220.19 ± 48.46) [66].

With regard to its influence on male fertility, the published impact on semen parameters is less robust. The World Health Organization (WHO) evaluated 190 men who were diagnosed with idiopathic deranged semen parameters. They compared the effect of clomiphene citrate 25 mg daily versus placebo for a 6-month duration. No significant changes in semen parameters nor pregnancy rates were recorded [67]. On the other hand, Hussein et al. investigated men with azoospermia due to spermatogenic dysfunction. They studied the effect of clomiphene citrate on sperm recovery in the ejaculate or successful microscopic testicular sperm extraction (mTESE) in this cohort of patients. The authors reported a 10.9% recovery of sperm to ejaculate with a statistically significant increase in mTESE sperm retrieval in those treated with clomiphene versus those untreated (57% vs 33.6%, respectively) [68]. A meta-analysis by Chua et al. including 11 randomized control trials studied the effect of estrogen antagonists (example: clomiphene citrate) in men with oligoasthenoteratozoospermia on semen parameters and pregnancy rates. After

pooling the data, the authors reported a statistically significant increment in pregnancy rates (pooled OR 2.42; 95% CI 1.47–3.94; $p = 0.0004$), sperm concentration (WMD 5.24; 95% CI 2.12, 88.37; $p = 0.001$), and motility (WMD 4.55; 95% CI 0.73, 8.37; $p = 0.03$) [69].

Aromatase Inhibitors (AIs)

Aromatase inhibitors exert their action on the aromatase enzyme, which is a cytochrome p450 enzyme present in multiple tissues including testes, ovaries, adipose tissue, liver, and brain. Aromatase catalyzes the conversion of androgen precursors such as testosterone to estradiol via the transformation of an enone ring to a phenol ring during the synthesis process [70]. Pharmacological inhibition blocks androgen conversion, decreasing estradiol production and limiting upstream HPG axis feedback inhibition. The decreased feedback inhibition results in upregulation of GnRH, LH, and FSH, which in turn endogenously stimulates Leydig cell and Sertoli cell function.

Aromatase inhibitors are a form of medications originally used to treat breast cancer in both postmenopausal women and men and also used to treat gynecomastia in men [71]. They are used off-label and non-FDA approved, for the treatment of hypogonadal infertile men. They are classified into nonselective steroidal inhibitors and highly selective nonsteroidal inhibitors. Anastrozole and Letrozole are competitive antagonists to the aromatase enzyme and belong to the former group [72]. Dosing regimens are highly variable. Current trends favor less frequent twice a week or three times a week dosing versus historic daily dosing to preserve a degree of aromatization and circulating estradiol rather than complete cessation.

In general, decreased bone maturation up to osteoporosis, arthritis, hair loss, liver and kidney dysfunction have all been associated with AIs usage. It is believed that low levels of estradiol due to the treatment with aromatase inhibitors is the main trigger for these effects. Treatment-related hypoestrogenemia and undetectable estradiol levels raised concern for decreased bone density and osteoporosis risk as was noted in AI use for postmenopausal women with breast cancer [73, 74]. In men the data on bone density is less conclusive [75–78].

Dias et al. studied the long-term effect of AIs and testosterone therapy in hypogonadal men. Their randomized, double blinded placebo-controlled study evaluated the change in cardiometabolic markers in 29 men divided into three groups. The authors concluded no difference between each group after 12 months of treatment in the cardiometabolic parameters including glucose homeostasis and insulin resistance, lipid metabolism, and inflammatory markers [79].

Multiple studies conducted in hypogonadal men have shown an increase of mean testosterone levels after treatment with AIs. They also observed an increase of the testosterone/estradiol ratio as compared to other treatment modalities due to its effect on decreasing estradiol synthesis and endogenously increasing testosterone levels [63, 80]. The effect of AIs on semen parameters is debatable. Some authors described improvements in sperm concentration and motility, sperm recovery in

ejaculate, increased chances of sperm retrieval in testicular surgical extraction, and pregnancy rates [81–86]. On the other hand, different conducted studies failed to show such changes [63, 87].

Gonadotropin Analogues

Another hormonal treatment form in hypogonadal patients, especially secondary hypogonadism, is gonadotropin analogues. This is achieved either by direct pituitary stimulation through pulsatile GnRH therapy or through injectable substitutions for endogenous FSH and LH hormones. Pulsatile GnRH therapy demonstrated efficacy for spermatogenic induction in hypogonadal patients [88]. However, the need of an external pump for pulsatile delivery and medication costs have greatly limited therapeutic implementation. GnRH therapy requires an intact pituitary gland for response to the exogenous hypothalamic hormone [89]. FSH analogues are presented in the form of recombinant FSH (rFSH) and human menopausal gonadotropin (hMG). The latter is a urine derivative that contains both bioactive FSH and LH components with different gonadotropin purity percentages [90]. LH analogues are presented in the form of human chorionic gonadotropin (HCG) which come in recombinant and urine derivative forms.

The mechanism of action of all previously mentioned gonadotropin analogues aims for HPA axis manipulation. Increased intratesticular testosterone levels with Leydig cell stimulation (LH effect) together with the activation of Sertoli cells (FSH effect), both promote the development of spermatogenesis [25]. Many studies conducted to investigate the effects of HCG, hMG, rFSH, and other analogues on male hormones and semen parameters in men presenting with hypogonadism and infertility. Different medication doses, frequency and combinations have been tested while their safety profile was monitored.

The published literature and clinical practice have significant heterogeneity in dosing protocols with initial doses typically starting at 1500–5000 IU 2–3 times per week for 3–6 months. When testosterone level is normalized but there are no signs of spermatogenesis induction, then addition of FSH (rFSH or hMG) 2–3 times per week with doses ranging 75–400 IU is warranted. Others suggest simultaneous therapy with both once treatment is initiated [91–94].

Boulox et al., in 2003, conducted a multicenter randomized study where he included 49 men diagnosed with hypogonadotropic hypogonadism. All patients were pretreated with HCG (1500 IU 2 times per week) for 4 months; 61% of patients reached eugonadism state but were still azoospermic on follow-up semen tests. They were then categorized into two groups, first group treated with rFSH 225 IU 2 times per week and the second group treated with rFSH 150 IU 3 times per week in addition to continued HCG for a total of 12 months. Mean testosterone increased from 1.08 ng/mL at baseline to 1.22 ng/mL after HCG alone, 6.24 ng/mL with group 1 and 4.52 ng/mL with group 2. At the end of the 12 months, there were no significant differences in achieving spermatogenesis between both treatment groups. Combined HCG and rFSH therapy resulted in the appearance of at least a sperm

concentration of 1×10^6/mL in 47% of patients over an approximate 5.5-month treatment duration [95].

In 2009, combined data from four different phase 3 open labeled multicenter studies investigating the effect of rFSH and HCG on spermatogenesis in hypogonadal infertile men was reviewed. A total of 100 patients were recruited, while 81 patients completed the 3–6-month pretreatment phase with HCG (1000 IU 3 times weekly or 2000 IU 2 times weekly). Once testosterone levels were normalized, rFSH 150 IU 3 times weekly was added for 18 months. Evaluation and medication titration was conducted every 3 months during the trials. Across the four trials, 84% of the 81 azoospermic men who completed the pretreatment phase showed evidence of spermatogenesis induction (sperm counts $>0 \times 10^6$/mL) after 6–9 months of treatment. Sixty-nine percentage of men had sperm concentrations above 1.5 million/ml after a treatment period of 9–12 months [96].

Novel Treatments

With an increased awareness of low testosterone at both a provider and consumer level, researchers and clinicians are in pursuit of therapies that provide improved symptomatic management of hypogonadism with the preservation of fertility potential. The rise of "Low T" centers has increased access to testosterone therapy, however, often without the proper evaluation and counseling of the negative impact on reproductive capacity.

Short acting forms of testosterone therapy are believed to have the least effect on the HPA axis and consequently sperm production. They have a shorter half-life necessitating more frequent dosing [97]. They mimic the pulsatile GnRH release and, hence, are hypothesized to maintain the FSH and LH production, fertility status, and testicular size. Intranasal testosterone is a rapidly absorbed gel form of testosterone, applied to the super permeable nasal epithelium, therefore bypassing the first pass metabolism, and providing very high bioavailability compared to other forms [98]. A study by Mattern et al. reported a 10–100 min half-life of intranasal testosterone with a maximum serum level reached in an hour [99].

Recent studies investigated the effect of 4.5% intranasal testosterone gel on both sperm and hormonal levels. Kavoussi et al. investigated the change in semen parameters and reproductive hormones in men changing to intranasal testosterone from clomiphene citrate. Sixty hypogonadal men were managed with 25 mg daily of clomiphene citrate, the dose was doubled at 1 month if testosterone levels were below 300 ng/dL. For men complaining of low libido despite clomiphene citrate treatment, they were offered to change to intranasal testosterone 11 mg, 2 pumps daily for a 3-month course. Medication doses were titrated based on a 1-month hormonal profile follow-up. Serum hormone levels and semen parameters were compared in both arms after completing a 3-month treatment course of intranasal testosterone group. They reported no statistically significant changes in the testosterone and LH levels in the intranasal arm compared to the clomiphene citrate arm. Sperm concentration, motility, morphology, and semen volume also showed no

changes between both treatments. FSH levels decreased significantly but remained in the low normal reference ranges, and symptoms of libido subjectively improved in men after intranasal testosterone application. However, given the established relationship of spermatogenic suppression from exogenous testosterone, the authors cautioned the need for long-term follow-up before suggesting safe use in patients actively attempting to conceive or those looking to maintain fertility potential [100].

Ramasamy et al. reported 2-year results of a single center, open-label study of men receiving intranasal testosterone gel. A total of 60 men were initially recruited, out of which 33 completed the 6-month treatment duration. Compared to the baseline, sperm concentrations showed no statistical significance after 3 and 6 months follow-up (mean difference of −4.1, $p = 0.193$ at 3 months; −5.5, $p = 0.081$ at 6 months). Total motile sperm counts were reported to be greater than five million in 93.9% of patients during the 24-week treatment period. 81.8% of patients maintained their baseline FSH levels and 72.7% maintained their LH baseline levels. 90.9% of patients who continued the 6-month follow-up, restored their testosterone levels to 300 ng/dL and above. Significant improvement on the international index of erectile function domains of sexual desire and overall satisfaction were documented. The authors concluded that despite the non-statistically significant decline in sperm concentration, motility and gonadotropin levels, there still could be potential benefits on fertility with the usage of intranasal testosterone but more long-term studies are needed at first [101]. It is believed that further future research interest will be directed in studying the effect on androgen receptors to continuous versus pulsatile testosterone stimulation.

Spermatogenesis Preservation

Patients presenting to the andrologist with symptoms and signs of hypogonadism must have a thorough discussion about their fertility plans before any management is outlined. Testosterone replacement therapy, while effective in addressing most hypogonadal symptoms, has detrimental effects on endogenous testicular function. Counseling should include a thorough discussion of risks and benefits with emphasis on spermatogenic suppression for men of reproductive age who wish to maintain reproductive potential.

Hypogonadal patients may be classified in various ways, one of which is based upon their reproductive goals: (1) those actively trying to conceive, (2) those who do not wish to conceive, and (3) those who are not actively trying to conceive but wish to preserve the option of doing so in the future. A separate cohort of patients is those who are currently on androgenic steroids, whether prescribed or not, and wish to recover spermatogenesis and reproductive capacity.

Anabolic androgenic steroids (AAS) are steroidal androgens that contain male testosterone hormone or synthetic analogues that perform the same action. These agents have been used and abused for the purpose of muscle bulking, enhanced performance, prolonged endurance, and body fat loss. The use of AAS has recently increased among adolescents and young men, reaching up to four million users in

the USA [102, 103]. The prevalence of lifetime usage of AAS was recorded up to 5% in the general population, with a predominate percentage in men (6.4%) [104–106]. Nevertheless, Kovac JR et al. reported that 15.2% of men regretted the use of AAS, mostly due to the lack of knowledge of its deleterious fertility effects [107].

Although there is a general understanding of the testosterone masculinizing effects, the understanding of the HPG axis, and its subsequent indications for therapy, have been found to be misguided among physicians. Samplaski et al. conducted a study to evaluate the frequency and reason for testosterone usage in infertile men and they found out that 59 (1.3%) out of 4400 men who followed in the fertility clinic received testosterone therapy before they started their fertility treatment. Among physicians prescribing those treatments, 24% were endocrinologists, 17% general practitioners, 15% urologists, 5% gynecologists, and 3% reproductive endocrinologists [108]. This uncovers a serious high percentage of misunderstanding physicians on the effects of testosterone replacement therapy on men seeking to father children.

Numerous detrimental effects with AAS/exogenous testosterone intake have been reported. Due to the suppression of the HPA axis from increased exogenous testosterone, low gonadotropin levels and decreased intratesticular testosterone production will eventually lead to germinal epithelium atrophy, and defective spermatogenesis [109]. The World health Organization task force in 1990 reported a multicenter study data conducted on 271 healthy men to study the effects of hormonally induced azoospermia. After 6 months of treatment with 200mg of testosterone enanthate weekly, 65% of men were azoospermic [110]. Anderson et al. reported a 55% of azoospermia in patients receiving exogenous testosterone for 18 months, while the remaining 45% suffered severe oligozoospermia during the treatment duration [111]. These percentages were reported even higher in a Chinese study that described a 93–99% azoospermia rate in men receiving monthly intramuscular testosterone during a 30-month trial [112]. The negative impacts of such treatments were reported to take up to 3 years to be reversed [113, 114].

Multiple groups have investigated protocols to maintain intratesticular testosterone levels and spermatogenic function. Coveiello et al. recruited 29 men in an experimental study conducted in patients with normal testosterone levels, seminal parameters, and testicular size. They received 200 mg of testosterone enanthate intramuscularly weekly for 3 weeks to induce gonadotropin suppression, and then, they were randomized into four groups: placebo, 125 IU hCG, 250 IU hCG or 500 IU hCG, all of them being used every other day. Intratesticular testosterone (ITT) levels were determined by percutaneous testicular fluid aspiration done at baseline and day 21 of treatment. Semen analysis and serum testosterone, LH and FSH were drawn at baseline, 3 weeks, and 3 months post-treatment. Compared to the mean baseline values, ITT was 94% suppressed in the placebo-control group, 25% decreased in the 125 IU hCG group, 7% decreased in the 250 IU hCG group, and increased 26% in the 500 IU hCG group. A statistically significant linear increase of ITT was observed with increased hCG doses. Authors concluded that maintaining normal ITT levels with gonadotropin suppression is possible with the addition of low hCG doses to testosterone therapy [115].

In 2012, Hsieh et al. retrospectively reviewed 26 men who had been on testosterone therapy (seven patients on daily transdermal gel and 19 patients on weekly intramuscular injections) with concurrent intramuscular hCG 500 IU injections every other day. Comparison of semen parameters and serum testosterone, FSH and estradiol levels at baseline and every 2–4 months during treatment was conducted. Men were followed at a mean of 6.2 months and up to 18 months. Results showed no statistically significant differences in semen parameters during follow-up other than a minor decrease in semen volume in the first 1–2 months. Moreover, types of testosterone therapy showed no statistically significant differences between them on sperm parameters. The authors stated that simultaneous intake of hCG and TRT preserves men's fertility potential by protecting spermatogenesis [116]. Ultimately such protocols may be considered for a degree of spermatogenic stimulation while undergoing testosterone therapy, but we would caution against any exogenous testosterone, with or without adjunct therapies, while a couple is actively trying to conceive. Such protocols may be considered when a man would like to preserve a degree of stimulation prior to future testosterone cessation and active attempts at conceiving.

Spermatogenic Recovery

There is heterogeneous data on the recovery of spermatogenesis with some suggesting return to the baseline semen parameters 1–2 years after cessation [117]. The WHO demonstrated 46% of men returning to baseline semen parameters after AAS discontinuation [118] A meta-analysis performed by Liu PY in 2016 studied different factors that could affect sperm recovery after several types of androgen replacement over a 16–78-week period. The authors stated that the median time to restore sperm concentration up to 3, 10, and 20 million/mL was 2.5, 3, 3.4 months, respectively. Their meta-analysis showed a 67% of sperm recovery up to 20 million/mL was reached in 6 months, which then increased to 90% after 1 year, 96% in 16 months and a 100% in 2 years [119].

It is well established that the first step in spermatogenic recovery is the cessation of any exogenous testosterone sources. As mentioned earlier, it is unpredictable whether spermatogenesis will restart or not and when that will occur. In addition to that, stoppage of testosterone supplementation means the return of hypogonadal symptoms, and sometimes even more devastating. Therefore, medical therapy aids in jump starting the testes to resume endogenous testosterone production, alleviate symptoms of hypogonadism, and expedite sperm recovery.

Wencker et al. were the first to investigate the effect of medical stimulation therapy on men with azoospermia or severe oligozoospermia (<one million/mL) due to testosterone supplementation therapy. Forty-nine patients with an average of 52.4 months of exogenous testosterone supplementation (45 were azoospermic while the remaining 4 were severe oligozoospermic) were recruited and upon enrollment all testosterone supplements were discontinued. Patients started a treatment protocol of HCG 3000 IU subcutaneously given 3 times a week to maintain a stable

level of HCG throughout the week (half-life 33 h approximately). Patients were co-administered selective estrogen receptor modulators or aromatase inhibitors for FSH support either in a solo or combined fashion. Approximately, 71% of patients were prescribed clomiphene citrate, 57% were prescribed tamoxifen, 20% were prescribed anastrozole, and 2% were prescribed recombinant FSH. The primary endpoint was the appearance of sperm in azoospermic patients or increased sperm counts in severe oligozoospermic patients, and the secondary end point was documented pregnancy. Of the 49 study patients, 47 had a return of sperm in the ejaculate or improvement in sperm density > one million, and one additional patient achieved pregnancy without obtaining a semen analysis. The average time of spermatogenic recovery was 4.6 months with a mean density of 22.6 M/mL at the time of initial recovery. The authors did not identify a correlation of spermatic recovery with the type of testosterone supplement administration (gel, injection, pellet) or the combination regimen utilized in the study period [120].

While there are not standardized regimens for the recovery of spermatogenesis, we favor discontinuation of testosterone therapy with initiation of 3000 IU of HCG intramuscularly or subcutaneously, for three times a week, along with clomiphene citrate 50 mg every other day, with frequent serum hormone monitoring and dose titration. If there is inadequate spermatogenic recovery after 6 months, the stimulatory regimen is adjusted to include an FSH analog.

In cases of hypogonadal patients whose fertility is still warranted, the authors advise to start with non-testosterone therapies (SERMS, AIs, HCG, and FSH analogues). If the couple does not desire children, then testosterone supplements may be prescribed only after proper counseling and explanation of the risks and benefits is achieved, and if desired, sperm cryopreservation. If the couple desire future fertility while treating hypogonadism, testosterone supplements could be given together with HCG aiming at sustaining intratesticular testosterone levels which should aid in sperm production once the decision to have children is agreed upon.

Summary

Men are currently more attentive to their general and sexual well-being than ever. This includes hypogonadism, sexual function, and fertility. With the increased prevalence of testosterone supplements prescription and abuse, an imminent need to improve awareness for both physicians and patients toward the reproductive implications of these agents. Patients seeking fertility while being treated for hypogonadism should be managed by experts familiar with these complex scenarios. Treatment algorithms are set only after a clear discussion about risks, benefits, alternative methods, and expectations are made. Finally, despite novel therapies being investigated to tackle both conditions, further randomized prospective studies are necessary to elucidate the most effective way in treating hypogonadal infertile men.

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Anabolic Steroid-Induced Hypogonadism

12

Alexandre Hohl, Simone van de Sande Lee, and Marcelo Fernando Ronsoni

Introduction

Anabolic-androgenic steroids (AAS) are a group of substances that promote muscle growth and the development of male secondary sex characteristics, and include endogenously produced hormones, such as testosterone, as well as synthetic derivatives. Androgen abuse is defined as the use of testosterone or other synthetic androgen without a clinical indication, for the purpose of improving athletic performance and physical appearance, and has been practiced as a variant of social drug abuse for decades [1].

AAS abuse is predominantly motivated by its effects on skeletal muscle. Androgens are particularly effective ergogenic drugs for strength sports, promoting competitive advantage. These ergogenic benefits derive primarily from increased muscle mass and strength, but are also potentiated by increased erythropoiesis, raising circulating hemoglobin. AAS are also widely used for cosmetic and recreational purposes such as bodybuilding and by security personnel to enhance job performance [2, 3].

AAS abuse can cause adverse effects involving all organs and tissues, notably cardiovascular, hepatic, neuropsychiatric, musculoskeletal, metabolic, and reproductive effects. The effect on the reproductive system in men is hypogonadism resulting from suppression of the hypothalamic-pituitary-gonadal (HPG) axis, with altered spermatogenesis, infertility, androgen deficiency, and sexual dysfunction [4].

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Scientific evidence on hypogonadism and other adverse effects induced by AAS abuse is limited, since it is not possible to ethically replicate the high doses and the various factors associated with the use of these substances in randomized clinical trials. Therefore, most of the scientific information in this area comes from observational studies.

The vast majority of AAS users are men, and this chapter deals exclusively with their effects on males.

Epidemiology

An estimated 2.9–4.0 million Americans have used AAS at some point in their lives. Of these, about 98% are men and approximately one third have some degree of steroid dependence. Although the use of AAS is often associated with sport, more than 80% of users have never used anabolic steroids for competitive athletic purposes. In this way, many users do not inform their doctor or do not undergo analysis like athletes, causing the epidemic of AAS use to remain hidden and receive less attention by public health authorities [5].

A meta-analysis of 187 studies estimated the overall prevalence of androgen abuse in the general population to be 3.3%, with a prevalence four times higher in men (6.4%) than in women (1.6%). The prevalence of androgen abuse varies by geographic location, with the highest prevalence reported in the Middle East and South America [3]. Abuse is more frequent among recreational athletes, some of whom have "muscle dysmorphia," a form of body dysmorphic disorder that causes individuals to see themselves as insufficiently muscular [5, 6].

Types of AAS

AAS are organic molecules that have in common the cyclopentanoperhydrophenanthrene nucleus, composed of four fused rings (A, B, C, and D). Synthetic AAS are derived from testosterone and, like the endogenous substance, act on androgen receptors and have variable anabolic and androgenic action. The relationship between the anabolic/androgenic actions of AAS is described in Table 12.1 [7, 8].

Table 12.1 Anabolic to androgenic ratios of AAS

AAS	Anabolic/androgenic ratio
Testosterone/methyltestosterone	1
Methandrostenolone	2–5
Oxymetholone	9
Oxandrolone	10
Nandrolone	10
Stanozolol	30

Source: Cunha et al. [7]

Table 12.2 Most commonly used AAS in oral and injectable formulations

Oral	Injectable
Ethylestrenol	Androstanolone
Stanozolol	Bolasterone
Formebolone	Boldenone undecylate
Fluoxymesterone	Clostebol
Gestrinone	Drostanolone propionate
Mesterolone	Methenolone enhantate
Methandienone	Nandrolone decanoate
Methenolone	Nandrolone phenpropionate
Methandrostenolone	Testosterone cypionate
Methyltestosterone	Testosterone enanthate
Methenolone acetate	Trenbolone
Mibolerone	Trenbolone acetate
Nortandrolone	Testosterone undecylate
Oxandrolone	
Oxymesterone	
Oxymetholone	

AAS differ in the type of chemical moiety attached to the main ring of testosterone. The 17β-esterified testosterone esters (cypionate, propionate, enanthate, and undecanoate) have greater lipid solubility and their release into the circulation is delayed, prolonging their action. The compounds are administered in injectable form. 17β-esterified nandrolone esters are also commercially available [7, 9]. The list of the most used AAS is described in Table 12.2.

The 17α-derivatives of testosterone (methyltestosterone, methandrostenolone, norethandrolone, fluoxymesterone, oxandrolone, and stanozolol) resist hepatic metabolism, therefore they are active when administered orally, and have high hepatotoxicity [7].

Patterns of AAS Abuse

The most frequent patterns of AAS abuse are the so-called cycles, in which high doses (up to 10–100 times the indicated doses for treating disease) are used for a period of 6–12 weeks, followed by a break for an equal or greater time interval and restart of use. Each cycle is typically done in a "pyramid" fashion, with sequences of gradually increasing the dose until reaching a peak amount, and then tapering off, supposedly to allow the body to adjust to these doses [10, 11].

In the "stacking" regimen, two or more AAS are used (for example, injectable and oral), to allow, according to users, a synergistic effect on muscle growth. In this scheme, in addition to AAS, other substances with anabolic potential can be associated. The overlapping or substitution between different types of AAS, called "plateauing," is also a frequent practice, in order to avoid tolerance. It should be noted that none of these usage patterns and their alleged effects have been scientifically tested [10, 11].

Pathophysiology of AAS-Induced Hypogonadism

The use of AAS results in the suppression of the HPG axis, by inhibiting the pulsatility of Gonadotropin Releasing Hormone (GnRH), and consequent reduced levels of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH), resulting in the development of hypogonadotropic hypogonadism [4, 12].

The duration of suppression and the signs and symptoms resulting from this modification of adequate physiology are highly variable and stem from the different AAS used, doses, duration of use and the number of times they were used [13]. Users often already have other impairments of the HPG axis function, such as cases of functional hypogonadism, which exposes them to a greater risk of damage to the gonadal function [14].

In addition, variable responses are identified in clinical practice among people exposed to AAS, and there may be different degrees of individual sensitivity to the magnitude of the impact on the hypothalamic-pituitary system and, consequently, the damage and recovery of the functioning of the gonadal axis in medium and long term. Some authors suggest that younger men may have a "more elastic gonadal axis" and be able to recover GnRH pulsation and gonadotropin secretion faster and more completely than older men. It is possible that cycles with shorter durations, lower doses, younger ages, and higher baseline total testosterone levels are associated with faster recovery of HPG axis function after AAS use [13].

Users often justify the use of AAS based on literature data regarding testosterone replacement therapy (TRT) in patients with hypogonadism. In TRT, testosterone formulations are used in fixed and safe replacement doses and the physiological and clinical response is more predictable. In individuals who use AAS, we are faced with more complex physiological changes, due to the overlap and cycling of various synthetic androgens at high doses with other auxiliary drugs, culminating in a unique pharmacological mixture [13].

Animal studies suggest that the use of AAS may also cause testicular toxicity [1]. However, the significance of these findings is not well established and the extent of damage that overuse of AAS may contribute to primary gonadal hypogonadism remains unclear, requiring further studies.

Diagnosis

Addressing AAS abuse cases is difficult because patients are often reluctant to report it to their physician. The approach must be careful, avoiding judgments and seeking to establish a trusting relationship [15].

The diagnosis of hypogonadism associated with the use of anabolic steroids is performed according to the same clinical and laboratory criteria used in other hypogonadism etiologies. However, normally the hormonal axis compromise in these cases is more intense and the laboratory results are more impressive. All patients seeking medical care with signs and symptoms consistent with hypogonadism should be asked about current or previous use of AAS [16]. Some signs and

symptoms may suggest AAS use, such as marked muscularity, truncal acne, gynecomastia, striae above the pectoralis muscle and testicular atrophy [15].

We recommend following the Endocrine Society guidelines and cut-off points for the diagnosis of hypogonadism. In its most recent publication, the Endocrine Society included, for the first time, the withdrawal from prolonged use of AAS among the list of clinical conditions with a high prevalence of low testosterone concentrations. Thus, patients who used AAS are candidates for serum total testosterone measurement [16].

Patients who have a desire or complaint regarding fertility should undergo a sperm analysis (at least two samples with an interval of two weeks between them). We emphasize that the diagnosis of hypogonadism is confirmed by the presence of low serum levels of testosterone and/or oligozoospermia or azoospermia associated with the presence of clinical signs and symptoms [16].

Management

Despite the increasing prevalence of androgen abuse, the literature on its adverse effects is limited largely due to its clandestine nature that leads to underreporting and a systematic limitation of the ability to establish causality from anecdotal and observational reports.

AAS users often report side effects they find esthetically unpleasant, such as testicular atrophy, water retention, acne, gynecomastia, and alopecia [5, 13, 17, 18]. Sexual dysfunction was reported by 25% of users and other symptoms of androgen deficiency, including fatigue and depression, are common complaints, especially during the period immediately following AAS use [13, 19].

Prolonged use can cause side effects such as liver, kidney, and cardiovascular disease. Hypertension and dyslipidemia are common among chronic users. Polycythemia is also a frequent finding, occurring in up to 40% of patients [13, 19, 20].

EAA abuse is typically done with high doses and often mixing of different substances, which has never been clinically tested in humans. This makes the side effects, both physical and psychological, extremely dangerous. These damages can be even more dangerous in young men who are in the developmental stage and can interrupt proper growth and sexual development [5, 13].

We recommend that all patients who are using AAS or who have a history of exposure in the past should be advised of the risks of its use and offered assistance in stopping the abuse. Psychological and psychiatric assessment should be evaluated individually due to the high rate of individuals with body image disorders or other associated diseases.

As previously discussed, the different combinations of AAS and the individual sensitivity associated with the duration and dose of use promote a huge variability of possible clinical situations. Thus, each case must be individualized and the approach must be rational and based on endocrine physiology. There is still a lack

of data in the medical literature to support the approaches and, in most cases, the level of evidence is expert opinion.

A systematic review and meta-analysis demonstrated normalization in LH and FSH levels between 12 and 24 weeks after discontinuation of AAS use while the testosterone level took longer to normalize, and recovery started only after the 16th week of discontinuation [21].

For severely symptomatic patients, a 4-week course of transdermal testosterone replacement therapy (TRT) may be helpful to immediately ameliorate the effects of testosterone deficiency and thereby reduce the chances of returning abuse (addictive cycle) and neuropsychiatric symptoms. We recommend the administration of transdermal testosterone gel at a dose of 25–50 mg, given as a single daily dose during the first phase of treatment. Figure 12.1 shows a suggested algorithm for the treatment of hypogonadism associated with the use of AAS. Simultaneous administration of a selective estrogen receptor modulators (SERM), such as clomiphene citrate, 25 mg daily or 50 mg every other day, assists in interacting with the hypothalamus, promoting LH stimulation and ultimately, an increase in testosterone [13].

Some patients are oligosymptomatic or have a previous history of AAS use and do not require initial TRT. In these cases, the administration of SERM alone can be effective and should be considered. In addition to SERM, aromatase inhibitors and human chorionic gonadotropin (hCG) are used in attempts to restore the HPG axis via estrogen suppression and, therefore, its negative feedback [13, 21].

After 4 weeks of treatment with SERM (with or without associated TRT), a new laboratory hormonal evaluation should be performed. If the patient has a deficient elevation of gonadotropins or persists with very low testosterone levels, the use of hCG (1000–3000 IU, three times a week, or 5000 IU once a week) is recommended, continuing daily treatment with SERM at the starting dose. After 8 weeks of using hCG and adjuvant treatment, hormone levels should be reassessed [13].

If testosterone levels are adequate (within reference levels for the method) and gonadotropin levels have been re-established, the SERM can be reduced to 50% of its initial dose at the tenth week of treatment while maintaining close hormonal monitoring, with reassessment between 12 and 16 weeks [13].

The recovery of hormonal function can be quite variable and even limited and incomplete in some men, and if total testosterone levels remain low after the 8th to 12th week of treatment, it is likely that we are facing a primary testicular failure and these patients may need longer treatment with TRT to avoid permanent hypogonadism secondary to the use of AAS [13].

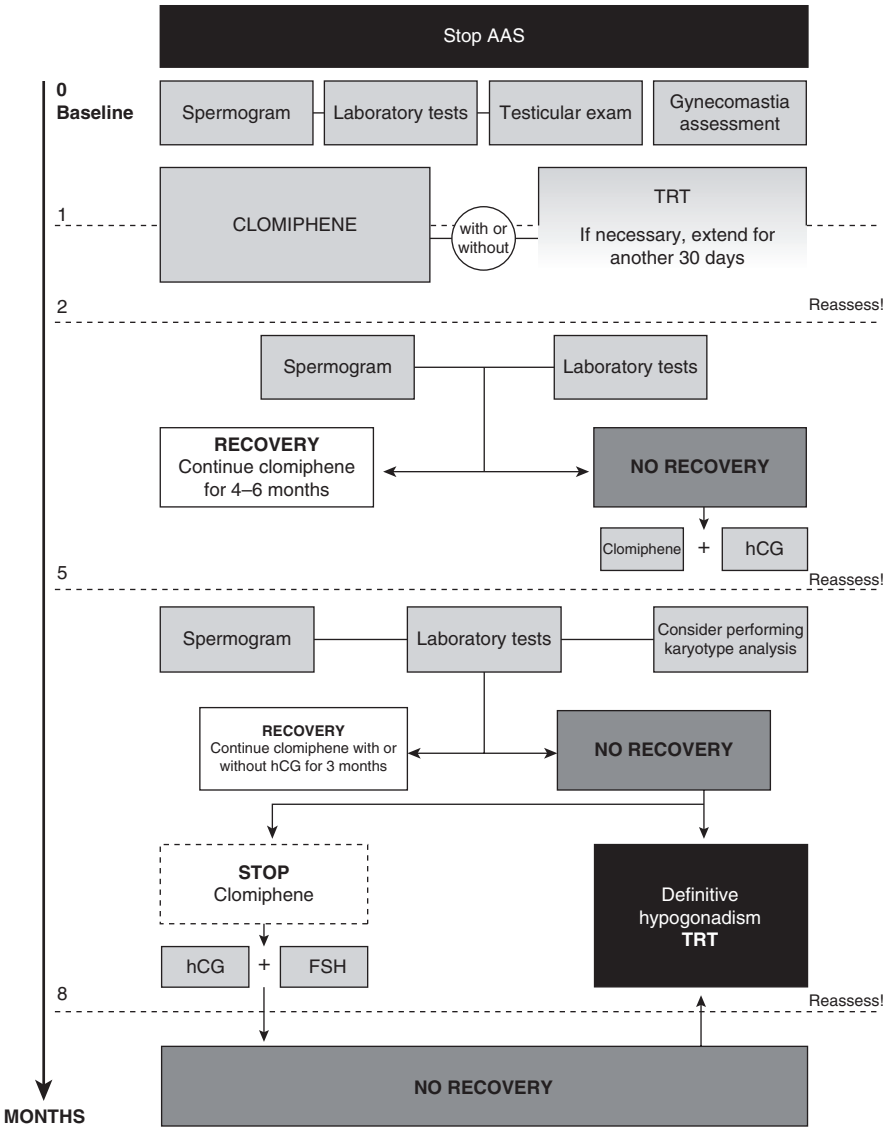

Fig. 12.1 Suggested treatment algorithm for symptomatic anabolic-androgenic steroid-induced hypogonadism

Associated Conditions

Sexual Dysfunction

Erectile dysfunction and decreased libido are common complaints from AAS users, especially after stopping use when endogenous testosterone levels are lower [5, 16].

Sexual complaints may also be associated with the type of AAS used. Nandrolone, for example, is linked to erectile dysfunction when used alone. This is likely due to an unopposed progestin-like action along with the relatively lower androgenic activity of its 5-alpha metabolite dihydronandrolone (compared to dihydrotestosterone). These effects are minimized with the concomitant administration of other AAS formulations with a greater androgenic effect [13].

Over 25% of users report using PDE5 inhibitors prophylactically or therapeutically for their erectile dysfunction [13, 18].

Gynecomastia

Gynecomastia (painful or not) is a common complication of AAS use and can occur in up to half of users. It occurs due to the imbalance of testosterone and estradiol levels in the breast in the same way as the other etiologies of hypogonadism. They may appear during or after the use of AAS and must be distinguished from lipomastia. Some patients may experience gynecomastia following hCG administration during treatment (and subsequent elevations in estradiol levels secondary to aromatization) with a decline in the entire endogenous androgen signaling system [5, 19, 22].

Some AAS have a greater effect on the breasts because they are more susceptible to aromatization [23]. Finasteride (used concomitantly in up to 10% of men to treat alopecia) may potentiate this effect and should be discontinued [13, 24]. Herbal supplements like Tribulus terrestris and Saw Palmetto (or Serenoa repens) extract have no proven benefit and can worsen gynecomastia. We emphasize that all patients being treated for hypogonadism secondary to the use of AAS should discontinue any use of supplements with no proven benefit [25, 26].

The response to gynecomastia treatment is also individual and the main prognostic indicator is its duration. Recent cases of gynecomastia are more responsive and often require a few months of treatment. In cases of persistent gynecomastia with more than one year of evolution, it is more likely to involve significant fibrosis and typically responds poorly to drug therapy. In these cases, surgical treatment is the best option for esthetic improvement [22, 23, 27].

Despite the lack of large clinical trials, tamoxifen appears to be the safest and most effective agent for the treatment of gynecomastia associated with AAS or hCG use [13, 22, 23]. The recommended starting dose is 10–20 mg daily, which can be increased to 30 mg if necessary.

The use of aromatase inhibitors such as anastrazole has been proposed as an alternative to the use of tamoxifen and there is evidence of its effectiveness in the treatment of gynecomastia. However, suppression of circulating estrogen levels with aromatase inhibitors can decrease libido, worsen erectile dysfunction, and increase body fat percentage in men [13, 28].

Infertility and Testicular Atrophy

Infertility has usually been a reason for seeking medical care in AAS users. Adequate intratesticular testosterone concentrations are required to maintain spermatogenesis and, with the use of AAS, there is suppression of the HPG axis and, consequently, a reduction in endogenous testicular testosterone production [29]. The main findings in the spermogram are azoospermia or oligozoospermia, as well as sperm dysmorphia and dysmotility [30].

The time to spermatogenesis recovery, with or without medical treatment, appears to be highly variable and difficult or impossible to predict. Withdrawal of AAS use may be sufficient to restore sperm function in some cases, which usually occurs within 4–12 months. SERM have been used successfully after much shorter AAS cessation intervals [13]. We recommend, for longer lasting cases or for those patients in need of faster recovery, the use of clomiphene 50 mg daily (or every other day) as a way of stimulating spermatogenesis [31].

Data on the use of hCG and gonadotropins are more limited, but therapeutic alternatives may be considered in selected cases. Rarely, restoration of spermatogenesis does not occur even after the measures described. In these cases, the search for fertilization services is guided by the techniques of in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI) or microdissection with testicular sperm extraction (micro-TESE) [13].

With regard to testicular atrophy, the use of SERM such as clomiphene or hCG tend to increase and, in some cases, restore testicular volume [13].

Polycythemia

Exposure to supraphysiological levels of plasma androgens stimulates the production of erythropoietin and can lead to clinically significant changes such as secondary polycythemia. The increase in plasma viscosity may be a factor associated with the increase in cardiovascular events in AAS users, especially in those patients with preexisting coronary risk factors [1].

Discontinuation of AAS and restoration of normal levels of endogenous hormones are critical to reducing the patient's risk of potential complications associated with polycythemia. However, in cases of severe polycythemia (hematocrit greater than 54%) the need for phlebotomy should be evaluated. Assess all patients with polycythemia for signs and symptoms of hypoxia or sleep apnea [13, 16].

Musculoskeletal Injuries

Tendon rupture, especially those of the upper body muscles, is 3–4 times more frequent in athletes who have used anabolic steroids and is a sign of suspected use of these substances. Tendon rupture probably happens because steroids stimulate muscle hypertrophy without having the same effect on tendons, generating a disproportion that increases the risk of tendon rupture [32]. Joint and juxta-articular soft tissue injuries are also described as potential adverse effects of AAS [5].

Cardiovascular Effects

A wide variety of cardiovascular effects have been associated with AAS abuse: arterial hypertension, lethal arrhythmias, cardiomyopathy, left ventricular hypertrophy, myocarditis, myocardial infarction, cardiac tamponade, thrombotic and hemorrhagic stroke, subdural hematoma, thrombosis (arterial and venous), and pulmonary embolism [33, 34]. As the causality of these effects remains speculative, the prospective risks are still difficult to define.

Liver Disease

Hepatotoxicity is a serious adverse effect especially with the use of oral 17α-alkylated androgens. Among the hepatic alterations described with the use of AAS, the following stand out: hepatic tumors (adenoma, carcinoma, cholangiosarcoma, angiosarcoma), peliosis hepatis, focal hepatic necrosis causing vascular cysts, and drug-induced hepatotoxicity (cholestatic pattern). Most liver tumors are slowly progressive and reversible after stopping androgen intake, but fatal cancers have been reported [1, 35].

Neuropsychiatric Changes

One of the most alarming adverse effects of AAS abuse is the risk of neuropsychiatric disorders that affect not only the individual but also their family and society [36]. Neuropsychiatric side effects can range from mild mood disorders, lack of judgment, uncontrolled aggression, hostility, sleep disturbances, mania, depression, to suicide risk [36, 37].

There is growing evidence that AAS abuse can involve addiction [38]. Also, anabolic steroid users are more likely to abuse other illicit substances concomitantly [5]. Psychological and psychiatric assessment should be encouraged with a view to counseling and understanding the reasons for the use and abuse of AAS and the identification of adjuvant psychiatric disorders or associated personality traits [13].

Nephrotoxicity

The use of AAS has also been associated with nephrotoxicity, although the clinical presentations are quite distinct and individualized. Focal segmental glomerulosclerosis with marked proteinuria has been reported after use of dietary supplements, testosterone, and growth hormone. With the increased use of various combinations of AAS and veterinary products, androgen-associated kidney damage has increased recently. Products with high doses of vitamin D can trigger hypercalcemia and, consequently, kidney damage. In the absence of specific findings, the use of AAS as a contributing factor to kidney damage cannot be ruled out [1, 39].

Other Findings

In addition to the adverse effects described, complaints of oily skin, skin and muscle abscesses, hair loss with androgenetic pattern baldness, and acne in the most different degrees are common. When used in pubertal periods, there is a higher rate of growth retardation [5]. There is also an increased risk of risky sexual behavior and of acquiring communicable diseases, such as hepatitis B and C and HIV, among AAS users through sharing needles and unprotected sex [5, 20].

Conclusion

The increasing use of AAS and its adverse effects have motivated the search for endocrinological care, especially among young adult men. Hypogonadism is a frequent adverse effect of the use of these drugs.

After a thorough endocrine and metabolic assessment, management strategies for male hypogonadism include the use of testosterone, SERM, and hCG on an individualized and, in many cases, transient basis. Responses to treatment can be quite variable, depending on the time of use, number of cycles, types, and doses of AAS used.

More scientific evidence on the subject is urgently needed. It is necessary to disseminate knowledge and the negative impact of AAS among health professionals, promote more interventions and educational campaigns to society, especially in groups at risk for abuse, create more stringent legislation and encourage the search for adequate anti-doping screening tests. These and other strategies may, in the future, reduce the abuse of AAS and minimize this public health problem.

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Testosterone Therapy: Oral Androgens

13

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Abbreviations

5-α-DHT	5-α-dihydrotestosterone
BPH	Benign prostatic hyperplasia
DHEA	Dehydroepiandrosterone
E2	17-β-estradiol
FSH	Follicle-stimulating hormone
HDL	High-density lipoprotein
HSDD	Hypoactive sexual desire disorder
IPSS-QL	International prostate symptom score and quality of life
IVF	In vitro fertilization
LDL	Low density lipoproteins
LH	Luteinizing hormone
LUTS	Lower urinary tract symptoms
MHT	Menopausal hormone therapy
PSA	Prostate-specific antigen
SHBG	Sex hormone binding globulin
TG	Triglycerides
Ultrasound	Ultrasonography

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History of the Creation and Use of Oral Forms of Testosterone

Adolf Friedrich Johann Butenandt and Leopold Ruzicka independently synthesized testosterone from cholesterol in 1935, for which they shared the Nobel Prize in 1939 [1, 2]. The interest in testosterone preparations has only increased since then.

Oral testosteroneis rapidly absorbed in the intestine, undergoes complete hepatic metabolism, and scarcely reaches its target cells. To achieve a physiological level of serum testosterone, it should be administered orally at a dose of 400–600 mg, which is 50–100 times higher than daily male secretion in the body. This treatment is very expensive and unsafe [3], wherefore an active search for modified testosterone molecule has been performed since the mid-1930s to obtain an effective and safe drug.

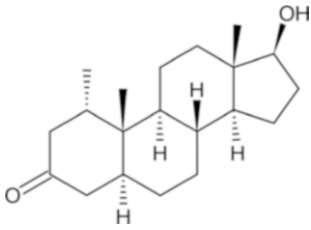
The first modified testosterone forms were oral forms: mesterolone (synthesized in 1934) and methyltestosterone (synthesized in 1935). In 1937, the first injectable testosterone (testosterone propionate) drug was synthesized.

Mesterolone

Mesterolone has the longest history of clinical use of all testosterone drugs and is still currently used [4, 5]. The drug is available in more than 30 countries worldwide under different trade names (Proviron, Mestoranum, Provironum, Plyuriviron, Vistimon, Restore), nevertheless it has never been approved for sale in the USA. It is prescribed for men at a daily dose of 25–75 mg. As a chemical, mesterolone it is not a product of testosterone aromatization, but a derivative of its most active metabolite-5-α-dihydrotestosterone (5-α-DHT), which makes it closer in structure to mestenolone because both have a nontoxic 1-methyl group which enhances resistance to disintegration in the liver; nevertheless such structure of the mesterolone molecule does not enhance the stability of its 3-keto group (Fig. 13.1).

Taking into account that mesterolone is a derivative of 5-α-DHT, it does not have the whole range of effects of natural testosterone, adjusting 5-α-DHT-dependent effects and being characterized by weak anabolic effects. In this regard, mesterolone is not currently used for replacement therapy of testosterone deficiency (hypogonadism), which needs all effects of both testosterone and its metabolites, including estrogens [6].

Fig. 13.1 Mesterolone chemical structure



Methyltestosterone

Methyltestosterone (17-α-methyltestosterone, 17α-methylandrosten-4-ol-17β--one-3) was first synthesized in 1935 and was the most widely used oral formulation of testosterone, according to its chemical structure it is a methyl derivative of testosterone.

The methyl group at the 17α-position of the testosterone molecule prevents its destruction in the liver ensuring drug efficacy when orally administered (Fig. 13.2).

Methyltestosterone has the entire range of anabolic and androgenic effects of the natural hormone (today we understand that androgenic effects include estrogenic effects via aromatization of testosterone to estrogens), and was easy to use in men in clinical practice and was available, and was inexpensive. Due to its high efficacy, the drug was actively used in women with good results to treat diseases such as breast cancer and excessive lactation, breast pain after pregnancy in non-breastfeeding mothers, functional dysmenorrhea, migraine in women, and osteoporosis [7–10].

In addition to standard tablets and capsules administered at a daily dose of 10–30 mg, methyltestosterone has been available in a dosage form for sublingual and buccal administration, specifically under the trade name Metandren, which was probably the most recognizable and popular steroid from 1950–1990. However, in 1981 the German Society for Endocrinology found that hepatocellular carcinoma occurred in association with methyltestosterone and made an official statement against the drug. As a result the drug was removed from all pharmacies in Germany. Soon the majority of European countries followed the example of Germany. Production and sale of methyltestosterone is currently banned in most countries worldwide [11–14].

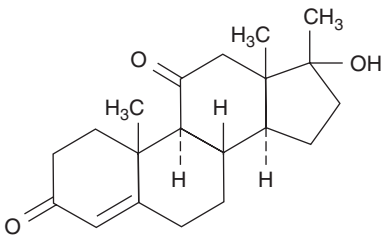


Fig. 13.2 Methyltestosterone chemical structure

Fluoxymesterone

A new oral testosterone preparation—fluoxymesterone was synthesized in 1956 (Fig. 13.3). It was a derivative of methyltestosterone, which differed in modifications at three positions: 17-α-methyl group of 11-β-hydroxy and 9-α-fluoro group that eventually made it essentially a halogen-testosterone derivative.

The first chemical modification made it possible to extend the half-life of the drug when administered orally. The second modification was aimed at preventing the enzymatic conversion of the molecule through attachment of aromatic rings, which made fluoxymesterone a non-aromatizing androgen. In addition, it involved the ability to block estrogen and prolactin receptors, which excluded the occurrence of gynecomastia, edema, and increasing fat mass.

The last modification of the molecule determined the drug name (fluoxymesterone) and was aimed at increasing its androgenic activity (facilitating the 5-α-reduction which leads to conversion to 5-α-DHT).

The new drug exceeded the anabolic activity of methyltestosterone by 20-fold. Compared with natural testosterone, the androgenic effects of fluoxymesterone were more pronounced, but at the same time its anabolic properties were inferior to natural testosterone [15, 16]. Fluoxymesterone was soon available on the US market under the trade names Halotestin and Ultradren (the daily dose was 5–20 mg) and was used for the treatment of male hypogonadism as well as other conditions. It was recommended for the treatment of burns, fractures, anemia, and for the treatment of consequences of glucocorticoid therapy [17, 18]. In the mid-1970s, control of drugs was tightened, and fluoxymesterone was prescribed only for the treatment of androgen deficiency in men and inoperable breast cancer in women [19–21].

Because fluoxymesterone, like methyltestosterone, has a 17-α-methyl group associated with hepatotoxicity, most countries declined both the drugs, although fluoxymesterone is still available in some countries under the trade name Halotestin.

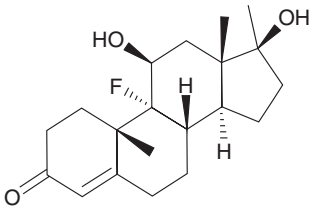


Fig. 13.3 Fluoxymesterone chemical structure

Testosterone Undecanoate

The revolutionary pharmacologic breakthrough in the modification of natural testosterone was a synthesis in the early 1980s, of a radically new oral testosterone drug—testosterone undecanoate, which is currently available for clinical use in many countries under different trade names (Andriol, Panteston, Restandol, Undestor, Virigen). Unlike all their "old" methylated predecessors, for the first time testosterone undecanoate was not a modified molecule of testosterone but a testosterone molecule identical to the natural one, as a fatty acid ester of the undecanoic acid salt (undecanoate), as illustrated in Fig. 13.4.

The creation a testosterone ester has opened new opportunities in oral therapy. The absence of hepatotoxicity significantly enhanced the effectiveness of the therapy due to the possibility of increasing the dose of the drug, as hepatotoxicity inevitably increased with the increase in the dose of the methylated derivatives. Moreover, testosterone undecanoate is absorbed from the intestine not into the blood, but into the lymph, which excludes the hepatic first-pass metabolism with generation of toxic hepatic metabolites typical of all previously used oral 17-C-methylated derivatives of testosterone [22–24].

Because testosterone undecanoate is an ester of natural testosterone, following its administration plasma levels of both testosterone and all its active natural metabolites, such as5-α-DHT and estrogens, increase due to its ability of aromatization by fat tissue (conversion to estrogens) [23, 25].

All the above beneficial properties of oral testosterone undecanoate made it widely used in many countries worldwide, although, for example, the drug has never been approved in the USA for clinical use [26] (Table 13.1).

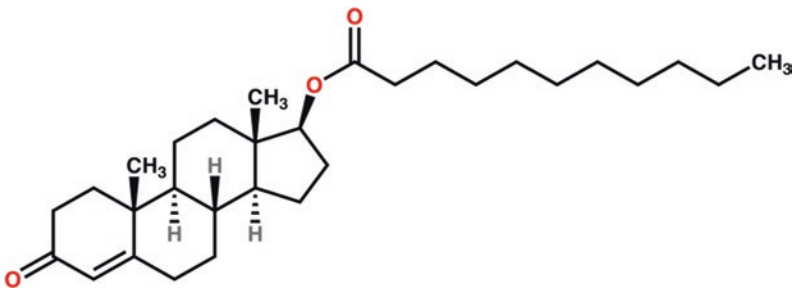


Fig. 13.4 Testosterone undecanoate chemical structure

Table 13.1 Comparison of oral (methyltestosterone, mesterolone, testosterone undecanoate), transdermal and injectable drugs (testosterone propionate and testosterone undecanoate)

Drugs/effects	Methyl testosterone	Mesterolone	Oral testosterone undecanoate	Testos-terone gel	Testos-terone propionate	Injectable testosterone undecanoate
Anabolic activity (% of testosterone)	100%	40%	100%	100%	100%	100%
Androgenic activity (% of testosterone)	100%	100%	100%	100%	100%	100%
Hepatotoxicity	Severe	Absent	Absent	Absent	Absent	Absent
Inhibition of LH, FSH secretion	High	Practically absent	Moderate	Mild	High	High
Aromatization to estrogens	Yes	No	Yes	Yes	Yes	Yes
Effect duration	4–6 h	12 h	8–10 h	24 h	2–3 days	8–14 weeks
Detection time	7–14 days	60 days	7–10 days	72–96 h	40 days	53–90 days

Testosterone Undecanoate: Pharmacokinetics and Pharmacodynamics

Currently, the only oral formulation of testosterone recognized in clinical practice is testosterone undecanoate, which is available as 40 mg capsules. Because testosterone accounts for 63% of the molecular weight, one capsule contains about 25 mg of testosterone.

Following oral administration, the essential part of testosterone undecanoate and lipophilic solvent is absorbed in the small intestine, then enters the lymphatic system, without the first-pass inactivation by the liver, resulting in the lack of the drug's hepatotoxicity.

The drug is administered during meals to improve absorption, as its bioavailability is quite low, about 7%. Gastrointestinal diseases (sialodenosis and xerostomia with saliva pH imbalance, intestinal dysbiosis, and hepatobiliary and pancreatic diseases) can significantly reduce the absorption and bioavailability of any oral preparations, including preparations of testosterone [27].

Testosterone undecanoate is absorbed with partial reduction and generation of 5-α-DHT. The drug is released into the plasma from the lymphatic system. In plasma and tissues, testosterone undecanoate is hydrolyzed to yield natural testosterone, which is further metabolized to 5-α-DHT and estradiol. Subsequently, testosterone, estradiol, and 5-α-DHT are metabolized via the normal pathways, and excretion takes place mainly via the urine as conjugates (etiocholanolone and androsterone). Single administration of testosterone undecanoate leads to a clinically significant increase of total plasma testosterone with peak levels reached 4–5 h after administration. The half-life of the drug is 3–4 h, therefore the drug is given at least 2–3. times a day.

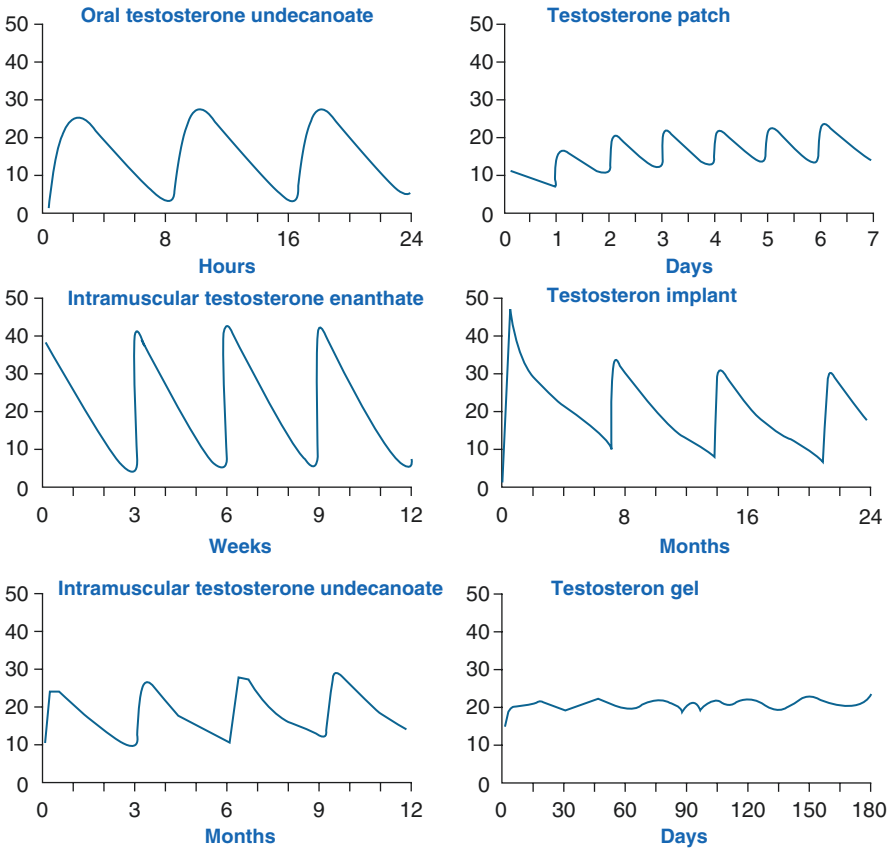


Fig. 13.5 Comparative pharmacokinetics of various formulations of testosterone

Figure 13.5 shows a patient receiving oral testosterone undecanoate has fluctuations in the plasma level of testosterone during the day [28, 29].

Oral Forms of Testosterone in Men: Indications for Use, Dosage, and Monitoring of Efficacy and Safety

The *main indication* of any form of testosterone (including oral forms) in men is testosterone deficiency (male hypogonadism) [30, 31]. Taking into account the latest safety data of androgen replacement therapy, including injectable drugs as well as a relatively small increase in testosterone level during the treatment of oral testosterone undecanoate, absence of significant decrease in luteinizing hormone (LH) and follicle-stimulating hormone (FSH), we believe that there are no contraindications for oral testosterone undecanoate therapy.

Oral testosterone undecanoate has been used in clinical practice for over 30 years and has been well studied. It has a mild androgen effect and is mainly used in cases of mild/moderate male hypogonadism in both boys and men. Several studies have shown that testosterone undecanoate therapy in hypogonadal men is able to recover the total and bioavailable testosterone level to normal, increase the serum level of 5-α-DHT and estradiol (E2) , reduce the plasma levels of sex hormone binding globulin (SHBG), LH, and FSH in a dose-dependent manner [32].

Interesting data were obtained during the use of oral testosterone undecanoate in the surgical treatment of severe forms of hypospadias in boys [33]. The efficacy and safety of testosterone undecanoate has been observed during the treatment of disturbance of puberty and induction of puberty in boys [34–37].

Oral testosterone undecanoate significantly improved the bone mineral density and increased the lean body mass in hypogonadal men in randomized studies, associated with normalization of plasma levels testosterone [38, 39].

The effect of oral androgens (specifically, testosterone undecanoate) on libido and erectile function in men has been investigated in many studies, giving ambiguous and sometimes contradictory results. Thus, some authors [40] obtained evidence of efficacy of testosterone undecanoate therapy in patients with type 2 diabetes mellitus, other researchers found no significant improvement in sexual function, including men of an older age group with borderline-low levels of testosterone [41–43]. It is obvious that such contradictory data are due to the pharmacokinetics of the drug, difficulty of dose adjustment and, thus, difficulty of comparing the results of studies in patients with different severity of hypogonadism.

Oral testosterone undecanoate in a dose-dependent manner reduces low-density lipoproteins (LDL) and triglycerides (TG) and improves blood perfusion in the coronary vessels in men [44, 45].

Patients with hypogonadism and diabetes mellitus showed improvement of the insulin-sensitivity of tissues, which was accompanied by a decrease in insulin resistance and hypoglycemic effect, associated with testosterone undecanoate therapy [46]. Additionally, this therapy was accompanied by improvement of psychoemotional background and quality of life of men [47, 48].

The available literature data also support the possibility of the effective use of oral androgens for the treatment of some forms of male infertility associated with hypogonadism [49, 50].

According to the large, randomized, multicenter, double-blind study that included 322 men older than 50 years with symptomatic hypogonadism and urination disorders, the oral testosterone undecanoate therapy at a daily dose of 240 mg for 1 year resulted in a significant improvement in urination according to IPSS-QL (International Prostate Symptoms Score-Quality of Life) without any significant effect on the prostate specific antigen PSA (Prostate Specific Antigen) level and prostate volume [51].

Selection of a dose of both oral and other forms of testosterone undecanoate is strictly done on an individual basis depending on the patient's age and severity of hypogonadism.

Typically, the starting dose is 80–120 mg (2–3 capsules) in 2–3 divided doses for 3–4 weeks, then if necessary, it can be increased to 160 mg/day in three divided doses, and upon reaching the clinical effect followed by an individual maintenance dose, which on average is 80–160 mg/day for men, however, some patients require higher doses—240–360 mg/day (6–8 capsules). It is recommended to take capsules with meals, swallowing them whole [52]. The fat content of food influences the drug bioavailability, the fattier the food, the higher the bioavailability. If an odd number of capsules is prescribed, the higher dose of testosterone undecanoate is taken in the morning. The testosterone undecanoate effects are dose-dependent, the dose linearity was shown for doses of 40–240 mg/day. The therapy with oral testosterone undecanoate with good tolerance and sufficient clinical efficacy should be long-term, and in case of age-related hypogonadism—for term of life.

Monitoring of efficacy of therapy with oral testosterone undecanoate, as with other forms of testosterone, is based on positive dynamics of clinical symptoms of male hypogonadism and a number of objective signs of testosterone deficiency which are identified during the general examination or the use of simple but informative investigation techniques.

Kalinchenko et al. believe that adequate drug therapy for hypogonadism in men should lead to complete liquidation of obesity, sarcopenia, nocturia, erectile dysfunction closely connected with hypogonadism which can be objectively evaluated using simple but informative diagnostic methods (bioimpedance analysis, contour analysis of the photoplethysmographic pulse [Angioscan], uroflowmetry, AMS (Aging Male Score), IIEF (International Index of Erectile Function) surveys) [53].

Monitoring of prostate safety during the use of all testosterone drugs includes regular examinations of patients and carrying out a minimum of investigations during the whole period of androgen replacement therapy (digital rectal examination [DRE], PSA test). During the first year of treatment with any testosterone preparations, these investigations are performed on a quarterly basis, during the second year—two times a year, during the third and subsequent years of androgen replacement therapy—once a year [30, 31]. We believe that patients receiving testosterone undecanoate therapy should undergo examination once a year, just as all men older than 45 years should also undergo examination, regardless of whether they receive androgen replacement therapy or not.

Summary

Oral androgens in men are apparently safe, have a very mild effect, do not suppress LH and FSH, and can therefore be used in infertile men. Oral androgens can also be used when intramuscular injections are impossible, for example, due to the patient's reluctance or coagulation disorders (for example, in patients receiving anticoagulants), or when a patient is unable (1–3 days) to have injections of intramuscular forms of testosterone administered at a clinic for a short period of time (e.g., holidays).

Potential Use of Oral Forms of Testosterone Undecanoate in Women

The possible indication for use of testosterone preparations in women, according to the available guidelines, should be the treatment of inhibited sexual desire (defined as hypoactive sexual desire disorder or HSDD) [54–56].

However, the testosterone effects may not just be limited to a positive effect on the libido and sexual function in women [57–61].

For the first time we have used testosterone undecanoate in a woman at the age of 81 years with hip fracture who has never received menopausal hormone therapy (MHT). Not only the repair of a bone fracture, but also an increase in muscle mass and strength were observed in association with the therapy, which allowed the patient to return to her usual way of life within a month after fracture.

Several studies have shown that testosterone therapy has a positive effect on bone health, while observational studies have suggested that higher testosterone levels are associated with a reduced risk of fractures [62]. Most observational studies have also shown that low blood levels of total, free, and bioavailable testosterone (free and albumin-bound testosterone) are associated with a higher probability of atherosclerotic disease of the carotid artery, cardiovascular complications, and total mortality [63, 64].

The interest in the problems of female hormonal deficiency has been growing recently, many clinical and experimental studies suggest a possible role of testosterone in women in the pathogenesis of central obesity, insulin resistance, sarcopenia, urinary disorders, nocturia, osteoporosis, and other contemporary socially significant women's health issues that may become the methodologic basis for potential extension of indications for use of natural testosterone preparations for women both concomitantly with traditional estrogen-progestin drugs and as monotherapy, depending on the personalized parameters of hormonal status [65–72].

The main contraindications of testosterone drugs for women are not officially formulated due to the continued lack of a single common point of view on both the pathogenic nature of possible female androgen deficiency and its diagnostics, and the specific features of hormone replacement therapy with testosterone preparations.

Recent studies show that androgens may have another field of application in women—as a pretreatment in poor ovarian responders before controlled ovarian stimulation (COS) during in vitro fertilization (IVF). COS is increasing the number of developing follicles and oocytes, thus improving the pregnancy rate in women undergoing IVF. Meanwhile, poor ovarian responders who have diminished ovarian reserve fail to respond adequately despite the big dose of gonadotropins administered. Despite the advancement in reproduction technologies, low response to ovarian stimulation is still considered one of the most challenging tasks in reproductive medicine. It affects a significant proportion of infertile couples, ranging from 9 to 24% [73–75].

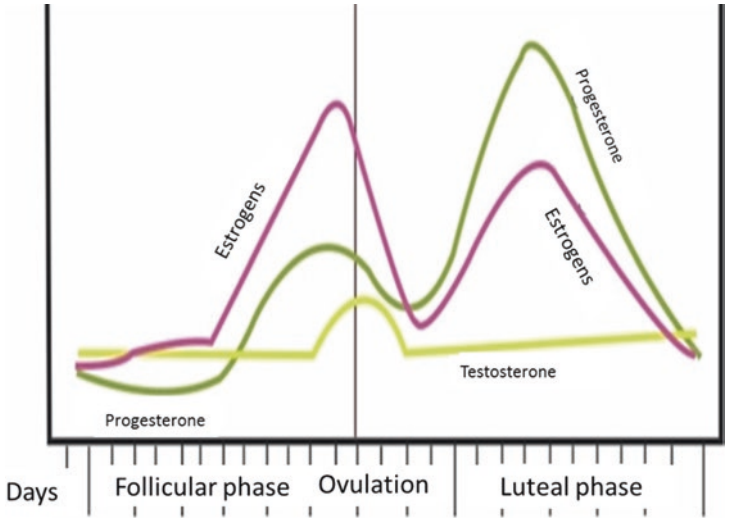


Fig. 13.6 Interaction of sex steroid hormones in the regulation of the menstrual cycle and ovulation in women of reproductive age [82, 83]

There is a slight age-related decline in serum dehydroepiandrosterone sulfate, dehydroepiandrosterone, and testosterone in women, which parallels the age-related decline in reproductive ability [76]. Some authors consider that poor response represents an androgen-deficient state [77].

Androgens play a fundamental role in follicular steroidogenesis, serving as a substrate for further estrogen synthesis. It has been shown in animal studies that androgens induce follicular FSH receptor expression in primate granulosa cells [78, 79], promoting the initiation of primordial follicle growth, resulting in the improved number of growing preantral and antral follicles [80, 81] (Fig. 13.6).

In humans, granulosa cells in vitro testosterone is able to positively modulate FSH receptor expression at the gene and protein level [84]. In a meta-analysis, testosterone treatment led to statistically significant increase in pregnancy and live birth rate in women with poor ovarian response [85].

Testosterone treatment with testosterone undecanoate in poor ovarian responders undergoing IVF increased the number of oocytes retrieved ($p < 0.05$) and the number of blastocysts per cycle ($p < 0.001$) when compared with the IVF cycle before treatment [86]. The clinical pregnancy rate was 27% per cycle and 43% per embryo transferred after administration of testosterone undecanoate.

In a study presented in 2015, testosterone undecanoate 40 mg pretreatment was used in women with DOR (diminished ovarian reserve) during the 48 days preceding COS for IVF. The clinical pregnancy rate was significantly higher in the testosterone treatment group (27%) than in control group (8, 9%, $p < 0.05$). Live birth rate was higher in the TU (testosterone undecanoate) group than in control group 16% vs 6.7%, respectively, although the difference was not statistically significant ($p = 0.18$) [87].

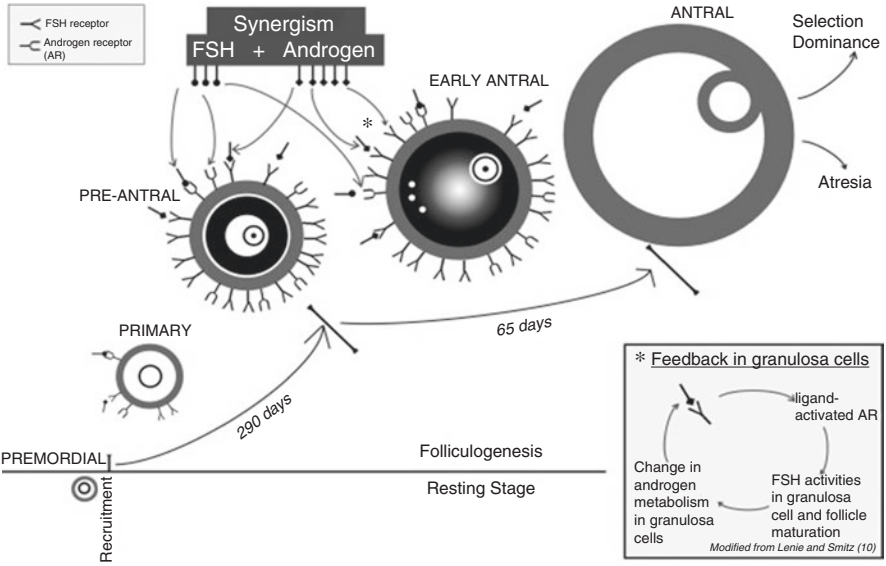


Fig. 13.7 Synergism between androgen and FSH in oocytogenesis [88]

Gleicher et al. proposed a new concept of ovarian aging: it impacts the ovarian environment, where follicle maturation takes place after recruitment whether oocyte at the primordial stage does not age [88] (Fig. 13.7).

In women following ovariectomy, oral testosterone undecanoate added to estrogen–progestin hormone therapy not only enhances libido and improves sexual function in general, but also promotes the increase in bone mineral density and decrease in fat mass [89–91].

Some of studies have shown that supplementation of oral testosterone undecanoate in a daily dose of 40 mg for 8 weeks in postmenopausal women has a significantly more pronounced positive effect on their sexual function compared with the placebo and standard estrogen–progestin therapy [92]. Although supplementation of oral testosterone undecanoate in a daily dose of 40 mg, according to some authors, may partially prevent the beneficial effect on cerebrovascular reactivity and lipid profile, the marked improvement in sexual desire and satisfaction is not denied by them [93].

It is difficult to agree with the conclusions of the authors of that study regarding some testosterone risks in relation to the endothelial function in women, as testosterone is a potent vasodilator in postmenopausal women [94]. A small study of testosterone therapy in women with congestive heart failure has demonstrated beneficial cardiovascular effects [95].

Combination therapy with transdermal estradiol and oral testosterone undecanoate (a daily dose of 40 mg) significantly improves the brain serotonin metabolism, the mood in postmenopausal women [96]. In addition, the relationship between testosterone levels and the severity of chronic fatigue syndrome in postmenopausal

women has been established [97] and the balance between estrogen, testosterone, and their metabolites was shown to be essential in maintaining the cognitive function in postmenopausal women [98].

However, according to other authors, the addition of oral testosterone undecanoate to estrogens in postmenopausal women can even worsen the verbal memory, in contrast to estrogen alone, while it has no negative impact on other memory types [99].

According to other data, additional administration of oral testosterone undecanoate at a daily dose of 40 mg concomitantly with estrogen therapy for 24 weeks has a more pronounced positive effect on inflammatory markers without any undesirable effects while estrogen monotherapy may increase the plasma level of C-reactive protein in postmenopausal women [100].

Experimental studies have shown that oral testosterone undecanoate added to estrogens in ovariectomized laboratory rats for 60 days had a more pronounced positive effect on the quantity and quality of muscles of the pelvic floor and bladder, and collagen ratio than the estrogen monotherapy [101, 102].

These experimental findings are confirmed by the pilot clinical study performed by Russian researchers who have observed a significant improvement and regression of urologic symptoms in 24 of 26 postmenopausal women at the age of 49–67 years (average age 57.2 ± 2.4 years) with androgen deficiency clinically confirmed by hormone tests, urination disorders and nocturia (in some patients associated with the systemic menopausal hormone therapy) on testosterone undecanoate at a daily oral dose of 40 mg continuously for 6 months [103].

It is evident that a not very favorable pharmacokinetic profile of oral testosterone undecanoate in men, which was described before and which makes it impossible to use successively in most patients with severe hypogonadism, can be rather successively adapted for use in women because oral testosterone undecanoate has a short life cycle and is excreted from the body without accumulation (good therapy control), and the necessity to take it several times a day cannot be an obstacle for women due to their lower demand for testosterone than for men. This makes the oral testosterone undecanoate a particularly "female" androgen—with gentle effects, which, though, can be sufficient enough to eliminate the clinical symptoms of female androgen deficiency. The treatment of testosterone deficiency in women can be limited to an increase of one capsule in 1–2 days. According to the recommendations of the International Menopause Society (IMS), testosterone therapy should be considered as a clinical study which should not be continued if a woman has not had significant improvement in months of therapy [A] [55].

Monitoring of efficacy of therapy with oral testosterone undecanoate in women, as in men, should be based primarily on the dynamics of clinical symptoms of low libido associated with the therapy.

The side effects of testosterone therapy are dose-dependent and may be prevented by using dosage forms and strengths suitable for women. Some data testify to the safe use of testosterone products in women with symptoms of androgen deficiency, in relation to the breast and endometrium in particular [104–107]. They are also confirmed by the results of long-term studies of the use of supraphysiological

doses of testosterone in female-to-male transsexuals which show no increase in the mortality rate, breast cancer, vascular disease, or other serious health problems in this category of patients [108, 109].

Most of the side effects attributed to testosterone occur because of the improper use of oral products or are secondary to the increased aromatase activity leading to excess activation of estrogen receptors by the estrogen excess generated by testosterone aromatization. Factors known to increase aromatase activity include age, obesity, insulin resistance, alcohol, certain drugs, hypodynamia, an unhealthy diet, and breast cancer. Although clinical studies do not often take into account or do not consider the possibility of testosterone aromatization in for the safe use of testosterone in both sexes [57, 110].

Summary

Therapy with oral testosterone undecanoate in women can be considered an option of pharmacotherapy of androgen deficiency in both women of the reproductive age and postmenopausal women as monotherapy or in combination with the standard estrogen-progestin drugs within the individually selected MHT.

Editor's note: However, further safety and efficacy studies are needed for this purpose, in addition to approval by health regulatory agencies in each country.

Optimization of Oral Forms of Testosterone and Future Perspectives of Clinical Use

Oral testosterone preparations have the longest history of application of all forms of testosterone preparations. However, pharmacological evolution has led to the fact that almost all of them (except for testosterone undecanoate) have gradually been removed from the pharmaceutical market and clinical practice because of a number of reasons: lack of clinical efficacy resulting from the inability to reach and maintain a constant level of plasma testosterone during the day, necessity of be taken several times a day, low compliance, and hepatotoxicity of methylated testosterone preparations.

In recent years a technical possibility has emerged of including a nature-identical testosterone molecule into cyclodextrins, which makes it possible to propose the so-called buccal forms of testosterone for clinical practice, while the carbohydrate matrix of dextrins makes lipophilic testosterone soluble in water [111].

Studies have shown that the buccal delivery of testosterone is characterized by a rapid rise and equally rapid decline in plasma testosterone concentration [112]. In this regard, the drugs for hormone replacement therapy must be taken several times a day, similarly to oral testosterone undecanoate [113]. The randomized double-blind crossover study has demonstrated the efficacy and safety of three tablets (90 mg) of buccal testosterone taken three times a day for the treatment of leuprorelin-induced hypogonadism in 24 healthy men [114].

However, without doubt, the positive aspect of this form of testosterone product is absorption of the hormone into the saliva, thus as with oral testosterone undecanoate, circumventing the first-pass inactivation by the liver, which is especially important if the patient has concomitant hepatobiliary diseases and/or hepatic dysfunction, and intestinal malabsorption associated with gastrointestinal diseases. There is evidence that buccal forms of testosterone lead to a lower increase in plasma testosterone level than oral TU is, raising the question about the feasibility of their usage in the treatment of male hypogonadism [115–117].

Another new direction in optimizing oral forms of testosterone is incorporation of testosterone in special PE (polyethylene) matrices having limited water solubility—the so-called testosterone mucoadhesive buccal systems (Striant) containing 30 mg of testosterone to be taken twice a day adhering to the inner cheek, with dose titration not required [118].

Serum concentrations of total testosterone, 5-α-DHT, and estradiol (E2) return to normal in hypogonadal men as a result of such therapy [119]. When using testosterone bioadhesive systems, testosterone is absorbed, as is the case with its buccal forms, through the mouth into the saliva and blood, bypassing the liver, which is a positive aspect of this type of androgen therapy, particularly for patients with chronic diseases and/or impaired hepatic function.

The buccal route of administration of testosterone may be a viable alternative for patients who require short-acting drug therapy and who have previously developed adverse skin reactions associated with administration of other forms of androgens. However, many patients find such variant therapy uncomfortable, because of the possibility of testosterone transmission to their partner through saliva. Moreover, buccal tablets may induce taste disorder and irritation of the mucous membrane of the gums. It is obvious that the most important condition for effective implementation of this type of androgen therapy is the absence of chronic diseases of the oral cavity and salivary glands with sufficient secretion of saliva. On the other hand, side effects for the initially healthy gums and oral mucosa occurring in 16–20% of cases can be considered the drawback of testosterone bioadhesive buccal systems [120].

Jatenzo® (oral testosterone undecanoate; Clarus Therapeutics Inc., Northbrook, IL, USA) is the first oral formulation of testosterone approved by the US Food and Drug Administration. Tlando® (oral testosterone undecanoate; Lipocine Inc., Salt Lake City, UT, USA), another oral testosterone formulation, has also recently been approved by the US Food and Drug Administration. Based on a self-emulsifying drug delivery system and lymphatic absorption, Jatenzo® and Tlando® address some of the limitations of other dosage routes, while providing a safe option with no evidence of liver dysfunction [121].

Conclusion

Oral forms of testosterone are the very first of all testosterone preparations, have gone their historical path in clinical practice, laying a strong foundation for further pharmacological studies, which at the end of the twentieth and beginning of the

twenty-first centuries ended up with the creation of new unique testosterone preparations with a good efficacy and safety profile. Presently, testosterone undecanoate is the main oral product used for the treatment of male hypogonadism, which has limited use in male hypogonadism therapy, but there will always be patients who will prefer, for whatever reason, oral testosterone forms. Oral administration of testosterone is promising for treatment of female androgen deficiency but has not yet been approved anywhere in the world for this purpose. Improved pharmacokinetics of oral forms of testosterone could be attributed to the development of new buccal forms and systems of the hormone; however, their efficacy, convenience, and usefulness in clinical practice need to be evaluated in the future.

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Testosterone Therapy: Transdermal Androgens

14

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Introduction

Transdermal administration of a medication is a method for delivering prescribed doses of drug through the intact skin. The drug can be introduced through an attached patch with a drug reservoir or through a permeable membrane or directly applied to the skin in the form of a gel or lotion. The outermost layer of the skin, *stratum corneum,* serves as a diffusion barrier and a depot. Small amounts of the drug pass through the skin layers in the epidermis to the capillaries in the dermis and the capillary plexus. The drug is constantly released into systemic circulation thus achieving sustained serum levels [1, 2]. There is a gradual increase in serum testosterone within the first few hours after gel application and then the transdermal testosterone preparation usually maintains serum testosterone within the adult male range for 24 h reaching steady state within a week or less. Transdermal delivery systems have been available as patches or spray for estrogen replacement in women

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and as patches, gels or lotions for androgen replacement in men [3, 4]. Transdermal testosterone gels are the most commonly used formulation to treat hypogonadism in the USA and several other countries [5, 6], while long acting injectables are more widely used in European and Australia. Acceptability studies have shown that men of different ages prefer topical gel products due to ease of use and avoidance of the more severe skin irritation seen with reservoir based "patch" delivery systems [7]. Currently available topical products vary by their application methods, dosage adjustment strategies, and are generally more expensive than older intramuscular preparations. Growing availability of generic agents may lead to decreased cost and improve affordability. This chapter describes advantages and disadvantages of currently available transdermal testosterone preparations, as well as recommendations for treatment and dosing strategy for hypogonadal men.

Advantages and Disadvantages of Transdermal Testosterone Compared to Other Delivery Systems

Table 14.1 illustrates the advantages and disadvantages of transdermal testosterone preparations. Transdermal testosterone preparations usually result in less fluctuation of serum testosterone levels compared to injectables or oral preparations [8, 9]. However, recent studies suggest that serum testosterone varied with fluctuations within a day in older men after testosterone gel application [10]. Furthermore, increases in serum testosterone levels may occur independently of time related pharmacokinetics in individual patients: these seemingly random measures may be related to changes in blood flow due to exercise and skin temperature. For some transdermal testosterone preparations depending on the time of gel application, serum testosterone profile mimics normal circadian variation observed in healthy young men [11]. Additionally, transdermal administration helps to avoid first-pass liver metabolism and has less effects on liver secreted proteins such as lipoproteins. Slow sustained delivery of testosterone may help to avoid adverse effects related to

Table 14.1 Advantages and disadvantages of transdermal testosterone for replacement in hypogonadal men

Advantages	Disadvantages
1. Ease of application	1. Possibility of skin transfer with gels and lotion on close skin contact
2. Availability of large skin surface area for application	2. Local site irritation mainly with patches compared to gels
3. Provides more steady and sustained release of testosterone into systemic circulation	3. Elevation of DHT due to high 5-alpha reductase expression in skin (most pronounced when applied on scrotal skin)
4. No hepatic first-pass results in higher bioavailability and less changes in liver dependent proteins	4. Variable rate of absorption
5. Mimics physiological testosterone secretion with some preparations	5. Expensive

peaks and troughs of testosterone concentrations commonly seen with injectables or oral administration, which may result in adverse effects, such as acne, mood swings, and erythrocytosis [8, 12]. It has been suggested that transdermal preparations may have a better cardiovascular safety profile than injectables [13]. Discussion on testosterone replacement therapy and cardiovascular disease risk is found in Chap. 17. Preliminary data also show lower levels of spermatogenesis suppression using transdermal androgens in comparison to injectable treatment [14, 15] but these findings need to be validated in larger cohorts.

A recent exploratory comparison of topical (gel) and oral testosterone undecanoate in self-emulsifying drug delivery system for over 3 months in hypogonadal men showed that the average serum testosterone over 24 h increased significantly over baseline in both groups but were slightly lower after daily gel application compared to oral testosterone undecanoate. Because after topical gel application, there is no first-pass effect of testosterone in the liver, serum SHBG was not changed, but SHBG was significantly decreased by 35% from baseline values after oral testosterone undecanoate therapy [16]. Additionally, LDL slightly decreased with topical *T* but increased with oral *T* ($p = 0.014$, which was not significant after correction for multi-comparison), and HDL decrease was less pronounced with topical *T* ($p < 0.001$). Safety profile was overall acceptable in both groups, with topical *T* showing slightly lower incidence of treatment emergent adverse events (14.5% and 18.7% for topical and oral *T*, respectively). Increased hematocrit, and 24-h ambulatory BP were more pronounced in oral T users (Table 14.2).

Skin irritation is a common side effect with all transdermal preparations but is much more pronounced with testosterone patches. Additionally, as the pricing level of these transdermal preparations is generally higher than commonly available short acting injectables (i.e., testosterone enanthate and cypionate), this makes these user-friendly methods less affordable to many hypogonadal men. Specific shortcomings for the different types of transdermal preparations will be discussed in detail in the respective sections.

Table 14.2 Comparison of the effects of oral vs. topical testosterone formulations

Parameter	Oral TU (*n*)	Topical T (*n*)	Raw *P* value between arms
HDL-C (mmol/L)	−14.2 ± 15.5% (154)	−2.4 ± 16.2% (49)	$P < 0.001$
LDL-C (mmol/L)	6.3 ± 26.5% (154)	−1.5 ± 18.0% (49)	$P = 0.014$
Triglycerides (mmol/L)	12.9 ± 46.5% (154)	10.4 ± 35.3% (49)	$P = 0.72$
Total cholesterol (mmol/L)	−4.3 ± 16.5% (154)	−4.6 ± 12.0% (49)	$P = 0.67$
Hematocrit (L/L)	6.0 ± 7.5% (154)	4.6 ± 6.4% (49)	$P = 0.15$
Glucose (mmol/L)	−1.9 ± 21.2% (154)	2.9 ± 16.8% (49)	$P = 0.16$
Systolic BP (mmHg)	2.6 ± 11.9 (153)	1.9 ± 9.8 mmHg (49)	$P = 0.30$
Ambulatory systolic BP monitoring (mmHg)	4.9 ± 8.7 (162)	0.2 ± 9.4 (45)	$P < 0.002$
Insulin (mIU/L)	34.5 ± 75.8% (26)	17.8 ± 55.1% (24)	$P = 0.46$
P1NP (mcg/L)	19.7 ± 61.6% (28)	18.8 ± 79.3% (33)	$P = 0.43$
CTX (ng/mL)	−7.3 ± 22.6% (27)	−0.8 ± 61.8% (33)	$P = 0.97$

Transdermal Patches

Scrotal patches were the first commercially available transdermal formulation but have since been phased out in the USA as newer more convenient alternatives became available. Thin, flexible, and self-adherent scrotal patches contain polymeric membranes impregnated with testosterone. When applied daily on the scrotal skin, the scrotal patch can consistently maintain testosterone level in mid-normal range. Testosterone levels peaked at 2–4 h and remained within the reference range of adult men for 24 h after the scrotal patch application. Application of the scrotal patch requires the clipping of scrotal hair and the large area of the patch does not allow application in hypogonadal men with small scrotum [17, 18]. Scrotal skin has high 5α-reductase activity which results in serum DHT levels in the high upper or above the reference range of adult men [19]. Although serum level of DHT does not correlate with the intra-prostatic DHT level and on long-term follow-up there is no known increase in the incidence of prostatic cancer [20], concerns of some clinicians or regulatory agencies with regards to slightly higher DHT levels remain [21]. Mainly because of inconvenience of poor adherence to skin scrotal patches are rarely used by patients.

Non-Scrotal patches (Androderm®) deliver 2 or 4 mg of testosterone. Usual starting dose is one 4 mg patch applied daily before bedtime. Testosterone is continuously released for 24 h with maximum concentration ranging within 4–12 h after application of the patch. After the removal of the patch serum testosterone decreases with an apparent half-life of approximately 70 min [11, 22]. Dosing should be adjusted based on morning testosterone levels after 2 weeks of usage. Values outside the adult male reference range require a dose decrease to 2 mg/day (one 2 mg patch) or increase to a maximum recommended dose of 6 mg/day (4 mg and 2 mg patches applied simultaneously).

Testosterone patches can be applied to healthy and clean skin on the back, abdomen, thighs or upper arms but not the scrotal skin or bony prominence. Application sites should be rotated and the same site should be avoided for 7 days. The patch consists of drug reservoir and a multilayer drug delivery system. In order to transport the required amount of testosterone through the skin, these systems are equipped with an enhancer which may lead to contact dermatitis [23]. Mild allergic skin irritation is noted in up to two thirds of patients, while up to 10–15% of subjects have been reported to discontinue the treatment. Topical corticosteroids have been suggested to decrease the discontinuation rate of the skin patches [23, 24]. More serious skin reactions are rare, however, localized skin necrosis has been reported [25]. As the patch contains aluminum it is recommended that prior to a magnetic resonance imaging procedure a patch is removed because it may cause skin burns.

Another larger non-scrotal matrix patch without enhancer was developed (Testoderm TTS®). Though this patch has caused less skin irritation than the reservoir patches, the problem of adherence to skin and frequent dislodgement led to discontinuation of marketing of this non-scrotal testosterone patch. Certain precautions are recommended to ensure maximum effect of patches, such as avoiding water contact for at least 3 h after application. Excessive sweating or physical

activity may lead to non-adherence of the patch. If the patch becomes dislodged, it is recommended to reapply by rubbing the finger around the edges. In case the patch falls off completely, it is advised to apply a new patch if this happened before noon, otherwise wait until regular evening application.

Transdermal Gels

Transdermal gels have been becoming increasingly popular and have surpassed injectable preparations as the most common form of testosterone replacement in the USA and United Kingdom in the past decade [5].

Testosterone gel is applied directly on the skin avoiding the requirement of a patch or a membrane and resulting in less skin irritation observed compared with transdermal patches. Testosterone gel is available as prepackaged single dose packets or multi-dose pumps. Some manufacturers provide both options (Table 14.3). Most testosterone gel preparations are formulated as hydroalcoholic gel, others use other enhancers in lotions. When applied to the skin, testosterone over time is absorbed into the *stratum corneum*, which serves as a diffusion barrier and reservoir. Testosterone is slowly released into the circulation over several hours resulting in steady state serum levels of the hormone [26]. The release of testosterone from the reservoir continues for about 24 h. Only approximately 10% of the testosterone applied on the skin surface is absorbed into the systemic circulation during a 24-h period.

The gel is applied to a large area of the skin usually on the arms and shoulders and the area of application may affect the absorption of testosterone [27]. Long-term studies with testosterone gel showed that steady and relatively consistent serum levels of testosterone levels are attained [9], which results in significant improvements of sexual and body composition parameters [28–30]. A landmark coordinated set of seven placebo-controlled trials evaluated effects of *T* gel in older men with unequivocally low *T* levels (The *T* Trials). It has shown that *T* gel was relatively easy to apply and was well tolerated (discontinuation rate in *T* gel group was lower than placebo gel group) [31]. The outcomes of the trials are summarized in comprehensive reviews [31, 32].

Several formulations of testosterone gels are available on the market [3, 4, 33]. Currently available gels vary in testosterone concentration and are usually applied once daily. Their pharmacokinetic profiles are also similar: Androgel 1%®/Testogel 1%® [9], Testim® 1% [34], Axiron® 2% [35] Fortesta Gel® 2%/Tostran® 2% [36], and Androgel 1.62%® [37]. These transdermal preparations have been proven to be efficient in normalizing serum levels, as well as reversal of androgen deficiency symptoms for long periods of treatment [28] and have been considered an acceptable form of testosterone substitution by users [7]. The maximum concentration of testosterone achieved is variable depending on the preparation but usually within 2–5 h of application and is maintained throughout 24 h. When applied in the morning a profile somewhat similar to circadian rhythm in healthy men is maintained. Recent studies in older hypogonadal men showed that after testosterone gel

Table 14.3 Characteristics of some testosterone gels (based on manufacturer's label)

Name, strength (manufacturer)	Packaging	Dosage	Time of testing	Application site	Sites to be avoided	Swim/ shower after
Androgel 1% (AbbVie Inc.)/ Testogel 1% (Besins Healthcare)	25 mg or 50 mg packet, multi-dose pump (12.5 mg/ actuation)	50–75–100 mg once daily	Not provided	Shoulders, upper arms, abdomen (coverable by short sleeve T-shirt)	Elsewhere (e.g., genitals, chest, back, abdomen, axillae, or knees)	5 h
Androgel 1.62% (AbbVie Inc.)	40.5 mg or 20.25 mg packet multi-dose pump (20.25 mg/ actuation)	40.5 mg (20.25–81) once daily	AM pre-dose morning blood draw ~14 and 28 days after start	Shoulders and upper arms bilat (area covered by short sleeve T-shirt)	Elsewhere (e.g., genitals, chest, abdomen, axillae, or knees)	2 h
Axiron 2% (Eli Lilly and Co.) Discontinued	Multi-dose pump (30 mg/ actuation)	60 mg (30–120) Once daily	2–8 h post application at least 14 days of constant use	Axillae bilaterally	Other parts of body	2 h
Fortesta 2% (Endo Pharmaceuticals Inc.)/Tostran 2% (ProStrakan)	Multi-dose pump (10 mg/ actuation)	40 mg (10–70) Once daily	2 h post application 14 and 35 days after start	Use one finger on front and inner thighs (not near scrotum) bilat	Genitals or other parts of body	2 h
Testim 1% (Auxilium Pharmaceuticals Inc)	50 mg in tube with emollient	50–100 mg Once daily	AM pre-dose *T* concentration 14 days after start	Upper arm, shoulder (coverable by T-shirt)	Abdomen, scrotum, penis	2 h
Vogelxo 1% (Upsher-Smith Laboratories, Inc.)	50 mg tube or packet Multi-dose pump (12.5 mg/ actuation)	50–100 mg Once daily	AM pre-dose T concentration ~14 days after start	Upper arm, shoulder	Abdomen, genitals	2 h

application there were large fluctuations in serum testosterone concentration both within and between patients [10]. Skin structural differences may be one of the causes of these significant variations in bioavailability of drug, which poses challenges in predicting effectiveness of medication and determining an adequate dose, as well as appropriate time for testing serum testosterone levels [10, 38]. Non-time dependent pulses of serum testosterone also occur in relation to exercise and skin temperature. Both factors may be mediated through changes in dermal blood flow. Another important issue is a possibility of blood sample contamination when it is drawn at the gel application site, which has led to spurious increase in measured

testosterone levels [39]. Sampling of blood after testosterone gel applications should be away from the application sites.

Different sites for drug application have been studied with various degree of success. Scrotal skin is thin and highly vascular hence it leads to better and sustained absorption of testosterone, which made it one of the early targets in the development of transdermal patch preparations. Scrotal application is not used for the gels because of the relatively small area where the gel can be applied. Application on axillary region may enhance the absorption and may have less skin transfer and has been shown to be beneficial to patients who fail other transdermal preparations in a single study [40]. However, due to sensitive skin in the area, skin irritation, edema, and erythema have been observed as in other transdermal preparations [41]. On the other hand, even though application of 1.62% testosterone gel on abdominal skin led to 30–40% lower availability than on upper arms and shoulders, application on all of these sites resulted in eugonadal testosterone levels [42]. While selection of application site may not be an issue for majority of patients, those failing to achieve sufficient systemic levels may benefit from a change of site.

Additionally, some gels include emollient that prevents skin drying and ensures better testosterone absorption. There are data that this may help achieve better bioavailability and higher serum concentrations [43]. Differences in gel formulations and their pharmacokinetic profiles are one of the reasons why gels cannot be used and dosed interchangeably. Therefore, it is recommended to follow specific instructions on sites for application and dosing of the drug provided in the labeling. Dosing information and recommendations for some of the preparations are presented in Table 14.3. It should be noted that the same gel is marketed in different countries under different names and in fact are produced by the same manufacturer.

As most of the gels contain alcohol, they are flammable, therefore precautionary measures are required. More importantly, there is a risk of skin transfer of gel to other persons on close contact. This is particularly important in women and children whose endogenous testosterone levels are low. To avoid this risk hands must be washed with soap and water after application of the gel. Once applied to the application site the gel dries within several minutes and should be kept covered with clothes at all times or washed thoroughly with soap and water to remove any residue of gel if close skin to skin contact is anticipated [44]. However, showering within short time (15–30 min) after application of the gel may result in lower serum testosterone levels [45] and should be avoided. Manufacturers recommended minimum time before washing after application varies among different formulations from 2 to 5 h (Table 14.3). Washing at those recommended times did result in approximately 30% decreased bioavailability of testosterone, however, serum testosterone levels within normal range were sustained. Even with these precautionary recommendations in place, skin transfer continues to pose challenges including infrequent case reports of virilization of prepubertal children [46–49]. Therefore, physicians prescribing transdermal testosterone gels or lotion must discuss with the patients the risk of transfer and the measures to prevent transfer as well as the other potential adverse events of testosterone discussed in this chapter and Chaps. 16 and 17.

Elevation of 5-alpha dihydrotestosterone (DHT) has been found to be more pronounced in transdermal gels compared to other formulations possibly due to high 5-alpha reductase expression in skin (especially when applied on scrotal skin) [9]. In contrast to transdermal patches, a much larger area of skin is exposed to testosterone, thus potentially leading to an increase in systemic DHT concentration. Because DHT is the main androgen in the prostate, it may have more stimulating effects on prostate growth. While serum DHT to *T* ratio is increased after transdermal testosterone application, there are no data showing association between higher DHT levels and adverse effects on prostatic hyperplasia or cancer of the prostate [21]. Elevation of DHT has been associated with higher risk of cardiovascular events in observational studies [50] but this needs to be systematically assessed in large-scale long-term studies. On the other hand, this moderate increase in DHT levels that is seen in transdermal gel users usually remain within the reference range limits in healthy adult men and has not been related to adverse effects on primary DHT targets, such as prostate. Moreover, a recent study has revealed no significant difference in DHT levels versus an oral T formulation [16].

Another important drawback of currently available testosterone gels is their cost. Compounded testosterone may be one of the alternatives but is not recommended as there is no quality control standard for compounded medications. A recent study in Canada reported large variations of testosterone levels in these preparations [51] and standardization strategies have been suggested [52]. Increasing availability of generic testosterone gels may lead to decreased costs and improve affordability in near future. As discussed above, there are distinct differences between the various transdermal preparations. Decision on the most appropriate treatment strategy should be based on individual patient profile and personal preferences after all available strategies are discussed. It is of utmost importance that patient is comfortable with the selected treatment as compliance is one of the major challenges with long-term treatment of chronic asymptomatic conditions [53].

For monitoring of testosterone concentrations after transdermal gel application, blood for dose adjustment should be drawn 2–8 h after the application following manufacturers recommendations. As significant day-to-day variability of serum *T* levels due to absorption or other reasons is likely, especially in older men [9], major dosing decisions should not be made based on a single measurement.

Other Topical Testosterone Delivery Systems

Similar strategies to those used in transdermal testosterone delivery systems have been employed in developing trans-mucosal preparations. Currently available systems include trans-buccal system and intranasal gel. Mucous membrane of the nose is much more permeable than skin, therefore due to higher level of absorption lower doses of testosterone are required. On the other hand, nasal application of testosterone results in quick onset and short duration of action, which leads to fluctuation of systemic testosterone levels and requires multiple daily applications (two or three times per day application for intranasal gel) [54]. However, based on the results of

a small single-institution, single-arm, open-label clinical trial, because of this, users of the formulation may experience less suppression on the hypothalamus-pituitary-gonadal axis and may thus have lesser impact on sperm production [55].

Trans-buccal system involves application of a tablet to the buccal mucosa, where the tablet forms a gel and delivers steady levels of testosterone for about 8–12 h [56]. Though there is no significant irritation to the gums, the tablet can be dislodged and some patients did not like to have a gel tablet in their mouth. There is also no dosing flexibility as all patients are required to apply one tablet twice a day and discontinue use if systemic testosterone level is outside normal range. A report of the safety and efficacy after 2 years continuous use of this buccal delivery system [57] showed that up to 62% of subjects had at least 80% of their testosterone measurements within reference range of adult men and the safety profile was generally favorable with local adverse effects (gum edema, blistering, and gingivitis) being mostly mild, leading to discontinuation in 4.3% of patients. This preparation is no longer available in the USA.

Conclusion

Transdermal testosterone delivery by applying gels or lotions on the skin is the preferred method of many men. This delivery method is user-controlled and does not require invasive injections or implants and can be administered in the patient's home environment. Significant skin irritation is not a common problem with gels and dose titration can be achieved by adjusting number of actuations of a canister number of sachets or tubes. The main issue of transdermal testosterone for replacement therapy is the potential of skin transfer of medication upon close skin contact. This can be largely avoided by showering or wearing protective clothing when skin contact is anticipated. The choice of which testosterone replacement is optimal for the patient depends on the patient's preference and whether there are contraindications to other therapy. In older men with co-morbidities, it may be prudent to commence treatment with lower doses of transdermal testosterone. If adverse events develop, the application of the gels or lotions can be stopped and the patient's testosterone will return to the prior levels within a short period of several days. With the emergence of more transdermal testosterone preparation options, the cost may be reduced making this delivery system more affordable for hypogonadal men.

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Testosterone Therapy: Injectable Androgens

15

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Abbreviations

AMS	Aging males' symptoms scale
AUC	Area under the curve
BMI	Body mass index
BPH	Benign prostatic hyperplasia
CC	Clomiphene citrate
CV	Cardiovascular
CVD	Cardiovascular disorder
EAU	European Association of Urology
FDA	Food and Drug Association
FSH	Follicle stimulating hormone
HDL	High density lipoprotein
IIEF	International Index of Erectile Function
IPSS	International Prostate Symptoms Score
LA-TU	Long-acting testosterone undecanoate
LDL	Low density lipoprotein
LH	Luteinizing hormone
LUTS	Lower urinary tract symptoms
MeT	Methyltestosterone
mg	Milligram
mL	Milliliter
MS	Metabolic syndrome
OSA	Obstructive sleep apnea
PSA	Prostate-specific antigen

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QoL	Quality of life
SARM	Selective androgen receptor modulator
SERM	Selective estrogen receptor modulators
T	Testosterone
TE	Testosterone enanthate
TRT	Testosterone replacement therapy
TU	Testosterone undecanoate

Introduction to Testosterone Preparations for Treatment of Hypogonadism

In males, testosterone (T) controls a number of important functions including sperm production, sex drive, muscle mass and fat distribution, bone density and red blood cell production, fat and sugar metabolism as well as mood and cognition. During puberty, luteinizing hormone (LH) and follicle stimulating hormone (FSH) start being produced by gonadotropes of the anterior pituitary gland. FSH is critical for spermatogenesis, while T production is regulated in the testes by LH. The action of T is via the androgen receptor located in the cytoplasm and nucleus of target cells. Starting with the fourth or fifth decade of life total T concentrations begin to decline progressively by approximately 1% per year from an average between 270 and 1070 ng/dL, while bioavailable testosterone is approximately 110–575 ng/dL in men aged 18–69.

Deficiency or absence of this hormone, which could either be of primary (originating in the testes) or secondary (a problem of the hypothalamus or pituitary gland) origin, seen in combination with characteristic symptoms such as impaired libido with loss of sexual function, regression of secondary sex characteristics, low muscle mass or decreased bone density is defined as hypogonadism. Apart from age-related reduction in testosterone concentrations, hypogonadism may also result as a consequence of autoimmune or genetic disorders, accidents, infection, prolonged exposure to heavy metals or alcohol, radiation, tumors and chemotherapy [1], and obesity [2]. A wide range of data from a number of cross-sectional studies indicate that hypogonadism may affect between 17.2% and 38.7% of middle- and older-aged men [3–5]. The primary approach for management of this condition is physiological testosterone replacement therapy (TRT).

Testosterone formulations have been available to patients since the 1930s when male hypogonadism was treated with exogenous testosterone in the form of implantable testosterone gel patches, followed in the 1980s, by injectable preparations. Other means of testosterone delivery use the transdermal route (genital or non-genital patches or gel) and offer numerous advantages over other delivery routes including ease of administration and/or cessation of therapy and the achievement of sustained drug plasma levels [1, 6]. These systems have the advantage of mimicking

the normal circadian rhythm of T, peaking in the morning and declining slowly toward the evening [7–9]. These transdermal delivery systems may, however, cause moderate to severe skin reactions due to the T delivery systems used, with regard to T patches, while caution is advised with regard to T gels in order to avoid inadvertent exposure to women and children [9–12]. Furthermore, T absorption, via the transdermal route, can vary greatly between individuals, and they require daily application, which some patients may not adhere to.

Some of the measures taken to overcome these limitations came in the form of chemical modifications of the testosterone molecule, which allowed for oral delivery routes such as testosterone capsules, transbuccal patches or sublingual administration. Some of these formulations proved ineffective due to the first-pass effect of the liver, or, in case of 17 alpha-alkylated derivatives such as methyltestosterone (MeT), caused hepatotoxicity. Oral T was reported to have stimulatory effects on hepatic microsomal enzyme systems in in vitro studies, and to be associated with the development of peliosis hepatis or hepatocellular carcinoma [13–16].

Testosterone injections delivered via the intramuscular route are absorbed directly into the blood stream and bypass the first-pass effect of the liver, thus avoiding hepatotoxicity. To date, these formulations remain the most cost-effective and widely used T therapy. The first preparations available were the short-acting formulations of T esterified with fatty acids dissolved in an oil-based vehicle, such as testosterone cypionate and testosterone enanthate (TE), testosterone propionate, and testosterone cyclohexanecarboxylate. However, they have an effective duration of action of 1–2 weeks which brings fluctuations in injection delivery and gives greater variability and subjectivity of symptoms in patients [17, 18]. Despite the pharmaceutical availability, of approximately 85 years, the therapeutic use of T has been hampered due to the low bioavailability following both oral and parenteral administration, associated with a short circulating half-life [19].

In search for a medium-term solution, with improved efficacy, balanced symptoms and reduced side-effects, long-acting testosterone undecanoate (LA-TU) with intramuscular administration was developed, initially in the 70s in China [20, 21], and subsequently, due to some problems at the injection site, redeveloped by Jenapharm GmbH & Co. KG, a subsidiary of Schering AG in Berlin, Germany [22]. Intramuscular TU is currently prescribed in the USA under the trade name Aveed® (Endo International plc, Dublin, Ireland), in Europe, Latin America, and Asia under the trade name Nebido® (Bayer HealthCare Pharmaceuticals, Berlin, Germany) and in Australia under the trade name Reandron 1000® (Bayer HealthCare Pharmaceuticals, Berlin, Germany). Reviews of the literature looking at the efficacy and safety of injectable TU treatment have concluded that this type of treatment has a significant positive impact on the quality of life (QoL), symptoms of hypogonadism and associated comorbidities in men. Injectable TU offers the possibility of a therapeutic intervention just four to five times per year freeing the patient, at least partially, from having a chronic condition, thus maintaining a positive, active role in self-caring [23, 24].

Pharmacology and Toxicology of Injectable Testosterone

Studies looking at the mode of action of LA-TU (molecular weight 456.7 Da) have shown that upon entry into the peripheral circulation, TU is hydrolyzed to T, which may then exert its androgenic role [25]. It is therefore believed that the toxicology of TU is the same as for other cleavable testosterone fatty acid esters such as T propionate (3 carbon atoms), -enanthate (7 carbon atoms), -cypionate (8 carbon atoms), and -undecanoic acid (11 carbon atoms). The use of T-undecanoic acid, which presents with a saturated aliphatic fatty acid, in contrast to using the fatty acid esters enumerated above, significantly improves the kinetics for side chain cleavage, thus permitting much longer injection intervals, while at the same time maintaining balanced serum T levels [22].

Animal studies focusing on the use of injectable TU as T replacement have shown that, in orchidectomized male rats, a single injection of 125 mg/kg body weight can induce physiological T levels for a minimum of 4 weeks, while a maximum injection of 500 mg/kg body weight resulted in supraphysiological T serum concentrations for up to 6 weeks in non-orchiectomized rats. When compared to other T-releasing formulations, such as subcutaneous T pellets, T-filled subcutaneous Silastic® (Dow Corning Corp.) implants or subcutaneous T-propionate, TU was clearly superior with regard to pharmacokinetic profile, safety, efficacy, and reduced side-effect profile [26]. Independent studies using cynomolgus monkeys (Macaca fascicularis) have addressed the pharmacokinetics of TU following administration of injectable TU 10 mg/kg body weight. One study revealed that with respect to pharmacokinetic and pharmacodynamic characteristics such as area under the curve (AUC), residence time, terminal half-life, maximal T concentration, and time to maximal T concentration, in contrast to the administration of TE 10 mg/kg body weight, TU showed clear superiority [27]. A second study, comparing TU dissolved in soybean oil, castor oil or tea seed oil, showed no significant differences in the pharmacokinetics of the three TU formulations with regard to plasma T and estradiol. The suppression of gonadotropin levels varied between individuals and despite increased prostate volumes after administration, these declined back to castrate levels after withdrawal [28].

In humans, a number of independent research groups have reported findings looking at pharmacokinetics of injectable TU in a variety of concentrations and using a number of delivery vehicles. The first pharmacokinetic investigation, lasting over 8–9 weeks, by Zhang and colleagues, concluded that, in hypogonadal men, administration of 500 mg injectable TU as first injection, followed by a 1000 mg injection 3 months later, provided more favorable peak testosterone values than when the 500 mg dose was administered as a second injection. The authors speculated that either long-term hypogonadism may induce faster cleavage or a clearance mechanism for TU and T by the time of the second injection or that residual endogenous T is suppressed by the first injection and that following the second injection, only exogenous T is measured [21].

The majority of pharmacokinetics studies of TU demonstrate that, after intramuscular injection of 1000 mg TU, serum T concentrations are still in the

physiological range. One exception, a study of 10 hypogonadal men reported that 500 mg TU every 6 weeks provided physiological androgen replacement with T levels within the normal range at all times, while administration of 1000 mg TU every 12 weeks and 750 mg TU every 9 weeks was reported to cause periodical supraphysiological plasma T levels. These findings with TU have not been replicated by other groups, and treatment with 1000 mg TU every 10–14 weeks postloading period is now used as the gold standard [29, 30].

With a view to establishing the most efficient vehicle of administration of TU in humans, Behre and colleagues, compared the Chinese preparation (TU 125 mg/mL in tea seed oil) injected in 2 volumes of 4 mL each at 2 sites, with TU 250 mg/mL in castor oil as a single 4 mL injection [31]. It appeared that when castor oil was used as a delivery vehicle, TU had a longer half-life than the tea seed preparation, an observation supported by another study showing that the bioavailability of the steroid in smaller injectable volumes (1000 mg nandrolone decanoate in 1 mL oily solution) was larger than in the larger volume (1000 mg nandrolone decanoate in 4 mL oily solution) [32].

A study by von Eckardstein and Nieschlag, examining suitable LA-TU injection intervals, concluded that, after initial loading doses at 0 and 6 weeks, injection intervals of 12 weeks established eugonadal values of serum T [33]. Consequently, in an open-label, randomized, prospective study, Saad and colleagues compared LA-TU (TU 1000 mg 3 times every 6 weeks, thereafter every 9 weeks) with TE (250 mg every 3 weeks) in 40 hypogonadal men [34]. Trough T levels, measured prior to every injection, remained within the physiological range in patients treated with LA-TU in contrast to the group treated with TE. 2.5 year follow-up data from this study demonstrated that both the group administered TU 1000 mg every 12 weeks (former LA-TU group) and the group administered 2× 1000 mg every 8 weeks followed by 1000 mg every 12 weeks (former TE group), resulted in stable mean serum concentrations of T and estradiol [35].

In summary, use of injectable TU has demonstrated a considerably better pharmacokinetic profile requiring only 2 initial 1000 mg 4 mL with a 6-week interval followed by injections every 10–14 weeks when T serum concentrations decrease to a range between 10 and 15 nmol/L. Another major advantage of LA-TU is that it requires only a few injections per year compared to 26 injections per year for testosterone esters taken at a dose of 200 or 250 mg every 2–3 weeks or orally administered TU which requires careful dosing at least twice a day, and a requirement to be taken with fatty meals in order to achieve acceptable plasma T levels [36].

Efficacy of Injectable TU and Comparative Studies

Though a number of T replacement therapies are currently available to patients, with varying degrees of efficacy and safety, the preferred T preparations include T gel and injectable T (TE and TU). A limited number of comparator studies exist. One such study examined T gel vs. injectable TU, in 27 hypogonadal men aged 47–74 years, indicated that while both preparations meet the requirements of

present day androgen treatment, higher plasma T levels are achieved with TU compared to T gel [37]. These include improved positive effects on the International Index of Erectile Function (IIEF), the Aging Males' Symptoms Scale (AMS), and International Prostate Symptoms Score (IPSS) in patients with metabolic syndrome (MS). Similarly, another comparative study showed that TU treatment generates higher plasma levels of T in contrast to treatment with T gel, which may explain the greater improvement in sexual and metabolic syndrome symptoms [38].

In comparison to TU injections, intramuscular TE administered at injection intervals of 2–3 weeks is the most commonly used form of therapy for hypogonadism. As previously discussed, this treatment is often associated with supraphysiological and subphysiological values of serum T shortly after and in the days before an injection, leading to mood swings and emotional instability. Additionally, elevated hematocrit values that may lead to thromboembolic events have been reported in 14 of 32 hypogonadal men receiving TE every 2 weeks [39]. Similarly, 30% of older men with low serum T receiving 200 mg TE developed hematocrit values of greater than 52% [40, 41]. Sommer et al. compared the efficacy of intramuscular administration of TU vs. TE (250 mg) in a randomized, controlled, prospective, parallel group study for a 30-week period followed by a long-term open-label study over 5 years [39]. During the first 30-week comparative phase, 40 hypogonadal men were randomly assigned to either 250 mg TE intramuscularly every 3 weeks ($n = 20$) or TU three times in 6 week intervals followed by a 9-week interval. Patients then received TU every 12 weeks in a 1-arm follow-up study over an additional 30 months. The authors reported that TU treatment had no serious side-effects and the slightly increased prostate-specific antigen (PSA) levels and prostate volumes observed in the first 30 weeks of treatment with either TE or TU remained stable over an additional 30 months on TU treatment. Additionally, both preparations improved sexual parameters of spontaneous morning erections, total erections, and ejaculations.

A number of independent studies have examined the effects of T therapy on anthropometric, endocrine, and metabolic parameters, and have reported a sustained and clinically meaningful weight loss in hypogonadal men, though the majority of these studies are of short duration [42–44]. A prospective registry study of 261 men treated with T has, however, provided long-term data on metabolic parameters following testosterone replacement [42, 43]. A significant weight loss of approximately 11 kg in 96% of subjects over the 5-year duration of the study was reported [42], possibly due to an increase in the overall level of vitality and physical activity subsequent to T treatment as also indicated by another study of more than 1400 hypogonadal men from 155 centers in 23 countries [44].

In a recent controlled study, Francomano et al. examined the effects of TRT on metabolic and hormonal parameters in hypogonadal men with metabolic syndrome. The group reported improvements in anthropometric parameters, such as BW and WC, in a stepwise yearly manner [44]. More interestingly, a continuous decrease in anthropometric parameters in these patients was observed when compared to the control group in whom no modification occurred [44]. Data from two previous independent studies observing more than 500 hypogonadal men, reported a significant

weight loss in over 95% of patients and changes in body composition, including a decrease in body fat concomitant with an increase in lean body mass [42, 45].

Finally, when TU administration was compared to the use of short-acting testosterone esters, the gonadotropins FSH and LH appeared permanently suppressed. Suppression of gonadotropins is desired for male contraception, for which TU is a potential candidate. However, further studies are required to establish a definitive regimen for male hormonal contraception.

In two long-term studies by Muenster et al., and Cologne et al., hypogonadal men were followed on TU (1000 mg) for up to 8.5 years or on a mixed TE and TU or TU regimen over 5 years, respectively [39, 46]. Muenster et al. reported that PSA concentrations did not exceed the normal range and that the prostate size remained below 30 mL in all patients. Hemoglobin and hematocrit increased initially during treatment but remained within the normal range over the entire treatment period. Overall, treatment with intramuscular TU demonstrated beneficial effects on body composition and lipid profiles that account for an observed decrease in body mass index (BMI) during the first 2 years of treatment that also concurred with slightly increased high-density lipoprotein (HDL) serum concentrations and decreased low density lipoprotein (LDL) serum concentrations over time. There were no relevant changes in blood pressure or heart rate. Similarly, Cologne et al. demonstrated that while serum PSA levels in both treatment groups had risen slightly, these values remained stable and within the normal range over the entire observation period. Decreases in total cholesterol, LDL, HDL, and triglycerides were observed. Extending the Muenster study, Cologne and colleagues reported that compared to TE treatment, TU treatment improved sexual parameters (spontaneous morning erections, total erections, and ejaculations) and psychological parameters for depression, fatigue, and anxiety. In addition, an independent study of 33 hypogonadal men treated with TU confirmed some of these findings where patients presented normal serum PSA levels and improved mood, sexual function, and quality of life [47].

Furthermore, in a study of 22 hypogonadal men treated with individualized injection regimens of 1000 mg TU, based on T serum concentrations, were followed for up to 8 years [48]. Consistently and in contrast to short-acting TE, TU treatment fluctuations in T serum concentrations were rarely observed and if so, it occurred during the last 2 weeks before the next injection. The authors recommend that transfer of hypogonadal patients on short-acting T injections (e.g., testosterone enanthate 250 mg) to treatment with TU be initiated with two injections of TU at an interval of 6 weeks, followed by injections every 10–14 weeks depending on T serum concentrations.

Safety and Tolerability of Injectable TU

Since testosterone is an endogenous protein, the pharmaceutically active component is testosterone itself, therefore injectable TU is well tolerated. Indeed, only minor complications with TU treatment have been reported and these are generally

limited to include local irritation at the site of injection, not usually lasting more than 3 days [22]. No patient reports of disrupted treatment due to problems or local discomfort have been reported. In contrast conventional injectable TE often lead to mood swings or emotional instability, most likely due to fluctuations in T values after injection and in proximal days before the new injection is due.

Another important consequence of the supraphysiological T levels seen following injections with TE is the elevation of the hematocrit, as reported by independent studies, where patients had received 200 mg of TE every other week [49–51].

Meta-analyses of clinical trials suggest no major adverse effects following TU administration on CVD and PCa and only a minority of patients reported any of the common side-effects of T administration that include gynecomastia, breast tenderness, and acne [52, 53]. With respect to the development of comorbidities, testosterone use has been associated with conditions such as prostate cancer, worsening benign prostatic hyperplasia (BPH), male breast cancer, polycythemia, an increased risk of obstructive sleep apnea (OSA) [54], and cardiovascular disorders (CVD). Indeed, the supposition that patients receiving T replacement therapy have increased the risk of prostate cancer is controversial. In this context, although there is no evidence that testosterone therapy increases the risk of prostate cancer, decades of physicians have been trained with the notion that testosterone is the fuel for prostate cancer as it is known to be driven through the AR. In order to address this, the incidence of prostate cancer was evaluated in three independent observational studies in more than 1000 hypogonadal men treated with testosterone therapy for up to 17 years [55]. From this cohort only 11 patients received a diagnosed of prostate cancer. Similarly, in a large meta-analysis of 18 prospective studies that included over 3500 men, there was no association between serum androgen levels and the risk of prostate cancer development, for prostate cancer, in a [56].

These data suggest that if EAU guidelines for prostate screening and monitoring are followed, T therapy should be a safe and effective treatment in hypogonadal men. Furthermore, large scale, randomized, controlled, long-term studies are needed to more completely address the linkage between testosterone levels and prostate cancer.

Increasing evidence suggests that testosterone replacement therapy does not increase lower urinary tract symptoms (LUTS) and is not contraindicated in men diagnosed with BPH. A randomized, double-blind, placebo-controlled trial of 44 hypogonadal men showed that T treatment for 6 months improves serum androgen levels, with little effect on prostate tissue androgen levels, tissue biomarkers, and/or gene expression [54]. An increase in PSA levels and prostate size has indeed been noted in several studies [57, 58], though PSA levels and prostate size remained within the normal range despite a significant increase being observed. This increase in hypogonadal men is associated with subnormal PSA values and small prostate sizes at baseline [59], and is observed with all testosterone preparations. A recent review and meta-analysis concluded that T therapy does not increase PSA levels in men treated for hypogonadism [60].

The association between T treatment and male breast cancer is yet to be fully understood despite the existence of several case reports [61] and one retrospective

review [62]. It is postulated that high levels of T may lead to increased aromatization to estrogen, which in turn may stimulate breast tissue growth via estrogen receptors [63].

While, through its erythropoietic function, T leads to an increase in hemoglobin by as much as 5–7% [64], thus exerting a positive effect on men with baseline anemia, it can lead to polycythemia in over 20% of men receiving T treatment [65]. Although complications such as an increased risk of vascular events, including stroke, myocardial infarction, and deep vein thrombosis with possible pulmonary embolus [65] are associated with polycythemia, an observation not yet made in men on T therapy [66]. Similarly, no documented evidence exists of polycythemia in studies using more traditional testosterone esters despite increases of erythropoiesis parameters to eugonadal values [35].

An examination of the literature reveals a wealth of evidence clearly suggesting that low T concentrations are associated with CVD risk and known risk factors for CVD, such as obesity, diabetes, and the metabolic syndrome (MS) [67, 68]. Of 11 longitudinal studies, 9 have demonstrated increased mortality rates in men with low T levels and improved survival in those with higher T [69], while 2 studies showed no effect [70]. In contrast, a recent study by Layton and collaborators investigating the CV safety of testosterone injections, patches, and gels revealed an association between T injections and an increased risk of CV events compared to T gels and patches. However, this study did not assess whether patients met the criteria for use of T and did not assess the safety of T among users compared to non-users [71, 72].

Two studies reporting risks with T gel preparations concluded that there is a significant direct correlation between T therapy and CVD risk [73, 74], although these studies should be interpreted with caution due to their study design limitations [75].

Impact of TU Therapy on Patient-Focused Perspectives

As androgen replacement therapy is normally associated with long-term medical conditions, therapy often extends over many decades, making patient compliance of utmost importance. Prior to TU administration, patients diagnosed with hypogonadism report a significantly reduced QoL, affected by symptoms including low libido, erectile dysfunction, infertility, gynecomastia, hot flashes, or as more non-specific symptoms such as low energy, sleep disturbance, depression or labile mood, impaired cognition, osteoporosis, and loss of muscle mass or increased BMI [69, 76, 77].

With regard to patient compliance and uptake, a major advantage of TU injections is the reduced frequency of visits allowing for reflection on efficacy and safety of TU therapy, when adjustment of the injection interval is required (most often by prolonging to every 13–14 weeks), as compared to almost bi-monthly visits for TE therapy. Furthermore, as TU only requires four injections per year compared to 26 injections per year with TE, there is a greater compliance rate in TU treated patients.

Future Alternatives to Injectable Androgens

Given that there is currently no global consensus on the medical approach to T deficiency, and that existing T replacement treatments are surrounded by conflicting efficacy and safety research and clinical reports, it comes as no surprise that alternative approaches to rectifying low T levels are increasing in number. Several decades of research, evaluating the field of selective estrogen receptor modulators (SERMs) and selective androgen receptor modulators (SARMs), have resulted in the use of clomiphene citrate (CC), an estrogen receptor modulator, in the treatment of male hypogonadism in an off-label capacity [78].

The mechanism of action behind CC involves the disruption of the LH and FSH release from the pituitary gland, thus stimulating the production of T in Leydig cells [79]. An initial study in hypogonadal men, comparing CC, T injections, and T gel, revealed comparable effectiveness with patients reporting similar satisfaction, although increased libido was indicated in the T injection group. While preliminary studies suggest that CC may not only be a suitable alternative to T supplementation and may be advantageous in terms of cost-effectiveness and reduction of side-effects [80] there is a clear need for larger randomized clinical trials to assess its safety and efficacy further, and to ascertain whether CC effectively mitigates the known side-effects of hypogonadism.

Alternatively, the discovery of steroidal and non-steroidal SARMs, used in the development of hormonal male contraception, could provide a promising alternative for T therapy. The identification of an orally bioavailable SARM with the ability to mimic the desired central and peripheral androgenic and anabolic effects of T in a tissue-specific manner and simultaneously avoid the undesirable side-effects, would represent an important step in androgen therapy [81–84].

The majority of these compounds are in the early phases of pharmaceutical development with combined research and clinical goals to produce reductions in catabolic consequences of hypogonadism and/or aging in order to preserve skeletal muscle and bone allowing the individual to maintain functional activities of daily living, reduce fall and fracture risk, and consequent disability. In light of recent guidance [85] on the restriction of exogenous testosterone administration, warranted by observational studies, indicating a potential increased risk of cardiovascular events [86, 87], in hypogonadal and/or aging men SARMs are promising candidates. Indeed pre-clinical models looking at SARMs have shown a positive levator ani/bulbocavernosus muscle complex/prostate ratio, demonstrating an improved anabolic/androgenic ratio with limited side-effects [88–90].

Conclusion

All testosterone preparations have, to varying degrees, favorable physical and metabolic effects. In view of its pharmacology, LA-TU presents with significantly improved efficacy and safety when compared to other conventional injectable T preparations (e.g., TE). Its advantages are obvious, from the reduced injection

frequency to a significant improvement in side-effects associated with fluctuations of plasma T seen with conventional TE.

As of January 2014 the FDA stated they are investigating the potential link between T therapy and several comorbidities, "FDA-approved testosterone treatment increases the risk of stroke, heart attack, or death," but have not yet concluded. Available evidence indicates that TU is largely considered to be safe in most hypogonadal men, with a small inherent risk of adverse events in some high-risk men with multiple comorbidities. T therapy has been associated with occasional modest increases in serum PSA and prostate size, yet within clinical safety limits, and without compelling evidence to support an increased risk of prostate cancer.

Indeed, when given to appropriately selected patients with vigilant monitoring, injectable T can produce improvements in QoL, energy level, libido, muscle mass, cognition, and bone density. Future research should focus on the evaluation of large, multiethnic cohorts of men through prospective trials to better elucidate both risk and hazard ratios of T as it relates to CVD and MS, prostate cancer, LUTS, OSA, erythrocytosis, and other yet-to-be-determined theoretical risks in men both with and without CV risk.

In parallel, progress is being made with respect to research looking at the use of SERMs and SARMs, as TU alternatives in the treatment of male hypogonadism. Larger randomized clinical trials are required to determine the proper use, safety, and efficacy of SARMs, but preliminary studies suggest that this is a cost-effective suitable alternative to T supplementation.

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Benefits and Adverse Events of Testosterone Therapy

16

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Introduction

Testosterone is a steroid hormone important in many aspects throughout men's development and life. Circulating testosterone levels and their actions differ in each stage of life. During the embryonic life, testosterone is responsible for sexual differentiation and development of the external genitalia (e.g., development of primary sexual characteristics). Neonatal testosterone levels seem to be important for penile growth and complete testicular descent [1, 2]. After 6 months of age, the gonadal axis enters into a state of quiescence that persists throughout childhood. Pubertal onset is marked by the reactivation of the gonadal axis, with an increase in the frequency and amplitude of secretory pulses of GnRH by hypothalamic neurons, with consequent increase of gonadotropins secretion by the pituitary induces maturation, secretion of sex steroids, and gametogenesis [3–5]. The progressive increase of testosterone levels and its active metabolites, estradiol, and DHT induces the development of secondary sexual characteristics (virilization) and other changes, including growth and development of the penis and testicles, testicular pigmentation, hair growth in androgen-dependent areas, deepening of the voice due to increase in the thickness of the vocal cords, growth of long bones and bone epiphysis closure, prostate enlargement, and seminal fluid production by seminal vesicle [6].

Clinical Conditions for Testosterone Treatment

The main and undisputable indication for testosterone treatment is androgen replacement in men with a confirmed diagnosis of hypogonadism, i.e., men with consistent symptoms and signs of androgen deficiency and unequivocally low serum

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testosterone levels [7, 8]. Other established or controversial indications include constitutional delay of growth and puberty (CDGP), men with sexual dysfunction, female-to-male transsexual persons, hypogonadism secondary to drugs or chronic illness, androgen deficiency in the aging male. Testosterone levels tend to naturally decrease in aging men, who may experience symptoms such as decreased strength, fatigue, and decreased libido. However, lower levels of testosterone do not necessarily mean a diagnosis of hypogonadism [7, 9]. With the aging of the population and the growing prevalence of obesity and diabetes, there is an increasing number of men presenting with low or low-normal testosterone levels without typical symptoms, not fulfilling the diagnostic criteria for hypogonadism. The laboratorial evaluation of testosterone levels needs to be careful. Serum testosterone levels exhibit a circadian variation with peak values in the morning; this circadian rhythm is blunted with aging. Because of this circadian variation in testosterone levels and the fact that normal ranges for serum testosterone are usually established using morning blood samples, testosterone measurement be performed in the morning [7]. If testosterone levels are low, the measurement of total morning testosterone should be repeated and, in some cases when total testosterone is near the lower limit of normal or in whom SHBG abnormality is suspected, measurement of free or bioavailable testosterone levels is then recommended [7]. Nevertheless, testosterone levels below which symptoms and signs of androgen deficiency occur are still not fully known. Likewise, it is not easy to determine a cutoff value below which testosterone therapy would be recommended and considered beneficial for improvement of symptoms. In a study involving healthy men as well as patients with different causes of hypogonadism, thresholds for androgen deficiency symptoms varied greatly among the individuals, showing a standard personal sensitivity to the various testosterone levels [10–12]. However, for most subjects, the testosterone threshold more likely to lead to symptoms corresponds to the lower limit for young men, i.e., approximately 300 ng/dL [10, 12].

The overall goals of therapy are to establish and maintain secondary sexual characteristics, sexual function, sense of well-being, and to improve body composition, muscle mass and strength, bone mineral density, and quality of life [7, 8, 13].

Treatment with testosterone should not be administered for the improvement of athletic performance, with esthetic goals, or for attempted anti-aging therapy. Androgen replacement for these purposes has not been proven to be effective and is not exempt from side effects.

Currently there are several available preparations of testosterone, with different routes of administration, pharmacokinetics, and half-lives, such as transdermal patches, gel, and the classical intramuscular injections (short-acting and long-acting formulations). Characteristics of each of them are detailed in Chaps. 13, 14, and 15.

A summary of the available preparations, their advantage and disadvantage and specific adverse effects is displayed in Tables 16.1 and 16.2, respectively.

Table 16.1 Advantages and disadvantages of available testosterone formulations for clinical use

Composition	Dose	Advantages	Disadvantages
Mixed testosterone esters	250 mg/2–3 week	– Low cost	– Does not mimic the circadian rhythm of testosterone release
		– One dose every 2–3 weeks	– Provides supra-physiological levels of testosterone within the first days after injection
Testosterone cypionate or enantate injection	200 mg/2–3 week	– Low cost	– Does not mimic the circadian rhythm of testosterone release
		– One dose every 2–3 weeks	– Leads to supra-physiological levels of testosterone within the first days after injection
Injectable testosterone undecanoate	1000 mg/12 week	–4 injections/year	High cost
		– Does not provides supra-physiological levels of testosterone	
Transdermal patch	5 mg/day	– Mimics the circadian rhythm of testosterone release	– Daily use
		– Moderate cost	– Often causes skin irritation at the site
		– Leads to physiological levels of testosterone	– High cost
Transdermal gel	50 mg/day	– Quick and efficient absorption	– Daily use
		– Maintains satisfactory levels of testosterone	– High cost
		– Does not cause skin irritation at the site	
Subcutaneous implants	600 mg/4–6 months	– Leads to stable and physiological testosterone levels	Possibility of extrusion and local infection
		– One implant every 6 months	
Oral testosterone undecanoate	80–160 mg/day	– The only effective and safe oral testosterone ester	– Daily use 2–4 daily doses
		– Does not cause hepatotoxicity	– Variability of absorption according to meals
			– Unstable testosterone serum levels
Oral system	30 mg/12 h	– Mimics the circadian rhythm	–2 daily doses
		– Leads to physiological levels of testosterone	– High cost
		– Does not seem to cause mucosal irritation	– Short experience of clinical use

Table 16.2 Testosterone formulations and specific adverse effects

Short-acting intramuscular testosterone esters (enanthate, cypionate, or mixed esters)	– Fluctuation in mood or libido
	– Pain at injection site
	– Erythrocytosis (especially in older patients)
	– Coughing episodes immediately after the IM injection[a]
Long-acting intramuscular testosterone undecanoate	– Pain at injection site
Transdermal patches	– Frequent skin reactions at application site[b]
Transdermal gel and solution	– Potential risk for testosterone transfer to partner or another person who is in close contact
	– Skin irritation
Buccal testosterone tablets	– Alterations in taste
	– Irritation of gums
Pellets implants	– Infection, expulsion of pellet
Oral tablets (17-α alkylated androgens)	– Hepatotoxicity
	– Decrease in the concentrations of HDL cholesterol

[a] The mechanism of cough, which has been reported rarely after IM injections of testosterone undecanoate and even more rarely after testosterone enanthate and cypionate, is unknown, but it has been attributed to oil embolization

[b] The frequency of skin reactions is higher with the testosterone patches than with the transdermal gels

Benefits and Indications of Testosterone Therapy in Different Conditions

Constitutional Delay of Growth and Puberty

Constitutional delay of growth and puberty (CDGP) is the single most common cause of delayed puberty in both sexes, being responsible for around 65% of the cases in boys [14]. CDGP is considered a variation of normal development and it is diagnosed when there is no testicular enlargement in boys or breast development in girls at 14 years in boys and 13 years in girls [14]. For boys with CDGP, the options for management include expectant observation or therapy with low-dose testosterone [14]. The decision regarding whether to treat should be made by the patient and his family and it is initiated mostly to reduce psychosocial difficulties, feeling of social inadequacy, low self-esteem, and anxiety about growth rate or body habitus [14]. It remains unclear whether adult bone mass is adversely affected by pubertal delay and whether this represents a medical reason to initiate sex steroid replacement [14, 15].

The studies in this field are largely observational, and some randomized-controlled trials involving a small number of subjects [16]. There are limited studies regarding oral and transdermal testosterone preparations in adolescents and intramuscular short-acting testosterone therapy remains the mainstay of therapy for pediatric patients because they allow us fractioning the dose. The data suggest that

short courses of low-dose testosterone (3–6-month course of 50–100 mg I.M. testosterone esters per month) lead to increased growth velocity and sexual maturation and positively affects psychosocial well-being, without significant side effects or excessive acceleration of bone age and prejudice to the final height without rapid advancement of bone age, or reduced adult height [14, 16–18]. Even at the low initial doses used for pubertal induction, there is a decrease in total fat mass, body fat percentage, and whole body proteolysis. Once started full-dose IM injections of testosterone, can be painful for the adolescent patient population [19]. In a study of transdermal testosterone delivered via a 5-mg patch, overnight use in boys with delayed puberty resulted in pubertal testosterone concentrations as well short-term growth [20]. Side effects of transdermal testosterone include local skin irritation. A recent observational retrospective study report 96 boys treated with oral testosterone undecanoate, 20–160 mg daily, for 0.5–1.3 years. Testosterone treatment was followed by pubertal development and a significant increase in growth velocity and predicted adult height without accelerated bone age advancement [21].

Congenital Hypogonadism (Hypogonadotropic and Hypergonadotropic Hypogonadism)

Testosterone is the primary treatment modality used in men with hypogonadism. In boys who have permanent hypogonadism, the need for therapy is lifelong. Initial sex steroid therapy is the same as for CDGP, but doses are gradually increased to full adult replacement levels over 3 years. Testosterone replacement therapy in hypogonadal patients should be started at 12–13 years of chronological age, preferentially before bone age reaches 14 years, when the critical period for bone mass gain starts. When therapy starts in adult men, testosterone replacement can be started at full dose [16].

Testosterone replacement in young, hypogonadal men increases hair growth in androgen-sensitive areas, fat-free mass, and muscle strength and decreases fat mass. It increases bone mineral density, although the effects on fracture risk are unknown. The treatment is also associated with overall improvements in the mood, energy, and sense of well-being, frequency and quality of sexual activity, sexual thoughts, and fantasies [7]. Exogenous testosterone does not induce testicular growth or spermatogenesis in men with hypogonadism [16]. When fertility is desired, testosterone replacement should be discontinued and gonadotropins should be initiated. The effects of testosterone on cognitive function are poorly understood; some studies report small effects on visuospatial cognition and verbal memory and fluency [7].

Testosterone Therapy in Men with Sexual Dysfunction

Androgen deficiency and erectile dysfunction are two independent clinical conditions with distinct pathophysiology, although they may coexist specially in middle-aged and older men. Men with sexual dysfunction should be evaluated for all possible

underlying causes, including low testosterone levels. However, mean serum testosterone levels are frequently in the normal range in men with erectile dysfunction [22]. Two meta-analyses showed that testosterone replacement therapy was associated with a large improvement in libido and a moderate effect on erectile function and overall sexual satisfaction in men with baseline serum testosterone concentrations below 300 ng/dL, whereas no effect was seen in eugonadal men [23, 24]. Some small trials reported a positive impact of testosterone therapy on other sexual outcomes, such as number of intercourses, orgasmic, and ejaculatory function [7].

Older Men with Low Serum Testosterone Concentration

Testosterone concentrations decline in average 1–2% per year with age [25–27]. A significant fraction of older men older than 65 years have levels below the lower limit of the normal range for healthy, young men [7, 28, 29]. In this group of patients low testosterone level may be associated with symptoms including low libido, decrease in sexual function, hot flushes, as well as less specific symptoms such as fatigue, loss of energy, loss of lean body mass, irritability, depressed mood, poor concentration, reduced physical performance, risk of falling, and sleep disturbance [7, 28]. The prevalence of symptomatic androgen deficiency in middle-aged and older men increases with waist circumference, diabetes, and poor self-reported health status, suggesting that testosterone deficiency may not be the sole cause of the symptoms [11].

In older men with symptomatic hypogonadism, testosterone replacement therapy should, at least in theory, improve the physiological manifestations of the condition. However, the benefits of treatment of late-onset hypogonadism are unclear due to the lack of controlled studies. A recent clinical trial has found that in men with 65 years of age or older with a serum testosterone concentration of less than 275 ng/dL and symptoms suggesting hypoandrogenism, testosterone treatment significantly increased libido, sexual activity, and erectile function [28]. However, it is important to note that increasing testosterone levels do not necessarily improve the symptoms of erectile dysfunction [30]. In several trials, testosterone therapy also has been shown to consistently increase muscle mass and to decrease fat mass. Some physical parameters such as grip strength and muscle mass may improve with testosterone replacement in frail elderly men with low testosterone levels [31]. On the other hand, effects on physical vitality and energy have been inconsistent, although men receiving treatment report slightly better mood and lower severity of depressive symptoms [28]. Elderly hypogonadal men have increased the risk of osteoporosis and testosterone replacement may reduce bone loss, although there are no studies evaluating the risk of fractures [32]. In this population, testosterone therapy should be offered on an individualized basis. A decision to treat older men depends on the physician's and the patient's assessment of risks and benefits of testosterone therapy. Older patients with a greater potential for adverse effects may opt to avoid testosterone therapy [7].

More detailed aspects of hypogonadism and aging are illustrated in Chap. 9.

Hypogonadism Associated to Chronic Illness or Drugs

Symptomatic androgen deficiency is common in men using corticosteroids and opioid analgesics, and in obese, diabetic, and HIV-infected patients, among other chronic conditions. Recommendation for testosterone replacement in these patients follows the same criteria as for classical hypogonadism, i.e., patients with symptoms of androgen deficiency and confirmed low levels of testosterone, and if well indicated, it may improve libido, quality of life, and body composition among other benefits.

There are many studies about hypogonadism associated with obesity, metabolic syndrome, and type 2 diabetes, but data showing an improvement in the metabolic parameters are still conflicting. Some studies suggest improvement in obesity, abdominal circumference, insulin sensitivity, and diabetes parameters, with different testosterone formulations [33–35], whereas others did not confirm these results [36–38]. In general, most studies demonstrated favorable effects in men with obesity or type 2 diabetes and confirmed hypoandrogenism [7]. A large trial of testosterone replacement therapy with a 2% gel showed beneficial effects on insulin resistance, lipid profile, and sexual health in men with type 2 diabetes, metabolic syndrome, or both [36]. Another study showed that intramuscular testosterone treatment in men with type 2 diabetes and HH increased insulin sensitivity and lean body mass (LBM) and decreased subcutaneous fat [39]. In a recent trial, testosterone replacement therapy was independently associated with reduced mortality in men with type 2 diabetes and low testosterone levels [40].

Functional hypogonadisms are discussed in Chap. 8.

In HIV-infected men, low testosterone levels are associated with weight loss, progression to AIDS, wasting, depression, and loss of muscle mass and exercise capacity. Testosterone administration in this group of patients increases lean body mass and muscle strength, with a moderate effect on depression, without significant adverse effects. In several clinical trials, changes in CD4+ T lymphocyte counts, HIV viral load, PSA, and plasma high-density lipoprotein cholesterol were not significantly different between placebo and testosterone groups [7].

There is a high prevalence of low testosterone levels in men receiving chronic glucocorticoid therapy due to glucocorticoid-induced suppression of all components of the hypothalamic-pituitary-testicular axis [41]. In two small-controlled trials, testosterone therapy of men receiving glucocorticoid treatment was associated with a significant decrease in fat mass, increase in LBM and lumbar bone mineral density, and a low frequency of adverse events in comparison with placebo [42, 43]. The Endocrine Society guideline for testosterone therapy suggest that testosterone therapy may be offered to men receiving high doses of glucocorticoids who have low testosterone levels to promote preservation of LBM and bone mineral density [7].

Opiates potently suppress the hypothalamic-pituitary-gonadal axis. There is a high prevalence of symptomatic androgen deficiency in men taking opioid analgesics [44, 45]. In addition, male chronic opioid users have higher prevalence of cardiometabolic abnormalities, so there is a potential concern with the cardiovascular

risks with testosterone therapy in this population. However, in a recent randomized-controlled trial, 14 weeks of testosterone replacement in men with opioid-induced androgen deficiency improved pain sensitivity, libido, body composition, and quality of life and was not associated with worsening of metabolic and inflammatory markers [45].

Female-to-Male Transsexual Persons

Testosterone treatment is essential for the induction and maintenance of virilization of female-to-male (FTM) transsexuals. Testosterone therapy is recommended for after the diagnosis is confirmed in 16 years or older patients [43, 44]. Medical conditions that can be exacerbated by testosterone treatment, such as breast or uterine cancer, erythrocytosis (hematocrit >50%), should be evaluated and addressed prior to initiation of treatment [43]. Clinical studies have demonstrated the efficacy and safety of several different testosterone preparations to induce masculinization in FTM transsexual persons. A long-term follow-up use of I.M. short-acting testosterone enanthate in a dose of 200 mg bi-weekly was effective and safe for FTM transsexuals treatment [44]. Either parenteral or transdermal preparations can be used to achieve and maintain testosterone values in the physiological male range. Regimens to change secondary sex characteristics follow the general principle of hormone replacement treatment of male hypogonadism [43]. The physical changes observed include by this hormonal transition are usually associated to an improvement in psychological well-being and in general life satisfaction [43]. In general, patients are very pleased with the virilization achieved. The I.M. short-acting testosterone cypionate in a dosage of 200 every 15–21 days determine interruption of menstrual cycles, breast atrophy, voice deepening, increased body hair, clitoris enlargement, libido improvement, redistribution of body fat, and increased muscle mass. After the induction of the virilization period, if no side effects are observed, higher doses (e.g., 200 mg of testosterone cypionate weekly) associated or not with dihydrotestosterone gel for 6 months may be used to increase clitoral size, facial hair, and muscle mass [44]. Undesirable side effects are generally not observed; there may be significant degrees of acne or seborrhea [43, 44]. In one recent study, 1-year testosterone administration to FTM transsexuals, both transdermal and parenteral depot preparations were associated with increased lean body mass and decreased fat mass, increased LDL, decreased HDL, and no change in insulin sensitivity [45].

Testosterone Therapy in Women

The primary indication for the prescription of testosterone for women is loss of sexual desire, which causes affected women substantial concern. Evidence supports the short-term efficacy and safety of high physiological doses of T treatment of postmenopausal women with sexual dysfunction due to hypoactive sexual desire disorder. However, no formulation has been approved for this purpose.

Further studies are needed to establish definitively whether an androgen deficiency syndrome exists in women and whether androgen therapy ameliorates this condition [46].

A detailed review of this subject is given in Chap. 20.

Benefits of Testosterone on Specific Organs and Systems

Sexual Function

It has been well established that testosterone treatment of hypogonadal men improves general sexual function, increases libido, sexual thoughts, response to erotic stimuli, and erectile function [8]. Testosterone therapy have been more consistently associated with improvement of libido than of erectile function [8]. In men with late-onset hypogonadism, older than 60 years, testosterone replacement does not show a strong positive association with erectile function, suggesting that other associated conditions may be involved in the origin of the erectile dysfunction [47]. This topic is detailed in Chap. 17.

Quality of Life, Mood, and Cognition

The exact role of testosterone therapy on quality of life and cognition remains unclear. Nevertheless, most studies reported improvement in mood, energy, well-being, physical function, and quality of life. The quality of life of older men with hypogonadism that use testosterone compared to those that do not, the improvement in the quality of life of the group using testosterone could be relative, i.e., determined by the decline in quality of life of the placebo group, suggesting a possible positive effect of testosterone on preventing the decline in quality of life with age. Moreover, the improvement of physical function and control of somatic and sexual symptoms with testosterone replacement improves the quality of life of patients with late-onset hypogonadism and may constitute an important treatment strategy in old age [28].

A meta-analysis showed that testosterone replacement had beneficial effects in depression scores [48]. Improvements in spatial and verbal memory have been seen after testosterone treatment, especially in older men with low testosterone and cognitive impairment [8, 49]. However, testosterone administration leading to supra-physiological serum levels negatively affected cognitive function [50].

Bone, Body Composition, Muscle Strength, and Physical Function

In hypogonadal men, testosterone replacement has a positive effect on bone mass and determines a significant improvement in bone mass in hypogonadal men of all ages [51]. Furthermore, testosterone replacement therapy is associated with

significant increases in trabecular microarchitecture and in spinal and hip bone mineral density with maximum improvement seen by 24 months [8].

Testosterone replacement therapy is associated with increased fat-free mass and decreased fat mass, increased lean mass, and muscle strength. Studies in frail men older than 60 years with low testosterone have reported improvements in muscle strength, body composition, and physical function [28, 52]. Direct evaluation of muscle size in elderly patients with hypogonadism and chronic diseases have shown that testosterone therapy in older patients leads to increase muscle size and strength improving performance in physical activities [53].

Contraindications

Testosterone replacement therapy is contraindicated in men with hormone responsive tumors, such as prostate or breast cancers. Patients with palpable prostate nodule or induration or abnormal PSA concentrations should be submitted to further urological evaluation before initiating testosterone administration. Other conditions that can be worsen by testosterone therapy include, untreated severe obstructive sleep apnea, severe lower-urinary-tract symptoms, uncontrolled or poorly controlled congestive heart failure, men in high risk for acute myocardial infarction, cerebrovascular accident, or acute coronary syndrome in the last 6 months [7, 8]. Baseline hematocrit above 50% is a relative contraindication to testosterone therapy because patients may develop a hematocrit above 54% when treated with testosterone. Men with hematocrit level above 50% should undergo further clinical evaluation before considering testosterone therapy [7].

Table 16.3 summarizes the main contraindications to testosterone therapy.

Table 16.3 Contraindications to testosterone therapy

Breast cancer
Prostate cancer
Nodule or induration on prostate examination (unless biopsy is negative)
PSA concentration >4.0 μg/L, or >3.0 μg/L in high-risk men (e.g., African Americans, first-degree relatives of men with prostate cancer) unless urological assessment is negative
Severe lower-urinary-tract symptoms associated with benign prostatic hypertrophy as indicated by AUA/IPSS ≥19
Hematocrit >50%
Untreated severe sleep apnea
Uncontrolled congestive heart failure

Adverse Effects and Treatment Monitoring

Testosterone therapy may be associated with increased risk of minor or serious adverse effects, the latter particularly in older men with previous disorders. Studies in young, hypogonadal men have found a low frequency of adverse events with testosterone replacement in physiological doses. Common drug-related adverse events include increase in hematocrit, acne, oiliness of skin, and breast tenderness [7]. The frequency of sleep apnea and prostate events is low in this population. Gynecomastia is relatively frequent in the beginning of the treatment with full dose short-acting IM preparations of testosterone, but usually resolves spontaneously. When breasts achieve Tanner stage IV or V, spontaneous regression does not occur and corrective surgery is necessary. Formulation-specific adverse effects are summarized in Table 16.3. Hepatotoxic effects are seen in men taking oral 17-α-alkylated androgens and these drugs should not be used in the treatment of hypogonadism [52]. Intramuscular formulations that elicit testosterone peaks (testosterone esters or testosterone cypionate and enantate) frequently lead to fluctuations in the mood and sex drive. In addition, intramuscular injections can cause pain at the injection site. Local skin irritation may occur with transdermal testosterone gels and patches. Skin reactions are more common with patches than with transdermal gel formulations. Local infection and expulsion of pellet can occur in approximately 10% of the cases with subcutaneous testosterone pellets [7].

Erythrocytosis

Erythrocytosis is the most frequent adverse event related to testosterone replacement therapy. Testosterone administration in hypogonadal men is associated with a dose-dependent increase in hemoglobin levels [7, 8]. The increase in hemoglobin is more frequent in men older than 60 years, probably because of reduced testosterone clearance [54]. Additionally, the frequency of erythrocytosis is higher with short-acting intramuscular testosterone preparations as compared to transdermal or long-acting IM testosterone administration, probably because of the peaks of testosterone concentrations in serum achieved with injections of testosterone esters [54]. It is not known whether testosterone therapy can increase the risk of erythrocytosis in men with other conditions that predispose to hypoxia, such as chronic obstructive pulmonary disease or obstructive sleep apnea. Testosterone stimulates bone marrow, promotes erythropoietin production, and suppression of hepcidin, a regulator of iron metabolism, which inhibits iron transport and absorption [55, 56]. If hematocrit rises to more than 54% during testosterone therapy, treatment should be discontinued and disorders that cause hypoxia should be investigated. Once hematocrit normalizes, we can consider resuming the treatment with lower doses or change IM to transdermic testosterone administration preparations.

Prostate

There is no evidence that serum testosterone concentrations increase the risk of prostate cancer. However, there is a concern that testosterone replacement therapy might stimulate the growth of pre-existing prostate cancer [8]. In men with benign prostatic hypertrophy testosterone therapy can increase the prostate volume, therefore in patients with severe lower-urinary-tract symptoms, careful monitoring is required [7]. This topic is detailed in Chap. 18.

Cardiovascular

Testosterone therapy and cardiovascular risk remains a controversial issue. The studies investigating long-term effects of testosterone on cardiovascular events have showed contradictory results [57]. A meta-analysis showed that men randomized to testosterone were nearly twice as likely to experience cardiovascular events as those receiving placebo [58]. A randomized trial showed an increased frequency of cardiovascular events in the testosterone group compared with placebo, however, in this trial most men were older than 65 years, with a high prevalence of underlying cardiovascular disease at baseline [52]. A more recent meta-analysis of controlled trials that analyzed 51 studies but did not include the trial with elderly men did not find any significant effect on mortality, prostate, or cardiovascular outcomes between testosterone and placebo groups [59]. A detailed review of this topic is discussed in Chap. 19.

Lipids

The effects of testosterone therapy on total cholesterol, LDL-cholesterol, and triglyceride concentrations have been mixed. In general, testosterone therapy is associated with a decrease in HDL cholesterol [59]. However, the effects of testosterone administration on the lipids profile depend on the dose, route of administration, and whether the androgen can be aromatized. The use of non-aromatizable oral 17-α-alkylated androgens has been consistently associated with decreased concentrations of HDL cholesterol, whereas treatment with other testosterone formulations has been associated with no or only slight decreases in HDL cholesterol [58, 59]. Ultimately, the effects of testosterone on lipid metabolism are still uncertain.

Sleep Apnea

The association between obstructive sleep apnea and testosterone replacement therapy is based in small uncontrolled studies that used supra-physiological doses of intramuscular testosterone [8]**.** The frequency of sleep apnea is low in trials of young, hypogonadal men [7]. In a systematic review of 19 randomized trials to determine the risks of adverse events associated with testosterone therapy in older

men, the frequency of sleep apnea did not differ significantly between groups [60]. Although the risk of worsening of sleep apnea is still unclear, it is not recommended to start testosterone replacement therapy in men with untreated severe obstructive sleep apnea [7].

Fertility

Exogenous testosterone exerts an effect of negative feedback in the gonadotropic axis, reducing the GnRH pulsatility and gonadotropins secretion. Consequently, testosterone therapy inhibits spermatogenesis, leading to a state of transitory infertility and is not appropriate in men with hypogonadotropic hypogonadism who desire fertility. This should be taken in consideration before testosterone replacement therapy is started in men with slightly low testosterone concentrations who plan to start a family in the near future [7].

Exogenous testosterone may cause atrophy of the germinative epithelium, suppressing spermatogenesis after approximately 10 weeks of use [61]. Testicular atrophy is not common, but it can occur as a reflection of the loss both Sertoli and Leydig cells. In normal men, recovery of testicular function usually occurs in 6–18 months. In some patients azoospermia can be persistent, with significant negative consequences for fertility in the future [61, 62].

Table 16.4 displays the potential adverse effects of testosterone replacement.

Table 16.4 Potential adverse effects of testosterone replacement

Erythrocytosis
Acne and oily skin
Detection of subclinical prostate cancer
Growth of metastatic prostate cancer
Reduced sperm production and fertility
Gynecomastia
Male pattern balding (familial)
Growth of breast cancer
Induction or worsening of obstructive sleep apnea

Conclusions

Since 2000, the number of men started on testosterone therapy has increased considerably in the USA [63]. In the last years, middle-aged and elderly men have been increasingly submitted to routine *serum testosterone testing in clinical practice.* Recent studies suggest that many patients that start testosterone replacement may not have a clear medical indication. The great majority of these patients have been submitted to only one testosterone test before starting treatment, without adequate follow-up. The increase in direct-to-consumer advertising and the availability of more convenient and easy to use formulations, such as topical gels, as opposed to bi-weekly intramuscular injections of testosterone esters, may have contributed to the increasing search for testosterone therapy.

Testosterone plays an essential role in several aspects of men's health. Untreated testosterone deficiency has serious consequences to physical and psychological health [64]. Nevertheless, it is important to remember that the diagnosis of testosterone deficiency can be done only in men with consistent symptoms and unequivocally low testosterone levels confirmed by repeated laboratory tests [64]. It is essential to always evaluate the risks and benefits of testosterone therapy in an individual basis before start treatment. Comparative analysis between the main modalities of androgen replacement therapy showed that all of them are safe and effective, although the transdermic formulations are more physiological.

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Testosterone and Sexual Function

17

Giovanni Corona, Giulia Rastrelli, Simona Ferri, Alessandra Sforza, and Mario Maggi

Introduction

Much evidence supports the concept that testosterone (T) represents the fuel of male sexual function [1–7]. Accordingly, data from the European Male Aging Study (EMAS), a population-based survey on more than 3400 subjects across eight European centers showed that among the different symptoms, sexual dysfunction represents the most important determinant for medical consultation and the most specific symptom associated with low T [8]. In particular, it was recognized that a triad of sexual symptoms (low libido and reduced spontaneous and sex-related erections) is the only syndromic association with decreased T levels [8]. In that large European survey, the simultaneous presence of the three sexual symptoms (hypoactive sexual desire, erectile dysfunction [ED], and perceived reduced sleep-related erections) combined with a total T level of less than 11 nmol/L and a FT level of less than 220 pmol/L were therefore considered the minimum criteria for the diagnosis of late onset hypogonadism (LOH; [8]). In line with these data, by comparing the prevalence of endocrine abnormalities in two different cohorts from the general (Florentine spin-off of the EMAS cohort; $n = 202$) and the symptomatic populations of Florence (a series of $n = 3847$ patients attending our clinic for sexual dysfunction), we recently reported that subjects seeking medical care for sexual dysfunction represent a population enriched with LOH [9]. In the same symptomatic population

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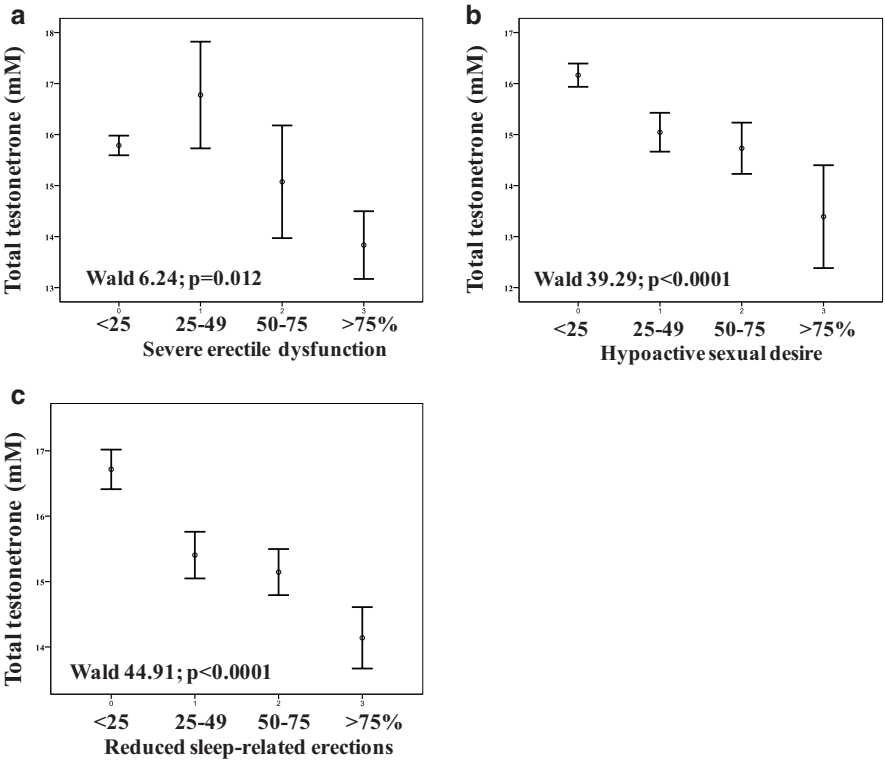


Fig. 17.1 Age adjusted relationship between total testosterone and severe erectile dysfunction (**a**), hypoactive sexual desire (**b**) and sleep-related erections (**c**). Data were derived from a consecutive series of 4793 subjects (mean age = 51.1 ± 13.3 years) seeking medical care at our Unit for sexual dysfunction

we, even more recently, confirmed that the simultaneous presence of reduced morning erections and desire is the cluster of symptoms that, along with total T < 10.4 nmol/L or cfT < 225 pmol/L, defines LOH in a specific, evidence-based manner [10]. The addition of a third symptom, ED, further improved the accuracy [10]. Accordingly, Fig. 17.1 shows the stepwise inverse relationship between T levels and the aforementioned sexual complaints as detected in a large (n = 4793 mean age = 51.1 ± 13.3 years) sample of our cohort of patients.

Despite this evidence, however, some data indicate that sexual activity per se can influence T levels. In other words, sexual inertia related to erectile dysfunction (ED) can impair T production.

In the following sections preclinical and clinical data supporting the role of T in regulating male sexual function will be analyzed in detail.

Testosterone Regulation of Male Sexual Response

Testosterone plays a crucial role in regulating male sexual response by acting on several levels.

Central Control

Androgen receptors (AR) are expressed in several distinct areas of the human brain, including the temporal, preoptic, hypothalamus, amygdala, midbrain, frontal and prefrontal areas, and cingulated gyrus (Brodman area 24, BA24; [5, 11–13]). Interestingly, the BA24 area, a part of the limbic cortex deeply involved in balancing emotional behavior and generalized arousal reaction, has been found to be activated by explicit erotic films in two different studies by using both positron emission tomography [14] and functional magnetic resonance imaging ([15]; see for review [16]). The role of T in BA24 is further supported by the observation that T supplementation to symptomatic hypogonadal men increases blood perfusion (as assessed by single-photon emission-computed tomography) in this area as well as in midbrain and superior frontal gyrus (BA8; [17]). Another androgen-sensitive brain area is represented by BA37 (middle occipital gyrus) which is involved in the processing of novel visual stimuli [16, 18].

Spinal Control

T acts at spinal cord level controlling ejaculation reflex [19]. The spinal nucleus of the bulbocavernosus muscle (SNB) is androgen-dependent [20]. Circulating androgens, in adult rats, can profoundly alter the expression of gastrin-releasing peptide in the lower spinal cord [21] that, by innervating the SNB, mediates the ejaculatory reflex [19]. Interestingly, bulbocavernsous muscle, like other muscles of the pelvic floor involved in the ejaculatory ejection of the seminal bolus (ischiocavernosus and levator ani muscle), is specifically androgen-dependent. In fact, hypertrophic action on the levator ani is a good predictor of effective anabolic androgens [19].

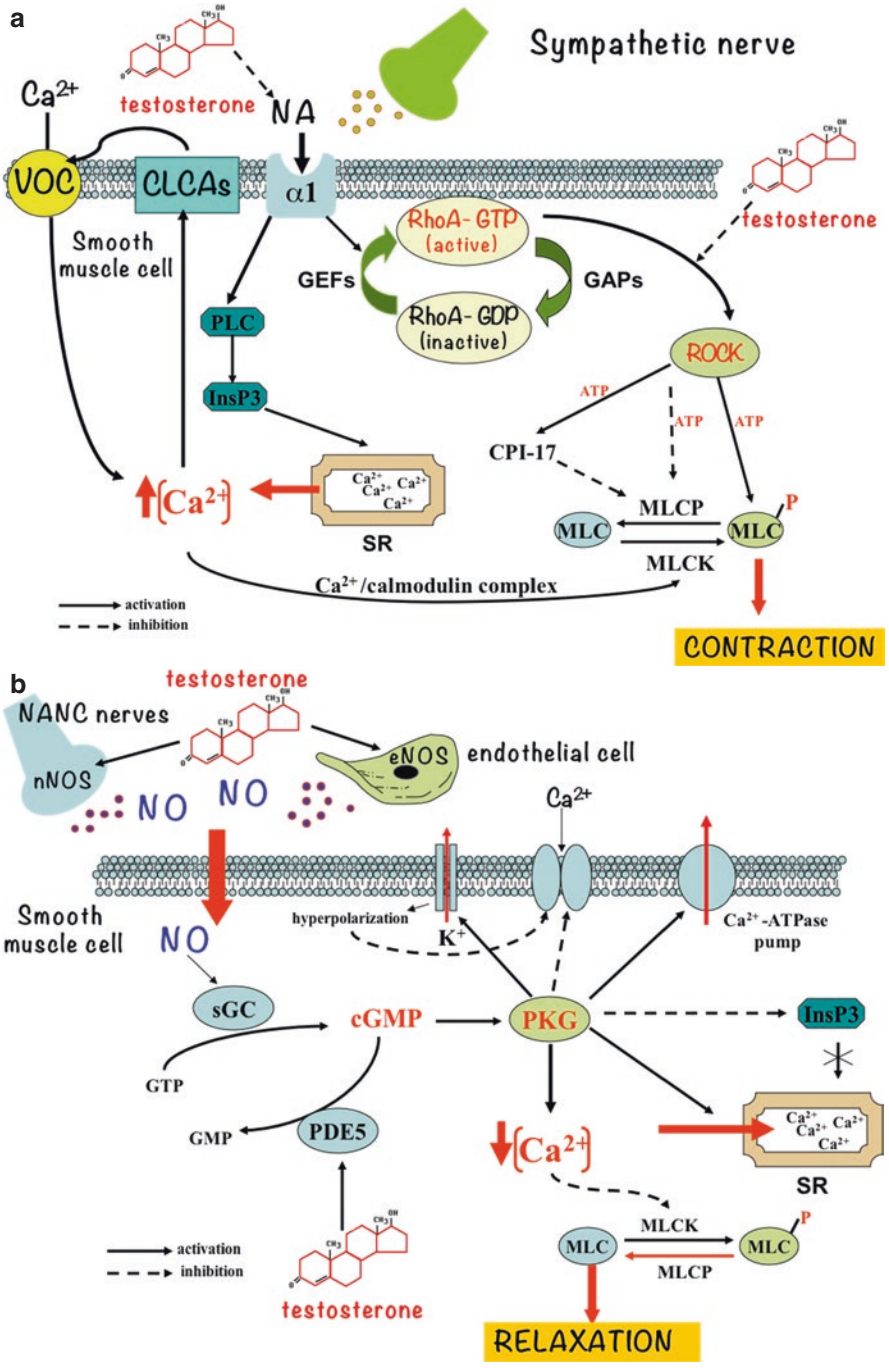
Peripheral Control

Experimental studies in animals and human cell cultures indicate that T controls, directly or indirectly, several mechanisms underlying erection and detumescence. In particular, T controls the commitment of penile cells to a smooth muscle phenotype favoring the functional and structural integrity necessary for penile erection [2]. Accordingly, androgen deprivation is associated with the accumulation of fat containing cells (fibroblasts or preadipocyte-like cells), especially in the subtunical region of the corpus cavernosum [2].

In addition, T controls numerous enzymatic activities within the corpora cavernosa (CC; Fig. 17.2). The role of T in regulating nitric oxide (NO) formation (acting on endothelial-NO and/or neuronal-NO synthases) has been demonstrated in numerous animal models ([22–25]; see for review 2; Fig. 17.2, panel b). Furthermore, T negatively regulates also the activity of the Ras homolog gene family member A/Rho-associated kinase (RhoA/ROCK) pathway, overall decreasing calcium sensitivity within penile smooth muscle cells ([26]; Fig. 17.2; panel a). Finally, T positively controls the expression and the activation of phosphodiesterase type V (PDE5; Fig. 17.2, panel b; [22–25]; see for review [2]).

Finally, another recognized mechanism of androgen action is the regulation of α1-adrenergic responsiveness of smooth muscle cells (Fig. 17.2, Panel a; see for review [2]). Consistent findings point toward an effect of T on the postganglionic parasympathetic neurons, or even further upstream, within the autonomic nervous system [2]. Accordingly, androgens appear necessary to support adequate neuronal stimulation to the corpora cavernosa, maintaining structural integrity in tissue as seen after the denervation following prostate surgery in men [2].

Fig. 17.2 Schematic representation of the biochemical events leading to penile flaccidity (upper panel) or erection (lower panel) along with the proposed events regulated by **testosterone**. **Panel** (**a**) Noradrenaline (NA) binding to its receptors generates inositol 1,4,5-trisphosphate (InsP3), which, by increasing intracellular calcium (Ca^{2+}) levels, activates Ca^{2+}-sensitive chloride channels (CLCAs) resulting in membrane depolarization, with the diffusion of the stimulus to the neighboring cells and the opening of voltage-operated channels (VOC). The increased Ca^{2+} flow promotes, through calmodulin, activation of myosin light chain (MLC) kinase and cell contraction. Cell contraction is also obtained by altering the Ca^{2+} sensitivity through a NA-induced activation of a second pathway, RhoA/ROCK, which increases, through a series of kinase activation, the sensitivity of MLC to Ca^{2+}. **Testosterone** is supposed to negatively regulate the latter event. **Panel** (**b**) Nitric oxide (NO) is generated by NO synthases in either nonadrenergic-noncholinergic (NANC) neurons (nNOS) or endothelial cells (eNOS). Both steps are positively regulated by **testosterone**. NO diffuses into smooth muscle cells and activates a soluble guanylate cyclase (sGC), which in turn transforms GTP into cGMP. CGMP activates protein kinase G (PKG), which, through the indicated pathways, finally decreases intracellular Ca^{2+} levels, leading to relaxation. Phosphodiesterase type V (PDE5) metabolizes cGMP into GMP, thereby limiting its effects. The latter event is positively control by **testosterone**. *SMC* smooth muscle cells, *CC* corpora cavernosa


a
Sympathetic nerve
testosterone
NA
Ca2+
VOC
CLCAs
α1
Smooth muscle cell
RhoA- GTP (active)
RhoA- GDP (inactive)
GEFs
GAPs
testosterone
PLC
InsP3
ROCK
ATP
ATP
ATP
CPI-17
MLCP
MLC
MLC
P
MLCK
[Ca2+]
SR
Ca2+/calmodulin complex
activation
inhibition
CONTRACTION
b
testosterone
NANC nerves
nNOS
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NO
eNOS
endothelial cell
Ca2+
Smooth muscle cell
NO
hyperpolarization
K+
Ca2+-ATPase pump
sGC
cGMP
PKG
InsP3
GTP
GMP
PDE5
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RELAXATION

Hypogonadism and Male Sexual Dysfunction

Sexual Desire

Much evidence has documented that hypogonadism represents a possible cause of reduced libido in men [27]. Accordingly, by performing the largest meta-analysis published so far scrutinizing the role of T treatment (TTh) on several aspects of male sexual function we confirm that TTh can improve sexual desire in hypogonadal (T <12 nM) subjects at baseline [3]. Conversely, the positive effect of TTh was not confirmed in those studies considering only eugonadal patients (T levels below 12 nM) at enrollment (Table 17.1). In line with these data, meta-regression analysis performed in the whole sample showed a trend toward an inverse relationship between baseline mean T levels and the amount of effect on the libido component, which reached statistical significance when studies enrolling eugonadal or mixed eugonadal/hypogonadal subjects at baseline were excluded from the analysis [3].

Despite this evidence the contribution of T in the age-related decline of male sexual desire in the general population is conflicting [28]. However, the incidence of secondary hypogonadism in a 4.3-year follow-up observational EMAS cohort was associated with new/worsening of low libido, along with ED and infrequent spontaneous erections [29] confirming the association between androgens and sexual desire in humans.

It is important to recognize that although T has a crucial role in regulating male sexual desire, its contribution is similar to that played by other intra-psychic and relational factors, as well as medical conditions [27]. For instance, a depressed mood or hyperprolactinemia have a greater deleterious effect on sexual drive than hypogonadism per se [27].

Table 17.1 Effect size (with 95% confidence interval [CI]) in several sexual parameters across randomized controlled trials evaluating the effect of testosterone substitution vs. placebo

Sexual parameter	Outcome
Erectile function component	
Overall erectile function component[a]	0.82 [0.47;1.17]*
Overall sexual-related function component[b]	0.75 [0.37;.1.12]**
Sleep-related erections	0.87 [0.47;1.27]**
Libido component	
Overall libido component	0.81 [0.47;1.17]**
Orgasm component	
Overall orgasmic component	0.68 [0.34;1.02]**
Other sexual parameters	
Frequency of intercourse	0.75 [0.33;1.16]**
Overall sexual satisfaction	0.80 [0.41;1.20]**
Overall sexual function	0.67 [0.22;1.12]**

Adapted from [3]. *$p < 0.001$, **$p < 0.0001$

[a] Including coital and non coital erections

[b] Only coital erections considered

Erectile Function

Since T positively controls both the enzymatic steps necessary for initiation (positive effect on NOS and negative on RhoA/ROCK) and the end (positive effect on PDE5) of the erectile process, its net effect on erection is rather modest. Accordingly, Rhoden et al. [30], in a large consecutive series of almost 1000 elderly subjects with or without ED, failed to find an association between T and the International Index of Erectile Function (IIEF-5). Hence, erections are indeed still possible in hypogonadal conditions, where a decreased 3′,5′-cyclic guanosine monophosphate (cGMP) formation, due to impaired NO production, is most probably counterbalanced by reduced PDE5 activity and cGMP hydrolysis. Accordingly, it has been reported since antiquity that eunuchs who were castrated after puberty were still capable of maintaining erections [31]. For that reason, it was a custom in ancient Rome for women to use more potent eunuchs for pleasure without the risk of procreation [31].

The main physiological action of T is therefore to timely adjust the erectile process as a function of sexual desire, therefore finalizing erections with sex [11, 28].

In line with the aforementioned evidence, data derived from studies evaluating the effect of TTh on patients with ED have yielded mixed results [2, 3, 32, 33]. Some of these trials enrolled only few men, and their inability to show an effect may reflect limited study precision. Similarly, previous meta-analyses on this topic have produced conflicting results. Jain et al. [34] included only five randomized placebo-controlled studies. Boloña et al. [35] found a small yet significant effect of TTh on erectile function in men with low-normal T levels, and a greater effect in the subgroup of younger subjects. In addition, the same Authors reported a small but significant effect of TTh on satisfaction with erectile function in those men with sexual dysfunction and a TT level >10 nM [35]. Conversely, the effect on the same parameter in hypogonadal men (TT <10 nM) was moderate, not significant and inconsistent, and there was no significant effect on overall sexual satisfaction whatever the TT level was at baseline. Finally, Tserstvadze et al. [36], did not document any effect on erections of TS alone or in combination with PDE5i. However, it is important to note that Tserstvadze et al. [36] analyzed only nine randomized controlled trials (RCTs) enrolling mixed eugonadal/hypogonadal subjects, which may have resulted in a possible inclusion bias. In fact our meta-analysis [3] in line with Isidori et al. [37] documented a positive effect of TTh on both sexual-related and spontaneous erections as well as sleep-related erections when only studies enrolling hypogonadal (TT <12 nM) men at enrolment were analyzed (see also Table 17.1). Accordingly meta-regression analysis showed an inverse relationship between baseline T levels and final outcome [3]. In addition, our data clarified that the effect of TTh on ED was less apparent in diabetic subjects. The effect of TTh alone on erectile function is lower in the presence of penile vascular diseases. Accordingly, it is well known that diabetes [38–41] and even the pre-diabetic condition [42, 43] can determine penile atherosclerosis and impair penile neurogenic control, through several mechanisms, many of which are testosterone-independent [44].

In complicated ED hypogonadal men the association between TTh and PDE5i is thus mandatory [2, 3]. In addition, since T regulates PDE5 expression, several studies

have also suggested that hypogonadism represents a risk factor for reduced PDE5i effect [2, 3, 45, 46]. All these observations emphasize the concept that hypogonadism must be ruled out and, if present, adequately treated, before the prescription of any PDE5i. Our meta-analysis, however, did not allow us to adequately clarify this point. In fact, although a positive effect of TTh and PDE5i combined therapy has been observed in uncontrolled studies, the results were not confirmed when only RCTs were considered. However, it should be recognized that 3 out of 5 [47–49] of the aforementioned RCTs enrolled mixed eugonadal/hypogonadal samples. In addition, in the large Spitzer's trial [50], although only hypogonadal subjects were enrolled, T supplementation was initiated after a sildenafil alone run-in period, at the end of which T increased up to the normal range (about 12.0 nmol/L). Accordingly, it has previously reported that sexual inertia is associated with functional hypogonadotropic hypogonadism and can be restored with the improvement of erectile function ([2]; see below). Hence more studies on hypogonadal men are advisable in order to better clarify the role of TTh as an add on to PDE5i in the treatment of ED.

Orgasm

In 2006, quite unexpectedly we originally reported that hypogonadism represented a risk factor for delayed ejaculation [51]. In a further study we documented that different T levels could be linked to different subsets of ejaculatory disturbances as they are higher in subjects with PE and lower in those with DE ([52]; see also Fig. 17.3).

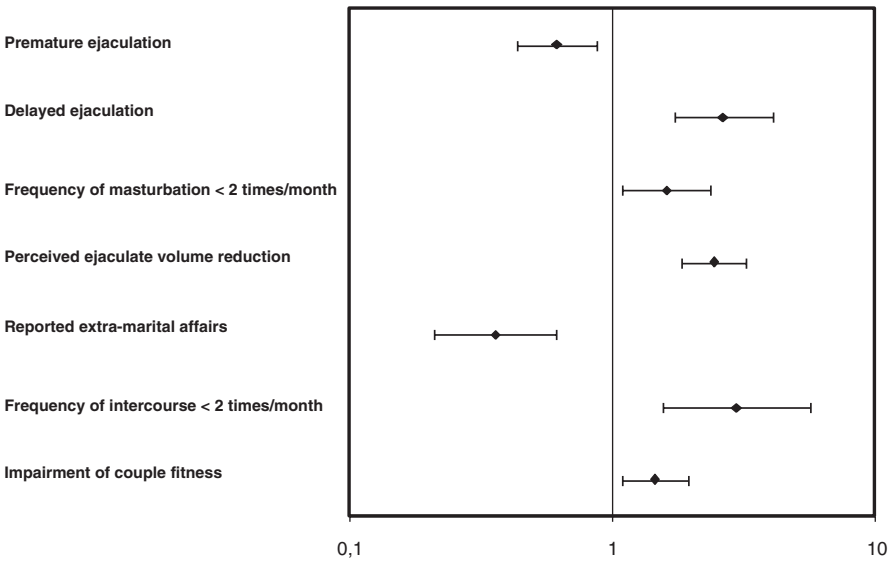

Fig. 17.3 Age adjusted risk of hypogonadism according to European Male Aging criteria (see [8]) of several sexual parameters. Impairment of couple fitness was evaluated using SIEDY Scale 2 score (see [53, 54]). Data were derived from a consecutive series of 4793 subjects (mean age = 51.1 ± 13.3 years) seeking medical care at our Unit for sexual dysfunction

Similar results were confirmed by other authors [55]. T might control ejaculatory reflex acting both at the central and peripheral level [19]. As reported above, AR are expressed in several supra-spinal and spinal areas involved in the control of ejaculation, including the medial preoptic area, the bed nucleus of the stria terminalis, the median amygdala and the posterior thalamus as well as SNB [19]. In addition, they can also regulate ejaculatory reflex acting at peripheral levels by modulating the integrated system NO-PDE5, involved in the contractility of the male genital tract [19]. Interestingly, our meta-analysis on the effect of TTh in placebo-controlled RCTs documented a positive effect of T also in ameliorating orgasmic function ([3]; see also Table 17.1). In addition, in line with what has been observed for libido and erectile function, meta-regression analysis documented an inverse relationship between baseline T levels and final outcomes [3].

Other Outcomes

T is important not only in controlling the mechanical process of penile erection but it also controls several other male sexual behaviors and attitudes. Figure 17.3 shows the age adjusted risk of hypogonadism according to EMAS criteria in a large series of subjects seeking medical care at our unit for sexual dysfunction.

Autoeroticism The practice of stimulating oneself sexuality is indeed androgen-dependent [56]. Accordingly, Fig. 17.3 confirms that masturbation is associated with a higher risk of hypogonadism.

Perceived ejaculate volume reduction As reported above, T is profoundly involved in the regulation of the growth and activity of male accessory glands, i.e., prostate and seminal vesicles, which contribute to more than 90% of ejaculatory volume [19]. Accordingly, we previously reported that the severity of the perceived ejaculate volume reduction (PEVR) was inversely related to T levels [57]. In line with these data we confirmed that hypogonadism represented a risk factor for PEVR (Fig. 17.3). Hence, hypogonadism can affect ejaculate volume interfering with either the production of the ejaculate bolus or its propulsion throughout the male genitalia tract (see above).

Unfaithfulness In line with other groups [57, 58] we confirm here that self-defined unfaithful men have a lower risk of hypogonadism ([59]; see also Fig. 17.3). It can be speculated that looking for additional partners, or the possibility of additional partners, is a competitive situation, which might be associated with higher T levels [60]. However, it is still unclear whether, in mating male individuals, T is higher to allow a better sexual and reproductive fitness (affecting libido/penile erections and/or spermatogenesis) or the reverse is true: sexual activity positively affects T production (see below).

Sexual Activity and Testosterone Levels

Some evidence suggests that sex is actually an excellent way to boost T levels. An often cited, single observation published in Nature almost 40 years ago [61] opened the possibility of this second scenario. An island resident observed an increase in beard growth on the day preceding, and during, his occasional visits to his mainland lover [61]. In 1992, Dabbs and Mohammed [62] evaluated salivary T concentrations in male and female members of four heterosexual couples on a total of 11 evenings before and after sexual intercourse and on 11 evenings on which there was no intercourse. They found that T levels increased on nights after sexual activity and did not on nights when there was no intercourse [62]. Accordingly, the anticipation of sex in animals increases T levels [63]. More recently, the group of Jannini robustly substantiated the hypothesis of a LH-mediated, sex-induced drive in T production [63–66]. In particular, they reported that the restoration of sexual activity in patients with ED ameliorated milder forms of hypogonadism. Interestingly, they showed that the T increase was independent from the kind of therapies employed, but strictly related to the successful outcome of therapeutic intervention. Hence, they speculated that sexual inertia resets the reproductive axis to a lower activity, somehow inducing a secondary hypogonadism, characterized by a reduced LH bioactivity [66]. Our data are in line with the latter hypothesis. We previously reported that the frequency of intercourse is directly associated with T levels [67, 68]. Accordingly, we here confirm that reduced frequency of sexual intercourse is associated with a higher risk of hypogonadism (Fig. 17.3). In addition, we found that the impairment of sexual activity due to relational complaints (as assessed by a higher score in Scale 2 of SIEDY structured interviewed) was associated with overt hypogonadism [67, 68]. Similar results were confirmed here in a larger sample of patients with sexual dysfunction (Fig. 17.3).

Conclusions

Testosterone plays a major role in regulating male sexual function. T replacement therapy is able to improve all aspects of male sexual function and should be considered the first line treatment in ED patients withovert hypogonadism. However, TTh as a mono-therapy might not be adequate in all cases because of the multifactorial nature of the pathophysiology of ED. In these cases a combination therapy with PDE5i may improve the outcome. In young uncomplicated individuals with milder forms of hypogonadism, the restoration of normal sexual function, however, obtained might improve T levels.

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Testosterone Therapy and Prostate Cancer

18

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Abbreviations

DHT	Dihydrotestosterone
EBRT	External beam radiation therapy
LH	Luteinizing hormone
LHRH	Luteinizing hormone-releasing hormone
PCa	Prostate cancer
PCPT	Prostate cancer prevention trial
PSA	Prostate-specific antigen
RP	Radical prostatectomy
SEER	Surveillance, epidemiology, and end results
T	Testosterone
TD	Testosterone deficiency

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Introduction

Testosterone deficiency (TD), also known as hypogonadism, is a clinical condition in which subnormal testosterone levels are associated with specific signs and symptoms that include decreased libido, erectile dysfunction, decreased sense of well-being, decreased muscle and bone mass, mood changes, and anemia, and it becomes more prevalent as men age [1]. It is estimated that TD affects 10% of men after 40 years of age, increasing to 50% after 70 years (defined by using a total testosterone less than 325 ng/dL). Although there is no consensus as to what threshold should be used to establish normal total testosterone concentrations, most clinical guidelines recommend a threshold in the range of 300–350 ng/dL [1, 2], with some experts advocating a threshold of 400 ng/dL in symptomatic men [3].

While the benefits of testosterone therapy are unequivocal in regard to improvement of the clinical signs and symptoms of TD, there remains a serious concern among the medical community that testosterone therapy may stimulate the emergence or progression of prostate cancer (PCa).

Clearly, PCa must be considered an androgen-dependent disease, at least in part. This is illustrated by the longstanding practice of treating men with advanced malignancy by androgen deprivation [2]. Although the actual effect of long-term T therapy on PCa risk remains incompletely established, so far the evidence fails to show significant prostate adverse effects. This calls into question long-held paradigms in the field.

Testosterone and Prostate Cancer

It is widely recognized that the presence of adequate serum levels of testosterone are necessary for the development of the prostate. Testosterone (T) can act directly on androgen receptors to exert their action or can be converted by 5-alpha-reductase into dihydrotestosterone (DHT) or to estradiol by the aromatase enzyme complex [1].

There are several reasons why it appeared logical to believe that testosterone therapy may stimulate prostate cancer. The prostate does not develop properly without androgen stimulation. Normal prostate glands undergo atrophy when serum androgens are greatly reduced via castration or administration of LHRH agonists. Furthermore, most prostate cancers are dependent on androgens in the early stages of progression and demonstrate regression with androgen ablation. Consequently, the presence of excessive androgenic stimulation as an etiologic factor in prostate carcinogenesis appears logical [1–5].

However, a number of studies have suggested that testosterone administration in supraphysiological doses to healthy men did not result in significant increase in prostate-specific antigen (PSA), nor prostate volume or urinary symptoms [6, 7]. PSA also does not seem be influenced by the circadian rhythm of testosterone levels [8].

A variety of studies in PCa, including experiments in animals, cell lines, and humans, indicate that prostate tissue (benign or malignant) responds quickly and vigorously to the addition of testosterone when it is in an androgen-deprived state. However, at higher concentrations, prostate tissue becomes unresponsive to this hormone [9].

The Testosterone-Prostate Dependence Myth

The concept that prostate cancer is androgen-dependent was established by the work of Charles Huggins in 1941, which demonstrated dramatic biochemical responses to castration in men with metastatic prostate cancer, earning him the Nobel Prize for Medicine in 1966. Orchiectomy rapidly became the first line treatment for men with advanced disease followed by the use of medical castration with LHRH agonists or antagonists when these agents became widely available in the 1980s. A small number of reports of adverse effects from T administration in castrated men reinforced the concept that prostate cancer was androgen-dependent, and that T administration was risky in men with PCa [9, 10]. Indeed, the transient initial increase in testosterone concentrations seen with LHRH agonists, called "testosterone flare," has been considered until the present time to be a concern with the use of these treatments, leading to strategies to add anti-androgens during the early phase of treatment to avoid adverse events due to cancer progression [5, 9].

This idea that testosterone behaved like "food for a hungry tumor" went unquestioned for decades and was termed the androgen hypothesis. The androgen hypothesis assumed a direct relationship between serum testosterone levels and risk of PCa, as well as rate of growth of existing PCa. The observation that malignant prostate tumors become increasingly prevalent as men age and experience a decline in serum testosterone levels was largely ignored as an inconvenient fact [1–10].

The androgen hypothesis only began to undergo scrutiny in the 1990s, with increasing awareness of the benefits of testosterone therapy and increased numbers of men receiving treatment. Since then, a large number of publications have shown physical and sexual improvements in hypogonadal men with T therapy. Surprisingly, these studies have failed to show increased PCa rates in subjects treated with testosterone compared with control groups or the general population or even in men considered as higher risk for the development of this neoplasia [11]. These observations have led to a reexamination of the relationship between testosterone and the prostate, especially PCa. Over the last 20 years we have witnessed a new era in the field of prostate physiology, carcinogenesis and its relationship with testosterone [5–8].

The Saturation Model

Endogenous serum testosterone appears to have limited effects on the prostate cells. Several studies suggest that testosterone stimulates the growth of PCa only at very low levels, and variations in the endogenous testosterone concentration within the

physiological range or above do not appear to influence prostate physiology. In other words, there appears to be a limited ability of androgens to stimulate prostate growth, or prostate cancer growth, with a maximum achieved at fairly low serum T concentrations. This concept of a finite stimulatory effect of androgens on prostate growth is called the saturation model. Multiple mechanisms may be contributory to the achievement of a maximal threshold effect; however, the most compelling is saturation of the androgen receptors in prostate cells [9]. The initial PSA elevation seen in hypogonadal men on T therapy is thus a physiologic response.

Monath et al. [8] investigated the correlation between serum levels of testosterone and PSA in 150 men without PCa, with a mean age of 60.1 years. The mean PSA was 2.0 ng/mL and the mean total testosterone 458 ng/dL. There was no correlation between serum testosterone and serum PSA concentrations ($r = 0.054$, $p = 0.515$). Similarly, The Massachusetts Male Aging Study [12], involving 1576 men, found no correlation between PSA levels and serum testosterone. Even exposure of men to supra physiological concentrations of T for periods up to 9 months failed to demonstrate a significant increase in either PSA levels or prostate volume [6].

These results convincingly demonstrate that variations in serum testosterone at physiological levels or above do not seem to influence the prostate, reinforcing the finite capacity of androgen receptors to bind to testosterone. However, at serum T concentrations below the saturation point, there is a relative shortage of testosterone or DHT, and in these cases the concentration of androgens becomes a limiting factor to prostatic tissue proliferation, whether malignant or benign. The administration of testosterone to these men will cause a rise in PSA, whereas administration of testosterone to men with baseline T levels above this point will result in minimal or no effect on the prostate (Fig. 18.1) [2, 9].

The saturation model explains the dramatic effect on PSA observed after reduction of testosterone to castrate levels as well as the lack of PSA response to treatment with supra physiologic T doses. Several studies have indicated the average saturation point to be approximately 250 ng/dL (8.7 nmol/L), although this is likely to vary from one individual to another. In the TriUS registry, men receiving T therapy showed a rise in PSA if their baseline total T was below 250 ng/dL, but not if it was higher than this value [13]. A similar result was obtained in a prospective,

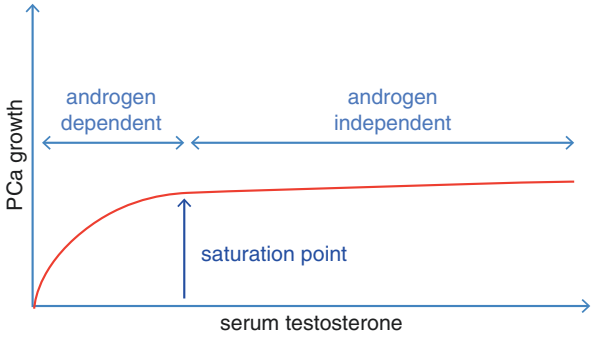

Fig. 18.1 The saturation model. Beyond a relatively low level of serum testosterone (the saturation point) all the androgen receptors are maximally bound to androgen, so there is no further ability of testosterone to stimulate prostate growth and PSA elevation

placebo-controlled 6-month study using 1.62% testosterone gel [14]. And in a study of 3156 men presenting to an Italian andrology center, PSA values showed a saturation curve identical to the theoretical one shown in Fig. 18.1, with a saturation point of approximately 250 ng/dL [15].

Other mechanisms may exist that contribute to the saturation effect of androgens on prostate tissue. Marks and colleagues [16] studied the effects of T therapy on prostate tissue of 44 men with androgen deficiency (68–70 years, assessed by questionnaire and serum total testosterone <300 ng/dL). The individuals were randomized to receive testosterone enanthate (150 mg intramuscularly every 2 weeks) or placebo. Interestingly intra-prostatic testosterone and DHT did not change, after 6 months of T therapy, despite the large increases in serum levels of these hormones. The prostatic hormonal milieu seems therefore differ significantly from the serum. In addition, there was no amplification of androgen-sensitive gene expression. These results suggest the prostate harbors some homeostatic mechanism regarding its hormonal milieu that is relatively insensitive to changes in serum androgen concentrations.

It is well recognized that eugonadal men present higher rates of PCa with aging, but only 20% express the disease clinically which demonstrates that testosterone by itself present a limited action in transforming histologic disease in a clinical condition. It is interesting also to observe that overall hypogonadal men over the age of 40, when undergoing prostate biopsy prior of entering on testosterone replacement therapy program, presented subclinical prostate cancer diagnoses in surprising 14%, and these rates increased to 29%, if only those aged of 60 or more years were considered. Although, the most interesting observation was that hypogonadal men receiving T therapy, however, had diagnosis of PCa in only about 1% when follow during the therapy, and not less interestingly is the fact that all other men who potentially had hidden prostate cancer did not develop clinically detectable disease based in the increase of serum levels of testosterone [13–17].

Endogenous Testosterone and Risk of Prostate Cancer

Historically, since the studies of Huggins et al. [10] demonstrating the effects of castration and regression of prostate cancer, there was a concern that testosterone may act as risk factor for the occurrence of such condition. Several theories tryed to explain the relantionship between testosterone and prostate cancer, but failed in demosntrating a consistent influence. Genetics, dietary, lifestyle, and hormonal factors certainly are involved in the development of prostate cancer. Serum testosterone does not have a direct relationship with prostatic tissue levels of such hormone. For years a number of studies have been conducted by different authors in different parts of the world trying to establish a causal relationship between serum testosterone levels and risk of PCa [17].

A study of pooled data from 18 prospective case-control studies assessed the relationship between serum hormone levels and the risk of PCa. This study involved 3886 men with PCa and 6438 men in control groups. The results of this study

showed no relationship between androgens, including total testosterone and DHT, and PCa risk. Specifically, men with the highest androgen concentrations were at no greater risk of developing PCa than men with the lowest androgen concentrations [18].

A meta-analysis of 25 retrospective studies comparing serum testosterone levels in men with and without PCa. In four of these studies men with PCa effectively exhibited higher serum levels of testosterone. However, in 15 of them testosterone levels were similar and interestingly in 6 of these, an inverse relationship was observed [19].

Testosterone Levels and Prognosis of Prostate Cancer

Contrary to longhand beliefs based on the androgen hypothesis, there is now a substantial body of evidence linking low serum T concentrations with poor prognostic factors for PCa [11, 20–23]. One recently published study showed that high-grade prostate tumors (Gleason pattern 4 and 5) were 2.4 times more frequently observed in men with lower T levels (OR: 2.4; 95% CI: 1.01–5.7) compared with men with normal serum testosterone [24].

Unfavorable prognosis of prostate cancer was also observed in a multicenter study published in 2003 by Massengill [25] including men undergoing radical prostatectomy. In this study, men with PCa at advanced stages showed lower mean serum levels of testosterone when compared with men with initial tumors. In addition, multivariate analysis showed subnormal levels of serum testosterone as independent predictors of disease at more advanced stage (pathologic stage T3 and T4). Positive surgical margins [26] as well as biochemical recurrence [27] after radical prostatectomy have also been more frequently observed in men with subnormal levels of testosterone.

In an interesting evaluation of 25 studies analyzing different stages of prostate cancer, the authors [28] observed that testosterone levels were not a prognostic factor of overall survival or biochemical recurrence. In advance disease higher levels of testosterone were associated with a better prognosis and reduced risk of death.

These findings have only recently gained appropriate attention from the scientific community, as the benefits of T therapy have become more established, and as evidence has mounted that the androgen hypothesis can no longer be seriously considered in light of so much contradictory evidence. Further study will be needed to definitively establish the biological relationship between serum T and PCa. However, it is clear that new perspectives are needed. In 1999, Prehn [29] suggested that prostate cells are prone to acquire characteristics that make them less androgen-sensitive in a low testosterone environment, and that tumors that develop under such hormonal conditions would be less differentiated and more susceptible to oncogenesis.

These data could possibly explain some findings of significant clinical trials such as PCPT (prostate cancer prevention trial), which evaluated the effect of finasteride, inhibitor of the enzyme 5-alpha-reductase, in the prevention of prostate cancer. Although its use reduced by 25% the overall rate of prostate cancer, tumors

diagnosed in the group treated with this drug had more unfavorable histological features [30].

Prostate Cancer in Men Receiving Testosterone Therapy

The incidence of PCa in men receiving T therapy has been demonstrated to be similar to that expected in general population. Mean PSA usually rises into the normal range in the first months of testosterone therapy and then stabilizes. As noted above, the rise in PSA is seen in groups of men with baseline T concentrations below 250 ng/dL, but not in men with less severe degrees of T deficiency [11].

In 2004 Rhoden and Morgentaler [17] published data showing that the PCa detection rate in patients undergoing clinical trials of T therapy was no higher than in individuals in placebo group. Specifically, only 5 (1.1%) cases of prostate cancer were observed among 461 men treated with hormone replacement testosterone when followed for 6–36 months. The cited above meta-analysis also found no greater increased risk of PCa in men receiving testosterone compared to men who received placebo.

More recently a systematic [31] review with strict inclusion criteria selected 11 studies involving men in T therapy placebo-controlled, showing that only 7 (1.3%) of 543 men in the treatment group developed prostate cancer while in the control group this event occurred in 5 (1.5%) of 333 men. These rates are low, considering that these individuals were followed continuously and more detailed than is usually performed in men in screening or routine evaluation programs for early diagnosis of prostate cancer.

Recently, Baillargeon et al. using SEER (surveillance, epidemiology, and end results)—Medicare dataset, identified 52,579 men diagnosed with incidental prostate cancer who had a minimum of 5 year continuous enrollment in such program before the diagnosis of PCa [32]. In that period 574 men received testosterone therapy. One an elegant statistical analyses using logistic regression for demographic and clinical characteristics, previous exposition of T therapy was not associated with increased risk of high grade prostate cancer (OR: 0.84, 95% CI: 0.67–1.05). Also, high-risk disease did not increase according to the total number of T injections (OR: 1.00, 95% CI: 0.98–1.01).

In a Canadian Institute for Health Information (CIHI) Discharge Database population-based matched cohort study of men aged 66 years or older newly treated with TRT and controls matched for age, region of residence, comorbidity, diabetes status, a cumulative effect of testosterone supplementation and prostate incidence was evaluated. This study evaluated 10,311 men treated with TRT and 28,029 controls over a period of 5 years. Interesting observation was the fact that the diagnosis of PCa diagnosis was decreased for those with the highest tertial of exposure (HR 0.60, 95% CI 0.45–0.80) compared with controls, but not for those with the shortest exposure [33].

In the testosterone trials, a highly anticipated multicenter randomized controlled trial, 790 men 65 years of age or older with a serum testosterone concentration of

less than 275 ng per deciliter and symptoms received either testosterone gel or placebo gel for 1 year. Only one case of prostate cancer was detected in men receiving testosterone. In the T4DM trial, a multicenter Australian trial in which 1007 men aged 50–74 years with abnormal oral glucose tolerance test were randomized to testosterone undecanoate injections or placebo injections for 2 years, 5 men in the placebo group and 4 men in the testosterone group developed prostate cancer [34].

Testosterone Therapy After Radical Prostatectomy

There is an increasing number of studies demonstrating that hypogonadal men previously treated for prostate cancer and no signs of recurrence may be candidates for T therapy.

The first study in this context was published by Kaufman and Graydon [35] in 2004, including seven patients treated for localized prostate neoplasia by radical prostatectomy (RP). Although all men presented favorable prognosis of cancer, no recurrence signal was observed during follow-up ranging from 1 to 12 years. The number of patients in this study was small, but the contribution in the development of understanding the relationship between testosterone and PCa was extremely relevant.

Perhaps one of the most relevant contributions of the literature in this context is the article published by Shabsigh [31], where the authors assessed biochemical recurrence in hypogonadal men previously treated for prostate cancer. In 111 men treated either by radiotherapy (EBRT—*external beam radiation therapy*) or radical prostatectomy, biochemical recurrence of the tumor in individuals in T therapy occurred in only 2 (1.8%) cases, a rate below the rates that are frequently observed in the majority of series involving RP results. However, one should take into consideration that this selected population is certainly different from most series that assess biochemical recurrence in men undergoing treatment with curative intent for PCa.

Similarly, a study conducted by Agarwal and Oefelein [36] included ten men with TD previously undergone retropubic radical prostatectomy and without biochemical recurrence signals. Most patients had PCa with favorable prognosis, but a man with Gleason score 8 was included. The mean duration of T therapy was 19 months and no case of biochemical recurrence was observed in the follow-up period in spite of serum total testosterone levels had categorical and sustained increase, from an average of 197 (95% CI: 145–248) to 591 ng/dL (95% CI: 469–713 the) after androgen supplementation.

In 2009, Khera and colleagues also published retrospective study of T therapy after RP. Fifty-seven patients with undetectable PSA and negative surgical margins received testosterone for a median of 36 months (1–136 months). The mean values of T increased from 255 to 459 ng/dL ($p < 0.001$). There was no increase in PSA levels after T administration and no diagnostic of biochemical recurrence, allowing the authors to conclude that the T therapy proved to be safe in patients treated for PCa [37].

A more recent study of men with PCa submitted to radical prostatectomy and observed for a median follow-up of 27.5 months included 103 men with TD treated

with transdermal testosterone that were compared with 49 eugonadal controls. A significant increase in PSA was observed in only individuals of the treatment group, but biochemical recurrence was defined in four patients of the treatment group and eight cases of the control group [38]. Several other studies [39–49, 57] are summarized in Table 18.1 demonstrating low rates of biochemical recurrence in men who were submitted to prostate cancer treatment and subsequently treated with TRT.

Table 18.1 Studies reporting TRT in patients with PCa

Author, year	PCa treatment	N of patients	Follow-up (months)	Variation of mean testosterone (ng/dL)	Variation of mean PSA (ng/mL)	PCa recurrence
Kaufman, 2004	RP	7	24	97–434	<0.1 to <0.1	0
Agarwal, 2005	RP	10	19	197–591	<0.1 to <0.1	0
Sarosdy, 2007	BT	31	54	188–498	5.3 to <1.0	0
Khera, 2009	RP	57	36	255–459	<0.1 to <0.1	0
Morales, 2009	EBRT	5	14.6	150–418	0.3–0.47	0
Morgentaler, 2011	AS	13	30	238–664	5.5–3.6	0
Pastuszak, 2013	BT, EBRT	13	45.6	178–368	0.3–0.66	0
Pastuszak, 2013	RP	103	27.5	261–460.5	0.004–0.007	4
Balbontin, 2014	BT	20	31	343–587	0.7–0.1	0
Pastuszak, 2015	BT, EBRT	98	40.8	209–420	0.075–0.09	6
Nabulsi, 2008	RP	22	12	228–427	NA	1
Davila, 2008	RP	14	12	291–630	0.1–0.1	0
Sathyamoorthy, 2010	RP	133	12	262–418	0.003–0.01	0
Isbarn, 2010	RP	69	19	NA	NA	0
Matsushita, 2012	RP	61	26	239–691	NA	1
Wynia, 2014	RP	57	24	265–550	0.003–0.0019	(1.8% TRT and 14.8% control group)
Kacker, 2014	RP	53	30	NA	NA	0
Ory, 2016	RP	22	41	190–383	0.0–0.0	0
Morgentaler, 2018	RP	92	19	NA	NA	6 (6.5%)
Ahlering, 2020	RP	152	40.8	NA	NA	11 (7.2% TRT and 12.6% control group)

BT brachytherapy, *EBRT* external beam radiation therapy, *RP* radical prostatectomy, *AS* active surveillance, *NA* not available, *TRT* testosterone replacement therapy

An interesting evaluation was performed by Kaplan and Hu [50] using SEER data to identify 149,354 men diagnosed with PCa. Of those group of men 2237 (1.5%) received T therapy before the diagnosis of PCa, although there was no association with aggressive PCa and no influence of overall or disease-specific mortality when individuals treated with testosterone were compared with those who did not receive such therapy, supporting the growing evidence that T supplementation is safe with respect to PCa.

Testosterone Therapy After Radiation Therapy

The contemplation of androgen supplementation in patients treated by non-surgical methods with curative intent (radiotherapy and brachytherapy) seems even more complex and its prescription may appear more risky than after RP. As the gland is not removed, viable prostate tissue might remain biologically responsive to testosterone. The unknown status of lymph nodes and the absence of pathology data of the whole gland generate uncertainty about the time after treatment and serum PSA levels that ensure control of neoplasia and safety to start T therapy.

The first study that reported T administration after treatment of prostate cancer with brachytherapy was published by Sarosdy [51] and comprised a series of 31 men with serum testosterone levels ranging from 30 to 255 ng/dL and symptoms consistent with TD who have received exogenous testosterone for 0.5–4.5 years after radiation therapy. This study aimed to assess the risk of biochemical recurrence of PCa. The median serum PSA level was 5.3 ng/mL previous radiation, the most common Gleason score was 6 (19 of 31 men—61.3%) and the most frequent clinical stage was T1c (20 of 31 patients—64.5%). The temporary androgen blockage was used for 8–12 months in 14 patients with high-risk disease. The latest PSA was <0.1 ng/mL in 23 patients (74.2%), <0.5 ng/mL in 30 patients (96.7%), and <1 ng/mL in 31 patients (100%). No patient discontinued T therapy because of biochemical recurrence or clinical progression. The authors concluded that testosterone can be safely administrated in men with TD who underwent brachytherapy as a treatment of PCa located but close monitoring should be provided.

More recently, another study was published including 20 men treated with brachytherapy for PCa cancer who received T therapy for symptomatic testosterone deficiency, showing significant clinical improvement and no cases of rising PSA and cancer recurrence [52].

Morales and colleagues [53] described five men with signs and symptoms of TD after treatment for localized PCa with external beam radiation therapy (EBRT) who received testosterone after PSA nadir had been achieved. All men had histological confirmation of PCa without evidence of locally advanced or distant metastasis disease. The mean Gleason score was 7 (6–8), the mean pre-EBRT PSA was 12.8 (3.8–28), and the mean pre-T therapy PSA was 0.3 ng/mL (<0.1 the 0.97). The duration of follow-up during the T supplementation was 14.6 months (6–27). Mean testosterone levels before T therapy was 5.2 nmol/L (1.1–9.2); at the last visit the levels were 17.6 nmol/L (8.5–32.4). One of the five patients experienced a transient

increase in PSA, but none showed higher level to 1.5 ng/mL. All patients reported significant improvement in clinical symptoms of TD and no cases of clinical recurrence or biochemical PCa was detected in the period of follow-up.

Pastuszak and colleagues also reported their experience with 13 men with testosterone deficiency who received testosterone after EBRT or brachytherapy for PCa for a median follow-up of 29.7 months, with improvement in hypogonadal symptoms and total T levels without significant increase in serum PSA and no diagnosis of PCa recurrence [54].

A recent multi-institutional study including 98 TD men treated for PCa with radiotherapy showed a significant increase in serum testosterone after T therapy (209–420 ng/dL, $p < 0.001$), but no change in PSA for low- and intermediate-risk PCa groups. However, PSA significantly increased in high-risk PCa patients (0.1–0.36 ng/mL, $p = 0.018$). The mode of treatment (EBRT, brachytherapy or combined) did not influence on endpoints [55].

In a meta-analysis published in 2010 which included 51 randomized controlled trials and assessed mortality, cardiovascular events, prostatic events, and erythrocytosis in patients undergoing T therapy, also no significant differences were observed regarding the incidence of PCa, the need for prostate biopsy, increased PSA or change in symptoms related to lower urinary tract compared intervention and control groups [56].

More recently, a retrospective study evaluated 40 men with PCa treated with either external radiotherapy or brachytherapy who received testosterone therapy, and in a follow-up of 26.7 months a rate of 6.7% of biochemical recurrence was noticed. Even with no Gleason score evaluated in the study the authors believe that the rates of recurrence were not increased with testosterone supplementation [57].

Studies involving PCa and testosterone have an obvious limitation which certainly limits the validity of the data for all patients treated for the disease, such as the small number of patients and selection of men with localized and low volume disease, and low to moderate histologic grade.

The majority of the studies recommended a close monitoring performing digital rectal examinations, hematocrit, and PSA. European Association of Urology (EAU) and American Association of Urology (AUA) recommend a 3, 6, and 12 months evaluation after starting TRT and 6–12 months thereafter [58].

Testosterone Therapy in Men with Untreated Prostate Cancer

With increasing recognition that men with low-grade PCa are at low risk for morbidity and mortality, there is a growing practice of deferring treatment until there is evidence of more aggressive pathologies. This practice is called active surveillance, and these men generally undergo regular PSA testing and follow-up prostate biopsies at regular intervals. Some of these men have symptomatic T deficiency and desire treatment. The use of T therapy in these men is highly controversial.

In a study from Morgentaler [59] reported on a 2-year history of testosterone therapy for sexual symptoms in an 84-year-old man on active surveillance for Gleason 6 PCa. His PSA was greater than 8 ng/mL at the time of diagnosis. Over 2

years, his PSA declined into the 6's. He was never biopsied again due to his age, however, he remained on T therapy without a significant rise in PSA for a total of 6 years, until he developed dementia at 90 years. Subsequently, Morgentaler et al. [60] reported on 13 patients with symptomatic testosterone deficiency who received testosterone for at least 12 months after the diagnosis of the neoplasia. The average duration of T therapy was 3.1 years. There was no significant increase in mean PSA or prostate volume in these men. All patients underwent at least one more prostate biopsy, with a mean of two sets of biopsies per individual. No definite cancer progression was noted in any patient. No cancer was found in 54% of follow-up biopsies in these men. All patients showed significant improvement in libido, sexual performance, humor and energy.

A more recent study investigated rates of progression during active surveillance in 28 men who received T therapy compared with 96 men who did not receive T therapy despite similarly low levels of serum T. Rates of progression were no different in these two groups [61].

Testosterone Therapy in High-Risk Prostate Cancer

In 2013 a retrospective review involving 103 hypogonadal men treated with testosterone after radical prostatectomy between 2007 and 2011 was published. Twenty-six of them present high-risk disease (Gleason score greater than 8, positive surgical margins or positive lymph nodes). During an average follow-up of 27.5 months, a significant increase in PSA was observed only in this high-risk group (0.004–0.14 ng/mL, $p = 0.017$), but no patient met criteria for biochemical recurrence [62].

The authors concluded that T therapy is viable treatment for patients treated for PCa, even when the disease has high-risk characteristics, since the incidence of recurrence in these groups proved to be much smaller than expected.

Testosterone Therapy in Advanced Disease and Bipolar Androgen Therapy

For eight decades androgen deprivation therapy (ADT) has been the standard treatment for men with advanced prostate cancer [63, 64]. Yet it is well recognized that eventually a majority of men develop castrate-resistant prostate cancer after ADT, usually associated with more rapid disease progression. Several theories have been proposed to explain this process involving molecular biology and also androgen receptor activity [65, 66]. Surprisingly, administration of high-dose testosterone alternating with castrate testosterone levels in men with castrate-resistant prostate cancer has been shown to restore some androgen sensitivity in some men [64]. Another study suggested that prolonged exposure of prostate cancer cells to castrate levels of testosterone increased the number of androgen receptors and this was suggested to contribute to androgen resistance.

Administration of testosterone in high doses may cause apoptosis of prostate cancer cells [67]. However, it is also evident that ADT causes several bothersome

and deleterious effects for men, including increased fat, decreased muscle mass and strength, impaired mood, bone mineral loss, and reduced libido and sexual activity. Normalizing testosterone levels may improve these symptoms and signs, with beneficial effects in quality of life [63].

Schweizer et al. [68] treated 16 asymptomatic metastatic castration resistant prostate cancer with bipolar androgen therapy (BAT) consisting of 4 week cycles of a high-dose (400 mg) injection of testosterone cypionate in men on LHRH agonists and also receiving etoposide. This produced alternating periods of supraphysiologic and then castrate levels of serum testosterone. The investigators observed a significant response in decreasing PSA levels as well as improving radiographic appearance of evaluable disease. The BATMAN study [68] included men with prostate cancer androgen sensitive, who received during 6 months with ADT and levels less than 4 ng/dL received testosterone administration in three cycles. Seventeen out of 29 cases presented a reduction in 50% of the levels of PSA. The possibility of administration of other drugs associated to bipolar testosterone administration such as enzalutamide was explored by other study with interesting results such as reduction of more than 50% of the levels of PSA levels in majority of the cases [69].

Morgentaler [64] published a variety of testosterone therapy approaches in 3 men with metastatic prostate cancer, including continuous testosterone injections, testosterone combined with enzalutamide, and a modified BAT protocol termed mBAT consisting of 8 weeks of high-dose injections of testosterone cypionate followed by 4 weeks of enzalutamide, a potent androgen blocker [46, 57]. In a subsequent series, Morgentaler and colleagues reported on the results of testosterone therapy in 20 men with advanced prostate cancer, consisting of 7 men with biochemical recurrence (BCR) and 13 with metastatic disease [63]. Eight of the metastatic group were treated with at least one cycle of mBAT. Mean duration of testosterone therapy was 33 months in the BCR group and 10 months in the metastatic group. There was only one prostate cancer-specific death. All of these men experienced improvements in quality of life.

These small studies with promising results augur a new era in the field of testosterone therapy and prostate cancer, deserving large-scale clinical and randomized studies to assess the benefits and risks of this approach. However, it is already clear that the overly simplistic notion taught for generations that testosterone is highly dangerous for men with known or suspected prostate cancer requires serious re-evaluation.

Conclusions

Testosterone therapy is highly effective in controlling symptoms of hypogonadism and improving quality of life. Recent clinical experiences in men with PCa have suggested that T therapy is not as risky as once believed. The actual risk of developing prostate cancer in men receiving testosterone is, however, unknown. The current scientific evidence comes from clinical studies that included few hundred men, and these studies were not designed to establish the risk of developing PCa. It is estimated that approximately 6000 randomized men be required to receive testosterone

or placebo for 5 years to assess whether the testosterone increases the incidence of PCa by 30%. None of the cited randomized placebo-controlled trials postulated prostate biopsies at the beginning and the end of the study. Thus, the prevalence and incidence of occult prostate carcinoma have not been accordingly studied.

Although the available information is coming from small clinical studies, data demonstrate the safety of T therapy after treated PCa, even in men with high-risk prostate cancer, leading to an increase in its prescription, which in turn results in the production of even more scientific evidence supporting testosterone replacement as safe for such patients.

Still, the emergence of publications on active surveillance in PCa surely bring important contributions to the biological behavior of this neoplasm forward to T therapy, since the follow-up protocols include prostate biopsies at regular intervals. Although, before definitive conclusions, individuals who meet criteria for inclusion in T therapy programs should maintain strict monitoring with regard to prostate evaluation by medical history, measurement of PSA, and digital rectal examination.

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Testosterone and Cardiovascular Effects

19

Bu B. Yeap

Abbreviations

ARIC	Atherosclerosis Risk in Communities Study
BHS	Busselton Health Survey
BMI	Body Mass Index
CHD	Coronary heart disease
CHS	Cardiovascular Health Study
CI	Confidence interval
CVD	Cardiovascular disease
DHT	Dihydrotestosterone
E2	Estradiol
EMAS	European Male Ageing Study
GC	Gas chromatography
HIMS	Health In Men Study
HPT	Hypothalamic-pituitary-testicular
HR	Hazard ratio
IHD	Ischaemic heart disease
LC	Liquid chromatography
LH	Luteinising hormone
LTL	Leucocyte telomere length
MACE	Major cardiovascular adverse events
MI	Myocardial infarction
MR	Mendelian randomisation
MrOS	Osteoporotic fractures in men
MS	Mass spectrometry

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Q	Quartile
RCT	Randomised controlled trial
SHBG	Sex hormone-binding globulin
SNP	Single nucleotide polymorphism
T	Testosterone
T4DM	Testosterone for the Prevention of Type 2 Diabetes Mellitus
TRAVERSE	Testosterone Replacement therapy for Assessment of long-term Vascular Events and efficacy ResponSE in hypogonadal men

Introduction

Demographic change is reshaping population structures, with an increasing proportion of older adults found in countries around the world [1]. This has important implications as the incidence of cardiovascular disease (CVD) manifesting as myocardial infarction (MI) or stroke increases with age [2]. A characteristic of male ageing is a decline in circulating testosterone (T) [3, 4]. Thus advancing age is associated with both lower circulating T and increasing manifestations of CVD, raising the question as to whether reduced exposure to T is a biomarker or risk factor for CVD in the increasing population of older men [5]. This chapter examines, firstly, the epidemiological evidence that associates endogenous T concentrations with incidence of cardiovascular events and mortality in community-dwelling men. Secondly, the results of key randomised controlled trials of T treatment in men and meta-analyses of T RCTs which reported cardiovascular adverse events. Thirdly, the results of retrospective case-control studies of men who were prescribed T treatment compared to those who were not, in relation to risk of CVD events and mortality. Finally, there is a brief discussion of Mendelian randomisation studies of T which have examined cardiovascular disease as an outcome, followed by conclusions as to the effect of T on cardiovascular risk in men.

Testosterone and Male Ageing

T is produced by the testis under the stimulation of pituitary luteinising hormone (LH) and circulates largely bound to sex hormone-binding globulin (SHBG) and albumin, with a small fraction unbound or free [6]. T undergoes conversion by 5α-reductase to dihydrotestosterone (DHT), a more potent ligand for the androgen receptor, and by aromatase to estradiol (E2) a ligand for oestrogen receptors [7]. Therefore, biological actions of T derive from the function of the hypothalamic-pituitary-testicular axis, circulation of T and its conversion to bioactive metabolites DHT and E2, and tissue effects which regulate male sexual development, virilisation, and body composition in adult men [5]. The presence of obesity or accumulation of morbidities can be reflected in lower T concentrations [8–11]. However, even healthy older men have lower circulating T compared to reproductively normal

younger men [12, 13]. In 124 healthy, reproductively normal men aged 21–35 years the reference interval for T assayed using mass spectrometry was 10.4–30.1 nmol/L [12]. By contrast, in 394 men aged 70–89 years who reported excellent or very good health with no history of smoking, diabetes, cardiovascular disease (CVD), cancer, depression, or dementia, the reference interval for plasma T assayed using mass spectrometry was 6.4–25.6 nmol/L [13]. Of note, circulating concentrations of DHT in men parallel those of T declining with increasing age, while E2 concentrations tend to be more stable [13–15]. The longitudinal decline in circulating androgens is accentuated in older men, where it is accompanied by an increase in LH, suggesting impairment of Leydig cell function [14, 16].

Sex Hormone Assays and Calculation of Free T

Circadian variation affects T concentrations, which are higher in the morning and lower in the evening [17, 18]. The diurnal variation in DHT and E2 is much less compared to T in men in both middle aged and older men [17]. Thus early morning sampling is optimal, and where possible in the fasting state [9, 19, 20]. Automated immunoassays of T tend to exhibit non-specificity and method-dependent bias [12, 21]. Therefore, mass spectrometry is the preferred assay methodology for accurate measurement of sex steroids [22, 23]. SHBG increases with age and is lower in the setting of insulin resistance and obesity, thus there are scenarios where consideration of unbound of free T may be informative on an individual level [24]. However, measurement of circulating free T by equilibrium dialysis is labour-intensive and not routinely performed, instead free T is commonly calculated using mass action or empirical equations [25, 26]. Depending on the method of calculation, calculated free T can vary from measured free T representing a potential limitation for its use [25].

Epidemiological Studies of T, DHT, E2, and Cardiovascular Events

Longitudinal cohort studies examining the association of sex hormones measured using immunoassays at baseline with the incidence of CVD events during follow-up are summarised (Table 19.1, part A). Three studies in predominantly middle-aged men did not find any association of T with incidence of CVD events [27, 28, 30]. Of studies measuring E2 one study found that higher E2 was associated with lower incidence of CVD events [28], but another study in older men associated higher E2 with increased risk of stroke [29]. In a large population-based cohort of older men, total or free T in the lowest quartile of values predicted an increased incidence of stroke or transient ischaemic attacks [31], while higher LH was associated with incidence of ischaemic heart disease (IHD) events [32]. A smaller study in older men found no association of baseline T or E2 with incident CVD events [33], but another study also in older men reported T in the lowest and highest quintiles to be

Table 19.1 Cohort studies examining associations between sex hormones with cardiovascular events in middle-aged and older men. *IHD* ischaemic heart disease, *CVD* cardiovascular disease, *MI* myocardial infarction, *TIA* transient ischaemic attack, *CHD* coronary heart disease, *RCT* randomised controlled trial, *MACE* major adverse cardiovascular event. A: Total T, DHT and E2 were measured by immunoassay; free or bioavailable T and free E2 were calculated. B: Total T, DHT, and E2 were measured by mass spectrometry

Study author and year	Size (*n* of men)	Follow-up (years)	Age (years)	Results
A				
Smith et al. (2005a) [27]	2512	16.5	45–59	482 deaths, 192 fatal and 128 non-fatal IHD events. Higher cortisol:T ratio associated with IHD deaths and IHD events in age-adjusted but not multivariable adjusted analyses
Arnlov et al. (2006) [28]	2084	10	56	386 had first cardiovascular event. Higher total E2 at baseline associated with lower incidence of CVD events. T not associated
Abbott et al. (2007) [29]	2197	≤7	71–93	124 had first stroke. Baseline E2 in top quintile (≥125 pmol/L) associated with higher risk, total T not associated
Vikan et al. (2009) [30]	1318	9.1	59.6	146 men had first ever MI. No association of total or free T or total E2 with incident MI
Yeap et al. (2009) [31]	3443	3.5	≥70	First stroke or TIA occurred in 119 men. Total and free T in the lowest quartiles (<11.7 nmol/L and <222 pmol/L) predicted increased incidence of stroke or TIA
Hyde et al. (2011) [32]	3637	5.1	70–88	618 men experienced IHD event. Higher LH associated with incident IHD
Haring et al. (2013) [33]	254	5, 10	75.5	No associations of baseline total T or total E2 with incident CVD events
Soisson et al. (2013) [34]	495; 146	4	>65	495 controls, 146 men with incident CHD or stroke. Total T in lowest and highest quintiles associated with CHD or stroke
Holmegard et al. (2016) [35]	4602	20	57	560 stroke events. Total T in lowest decile (0–10th percentile) associated with stroke
Wang et al. (2019) [36]	5553	6	63.5	Post-hoc analysis of RCT cohort of men with impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes randomised to insulin glargine or standard care. 1028 men experienced a CVD event. Neither total T nor cFT predicted CVD events, higher SHBG predicted CVD events
Hatami et al. (2020) [37]	816	12	46.1	121 experienced a CVD event. Total T was not associated with risk of CVD events
Zhao et al. (2020) [38]	4107	19.2	63.2	873 new cases of heart failure. Lower total T associated with increased risk of incident heart failure
Schafer et al. (2021) [39]	3865	13.8	48.2	319 incident heart failure cases. Lower total T did not predict incident heart failure

Table 19.1 (continued)

Study author and year	Size (*n* of men)	Follow-up (years)	Age (years)	Results
Yeap et al. (2022) [40]	210,700	9.0	58.0	8790 incident cardiovascular events. Total T was not associated with risk of incident MI, stroke, heart failure, or MACE. Lower SHBG was associated with higher risk of incident MI, but lower risk of heart failure
B				
Ohlsson et al. (2011) [41]	2416	5	69–81	485 CVD events. Men with total T[a] in highest quartile (≥19 mol/L) had lower risk of CVD event. E2 was not associated
Shores et al. (2014b) [42]	1032	9	76	436 men had a cardiovascular event. Total T[b] not associated with cardiovascular events, DHT <1.7 or >2.6 nmol/L associated
Shores et al. (2014a) [43]	1032	10	76	114 men had ischaemic stroke. Total T[b] not associated with stroke, DHT <1.7 or >2.6 nmol/L associated
Yeap et al. (2014b) [44]	3690	6.6	70–89	Incident MI occurred in 344 men, stroke in 300. T[c], DHT, and E2 not associated with MI. Higher total T (>12.6 nmol/L) or DHT (>1.34 nmol/L) associated with lower incidence of stroke
Srinath et al. (2015) [45]	1558	12.8	63.1	287 men had a CHD event. T[d] was not associated with incidence of CHD events
Chan et al. (2016) [46]	1804	14.9	50.3	399 men experienced CVD events. Total T[c], DHT, and E2 were not associated with CVD events
Srinath et al. (2016) [47]	1558	14.1	63.1	79 men experienced incident ischaemic stroke. T[d] was not associated with incident stroke
Gyawali et al. (2019) [48]	1492	4.9	54.2	101 men experienced an incident CVD event. Higher SHBG, or lower total T[c], was associated with higher risk of incident CVD events. E2 and DHT were not associated
Collet et al. (2020) [49]	552	7.4	≥65	137 men had at least one CVD event. Neither T[e] not E2 was associated with incident CVD events

[a] T and E2 assayed using gas chromatography-mass spectrometry (GC-MS)
[b] T and DHT assayed using liquid chromatography-tandem mass spectrometry (LC-MS)
[c] T, DHT, and E2 assayed by LC-MS
[d] T assayed using LC-MS
[e] T and E2 assayed using LC-MS

associated with CVD events [34] suggesting a U-shaped association. More recent studies did not find an association of total or calculated free T with risk of incident CVD events, including one study of men with impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes [36, 37]. Two further studies reported contrasting results: one finding an association of lower total T with higher risk of incident heart failure [38], whereas another study found no association of total T with risk of incident heart failure [39].

In the largest prospective cohort study to date, the United Kingdom (UK) Biobank involving over 200,000 men aged 40–69 years with more than 8000

incident outcome events, serum total T measured by immunoassay was not associated with incidence of myocardial infarction (MI), haemorrhagic stroke, ischaemic stroke, heart failure, or major adverse cardiovascular events (MACE) [40]. The size of the UK Biobank and the number of incident outcome events indicate that this finding of a null association is likely to be robust, at least for a generally healthy population of predominantly middle-aged men of this ethnic background. Interestingly, in UK Biobank men, lower SHBG was associated with higher risk of incident MI, but lower risk of heart failure events [40]. The finding for SHBG is concordant with an earlier study associating higher SHBG with risk of CVD events [36].

Large cohort studies (albeit smaller than UK Biobank) where sex steroids were measured using mass spectrometry have been informative (Table 19.1, part B). In the Osteoporotic Fractures in Men (MrOS) Study the risk of experiencing a cardiovascular event was 30% lower in men with higher total T (highest quartile Q4, T ≥19 nmol/L vs. other men Q1-3, T <19 nmol/L: hazard ratio [HR] 0.70, 95% confidence interval [CI] 0.56–0.88) [41]. In the Cardiovascular Health Study (CHS) involved T was not associated with cardiovascular death, or non-fatal myocardial infarction or stroke, but DHT was associated with higher risk of cardiovascular events for concentrations <1.7 or >2.6 nmol/L [42]. DHT (<1.7 or >2.6 nmol/L) was also associated with ischaemic stroke when that outcome was analysed separately [43].

In an updated analysis from the Western Australian Health In Men Study (HIMS) T, DHT, and E2 were not associated with incident MI [44]. By contrast, higher T or DHT was associated with lower incidence of stroke. For men with T in Q4 (≥15.8 nmol/L) compared to Q1 (≤9.8 nmol/L), the risk stroke was almost halved (fully adjusted HR 0.56, 95% CI = 0.39–0.81). A similar result was found for DHT (Q4 ≥1.8 nmol/L vs. Q1 ≤0.9 nmol/L; full-adjusted HR 0.57, 95% CI 0.40–0.81) [44]. The results for calculated free T paralleled those for total T. E2 was not associated with stroke. In an analysis from the Atherosclerosis Risk in Communities Study (ARIC) lower T was associated with adverse cardiovascular risk factors, but not with incidence of CHD events [45]. Of note, the initial and extended studies identifying low T as an independent predictor for higher incidence of stroke in older men [31, 44] have subsequently been confirmed by the Copenhagen Study [35] (Table 19.1, part A). A more recent study reported associations of higher SHBG, or lower total T, with higher risk of incident CVD events consistent with [48]. However, another study found no association of total T nor E2 with risk of incident CVD events [49].

Several conclusions can be drawn from these studies. Cohort studies based on the use of immunoassays for sex steroids (Table 19.1, part A) provide limited evidence that T is associated with incidence of MI per se. In particular, the UK Biobank analysis found null associations of total T with incident CVD events in men aged 40–69 years [40]. However, there is some evidence particularly in men aged ≥70 years, for an association of low total or free T with incidence of stroke and transient ischaemic attack [31, 35].

Considering cohort studies where sex steroids were measured accurately using mass spectrometry-based methods (Table 19.1, part B), several studies found no association of lower T with coronary or cardiovascular events [42, 45, 49]. The two

largest cohort studies which measured both T and E2 by mass spectrometry reported associations of low T but not E2 with CVD events in MrOS [41], and stroke in HIMS [44], both cohorts of older men. These findings were supported by a study in middle- to older-aged men [48]. Therefore, lower circulating T may be a biomarker for CVD risk in older men, particularly for an increased incidence of stroke which may drive associations of lower T with CVD events in general. An age differential may be present: in younger and middle-aged men lower T is associated with adverse cardiovascular risk factors (e.g. [45, 50]) rather than incidence of CVD, while in older men lower T or DHT is associated with increased incidence of CVD manifesting as stroke more prominently than MI [41–44].

Epidemiological Studies of T, DHT, E2, and Mortality

Longitudinal cohort studies examining the association of baseline sex hormones measured using immunoassay with the outcome of mortality are summarised (Table 19.2, part A). Cohort and case-control studies have reported associations of lower T with higher mortality [30, 51, 52, 55, 57–59]. Several studies have reported contrasting or equivocal results, or implicated other anabolic hormones in addition to T [33, 53, 54]. Two more recent studies provide a degree of clarity. In one study of men with impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes, neither total T nor calculated free T was associated with mortality risk, whereas higher SHBG was associated with all-cause mortality [36]. In an analysis from the UK Biobank cohort involving ~150,000 men aged 40–69 years in which 10,000 deaths occurred, men with lower total or calculated free T had higher mortality from any cause and from cancer, but there was no association of total or calculated free T with CVD-related mortality risk [60]. Of note, in that study, lower SHBG was associated with lower all-cause, CVD-related, and cancer-related mortality risk [60].

The relationship of E2 to mortality risk in men cannot be clearly defined with contrasting findings reported [56, 57]. Overall, these studies implicate lower T as a biomarker for mortality risk, albeit the studies are heterogeneous and causality remains to be proven [65]. While low T might predispose to dying, it is also possible that underlying ill-health could result in both low T and increased mortality risk. Interestingly, in two studies, one of which was the large UK Biobank cohort, lower SHBG was associated with lower mortality in men [36, 60].

Cohort studies in which the relationship between baseline sex steroids assayed using mass spectrometry and the outcome of mortality were studied are summarised (Table 19.2, part B). In the analysis from MrOS compared to men with total T in Q1 (≤11.7 nmol/L), those with T in higher quartiles had lower risk of dying from any cause (Q2 11.7–15.2 nmol/L, HR 0.71, 95% CI 0.53–0.96; Q3 15.2–19.2 nmol/L, HR 0.55, 95% CI 0.39–0.76; Q4 ≥19.3 nmol/L 0.59, 95% CI = 0.42–0.83) [61]. No significant associations were seen for T or E2 with cardiovascular mortality. In the mortality analysis from HIMS, compared to men with total T Q1 (<9.8 nmol/L), those with total T in the middle two quartiles had lower all-cause mortality (Q2 9.8–12.5 nmol/L, HR 0.82, 95% CI 0.69–0.98; Q3 12.6–15.8 nmol/L, HR 0.78,

Table 19.2 Cohort studies examining associations between sex hormones and mortality in middle-aged and older men. *IHD* ischaemic heart disease, *CVD* cardiovascular disease, *CHD* coronary heart disease. A: Total T, DHT, and E2 were measured by immunoassay; free T and free E2 were calculated. B: Total T, DHT, and E2 were measured by mass spectrometry; free T was calculated

Study author and year	Size (*n* of men)	Follow-up (years)	Age (years)	Results
A				
Shores et al. (2006) [51]	858	4.3	≥40	208 deaths. Men with two or more low T levels (total T <8.7 nmol/L or free T <0.03 nmol/L) had higher mortality
Khaw et al. (2007) [52]	825 and 1489	≤10	40–79	825 deaths, 1489 controls. Total T inversely related to mortality from all causes, CVD, and cancer
Araujo et al. (2007) [53]	1686	15.3	40–70	395 deaths. Higher free T associated with higher IHD mortality. Equivocal association of lower DHT with IHD mortality
Maggio et al. (2007) [54]	410	6	≥65	126 deaths. Combination of bioavailable T, insulin-like growth factor-I, and dehydroepiandrosterone sulphate in lowest quartiles associated with higher mortality
Laughlin et al. (2008) [55]	794	11.8	50–91	538 deaths. Total T in the lowest quartile (<8.4 nmol/L) predicted increased mortality from all causes and from CVD and respiratory causes
Vikan et al. (2009) [30]	1568	≤13	59.6	395 deaths (130 from CVD and 80 from IHD). Free T in the lowest quartile (<158 pmol/L) predicted higher overall mortality, total T not associated
Szulc et al. (2009) [56]	782	10	≥50	Higher total E2 predicted increased mortality after the third year
Menke et al. (2010) [57]	1114	18	≥20	103 deaths, 42 from CVD. Difference between 90th and 10th percentiles for free T associated with overall and CVD mortality in first 9 years of follow-up. Difference for total E2 associated with CVD mortality
Haring et al. (2010) [58]	1954	7.2	20–79	195 deaths. Total T <8.7 nmol/L associated with increased all-cause and CVD mortality and cancer death
Hyde et al. (2012) [59]	3637	5.1	70–88	605 deaths, 207 from CVD. Lower free T (100 vs. 280 pmol/L) predicted all-cause and CVD mortality
Haring et al. (2013) [33]	254	5, 10	75.5	Higher baseline total T associated with lower 5 year but not 10 year mortality risk. E2 not associated
Wang et al. (2019) [36]	5553	6	63.5	Post-hoc analysis of RCT cohort of men with impaired fasting glucose, impaired glucose tolerance, or type 2 diabetes randomised to insulin glargine or standard care. 951 men died. Neither total T nor cFT predicted mortality, higher SHBG predicted all-cause mortality

Table 19.2 (continued)

Study author and year	Size (*n* of men)	Follow-up (years)	Age (years)	Results
Yeap et al. (2021b) [60]	149,436	11.3	58.0	10,053 deaths. Men with lower total T had higher all-cause and cancer-related mortality. Men with lower SHBG had lower all-cause, CVD-related, and cancer-related mortality
B				
Tivesten et al. (2009) [61]	3014	4.5	75	383 deaths. Total T[a] and E2 levels in the lowest quartiles predicted mortality. Risk of death nearly doubled in men with low levels of both total T and E2
Yeap et al. (2014a) [62]	3690	7.1	70–89	974 deaths, 325 from IHD. Optimal total T[b] (9.8–15.8 nmol/L) predicted lower all-cause mortality. Higher DHT (>1.3 nmol/L) predicted lower IHD mortality. E2 was not associated with mortality
Pye et al. (2014) [63]	2599	4.3	40–79	147 deaths. Presence of sexual symptoms, total T[c] <8 nmol/L, and free T <220 pmol/L associated with mortality
Shores et al. (2014b) [42]	1032	9	76	777 deaths. Total T[d] not associated with mortality, DHT <1.0 nmol/L was associated
Srinath et al. (2015) [45]	1558	12.8	63.1	347 deaths, 29 from CHD. Total T[c] not associated with all-cause or CHD mortality
Chan et al. (2016) [46]	1804	14.9	50.3	319 deaths, 141 from CVD. Total T[c], DHT, and E2 were not associated with all-cause or CVD mortality
Boden et al. (2020) [64]	2118	3	≥40	Post-hoc analysis of RCT cohort of men randomised to niacin or placebo plus simvastatin. 643 men with total T <10.4 nmol/L had higher risk of combined endpoint of CHD death, MI, or stroke, compared with 1475 men with total T ≥10.4 nmol/L

[a] T and E2 measured using gas chromatography-mass spectrometry (GC-MS)
[b] T, DHT, and E2 measured using liquid chromatography-tandem mass spectrometry (LC-MS)
[c] T measured using GC-MS
[d] T and DHT assayed using LC-MS
[e] T assayed using LC-MS

95% CI 0.65–0.94), with no difference seen in mortality for men with total T in Q4 [62]. Similarly, mid-range DHT was associated with lower all-cause mortality (DHT Q3 1.3–1.8 nmol/L, HR = 0.76, 95% CI 0.63–0.91). Of note, higher DHT was associated with lower mortality from IHD (compared to DHT in Q1 ≤0.9 nmol/L: Q3 1.3–1.8 nmol/L, HR 0.58, 95% CI 0.42–0.82; Q4 >1.8 nmol/L, HR 0.69, 95% CI 0.50–0.96) [62].

In the European Male Ageing Study (EMAS) there was no significant association of quintiles of either total or free T with all-cause or CVD-related mortality, instead men with sexual symptoms and T <8 nmol/L had increased risk of death from any cause [63]. In an analysis from the CHS, neither total nor free T was associated with all-cause or CVD-related mortality, although a curvilinear association of

DHT with all-cause mortality was noted [42]. A study from the Busselton cohort of men spanning younger, middle, and older ages found no association of total T, DHT, or E2 with all-cause or CVD mortality [46]. By contrast, in a study of men aged ≥40 years who had been randomised to niacin or placebo plus simvastatin, men with total T <10.4 nmol/L had a higher risk of the composite outcome of CHD death, MI, or stroke, compared with men with total T ≥10.4 nmol/L [64].

MrOS and HIMS have provided significant and generally concordant results, which contrast to an extent with the findings of EMAS and CHS. EMAS spans a larger age range with more middle-aged men and shorter follow-up, thus has fewer outcome events ($N = 147$) which likely reduced the statistical power of the proportional hazards regression to detect associations of baseline T with mortality [63]. The ARIC study had only 29 cases of deaths due to CHD [45]. The CHS study, while smaller, had longer follow-up and accumulated a large number of outcome events which paralleled marked attrition of the cohort as a whole (777 deaths in 1032 men, 75% cumulative mortality) [43]. It is possible that with extended follow-up, attrition of a cohort through advancing age, or "drift" of biochemical variables away from the initial baseline value, might impair the predictive utility of baseline hormones for outcomes of interest. MrOS and HIMS are large studies in older men, with sufficient outcome events during defined periods of follow-up to enable robust longitudinal analyses using proportional hazards regression for the outcome of death from any cause ($N = 383$ and $N = 974$, respectively) [61, 62], and in the case of HIMS, for the outcome of IHD mortality ($N = 325$) [62]. Comprehensive adjustments were made for factors that could plausibly confound associations with mortality. MrOS indicates that higher total T or E2 are predictors of reduced all-cause mortality in older men, while HIMS found that an optimal rather than high total T was associated with the longest survival in older men [61, 62]. The findings from HIMS indicate that in older men higher DHT is a biomarker for lower risk of death from IHD [62]. Thus, higher circulating DHT may represent a survival or resilience factor following an IHD event.

Therefore, several epidemiological studies are consistent with a beneficial or protective effect of adequate sex hormone exposure particularly T and to an extent DHT on the risk of CVD, in older if not middle-aged men. However, causality, or proof that intervention with T supplementation would protect against CVD events, can only be determined conclusively by means of randomised controlled trials (RCTs).

The T Controversy: Results from Randomised Controlled Trials

Several small RCTs have shown effects of T supplementation to protect against myocardial ischaemia. In 46 men with stable angina, 12 weeks of transdermal T reduced exercise-induced myocardial ischaemia [66]. In 15 men with angina, intramuscular long-acting depot T over 12 months reduced time to ischaemia on exercise testing [67]. In a study of 87 older men with diabetes, oral T undecanoate over 12 weeks reduced the frequency of angina [68]. Therefore, the publication of the

Testosterone in Older Men with Mobility Limitations (TOM) trial sparked intense debate [69] (Table 19.3). Men ≥65 years of age, with total T of 3.5–12.1 nmol/L or free T of <173 pmol/L and evidence of limitations in mobility were randomised to 100 mg daily transdermal T or placebo for 6 months. The trial was discontinued early after 209 of the target sample of 252 men had been randomised, due to an excess of cardiovascular adverse events in the T arm [69]. Men were 74 years old on average, and half had pre-existing CVD. This contrasts with a comparable study in which 274 men aged ≥65 years who were frail or intermediate frail with total T ≤12 nmol/L or free T ≤250 pmol/L were randomised to 50 mg daily transdermal

Table 19.3 Selected randomised controlled trials (RCTs) of T supplementation in middle-aged and older men. *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *HOMA-IR* homeostatic model assessment-insulin resistance, *IM* intramuscular (injection), *Ex* exercise training

Study author and year	Population (men)	Formulation of T	N active	N placebo	Duration	Result
Basaria et al. (2010) [69]	≥65 years, T 3.5–12.1 nmol/L or free T <173 pmol/L, mobility limitation	Transdermal gel 100 mg daily	106	103	6 months	Trial stopped prematurely due to excess cardiovascular events in T arm
Srinivas-Shankar et al. (2010) [70]	≥65 years, T ≤12 nmol/L or free T ≤250 pmol/L, frail or intermediate frail	Transdermal gel 50 mg daily	138	136	6 months	T improved muscle strength and physical function, no signal for cardiovascular adverse events
Basaria et al. (2015) [71]	≥60 years, T 3.5–13.9 nmol/L or free T <173 pmol/L	Transdermal gel 75 mg daily	156	152	3 years	No difference in rates of change in carotid intima-media thickness or coronary artery calcium
Snyder et al. (2016) [72]	≥65 years, T <9.5 nmol/L, sexual dysfunction (A), diminished vitality (B), and/or mobility limitations (C)	Transdermal gel 50 mg daily	A 230 B 236 C 193	A 229 B 238 C 197	12 months	Modest benefit of T on sexual function, no signal for cardiovascular adverse events
Budoff et al. (2017) [73]	≥65 years, T <9.5 nmol/L, sexual dysfunction, diminished vitality, and/or mobility limitations	Transdermal gel 50 mg daily	73	65	12 months	Greater increase in non-calcified plaque volume in T group. No change in coronary artery calcium

(continued)

Table 19.3 (continued)

Study author and year	Population (men)	Formulation of T	N active	N placebo	Duration	Result
Mohler et al. (2018) [74]	≥65 years, T <9.5 nmol/L, sexual dysfunction, diminished vitality, and/or mobility limitations	Transdermal gel 50 mg daily	394	394	12 months	T treatment decreased total cholesterol, HDL cholesterol, LDL cholesterol, and slightly decreased fasting insulin and HOMA-IR
Wittert et al. (2021) [75]	50–74 years, waist ≥95 cm, T ≤14 nmol/L, impaired glucose tolerance or newly diagnosed type 2 diabetes	IM T undecanoate every 3 months	504	503	2 years	T reduced risk of type 2 diabetes at 2 years by 40%, no signal for cardiovascular adverse events
Chasland et al. (2021) [76]	50–70 years, T 6–14 nmol/L, no known CVD	Transdermal cream 100 mg daily	40 (+Ex in 21)	40 (+Ex in 20)	12 weeks	Exercise improved flow-mediated dilation, T did not improve flow-mediated dilation, nor was there any additive benefit

T or placebo for 6 months [70]. That study was completed successfully with no signal for adverse cardiovascular events, finding that T supplementation improved muscle strength and physical function. Neither of these RCTs, nor for that matter any preceding T RCTs [77], were designed to examine cardiovascular events as pre-specified outcomes, utilising reporting of adverse events. A 3-year RCT of transdermal T in 308 men aged ≥60 years found no difference in the rates of change of either common carotid intima-media thickness or coronary artery calcium score in men receiving T vs. placebo [71]. The authors concluded that while no difference in atherosclerosis progression was seen, the result should not be interpreted as establishing cardiovascular safety of T use in men.

The United States Testosterone Trials (T Trials) recruited 790 men aged ≥65 years, with self-reported sexual dysfunction, diminished vitality and/or mobility limitations, and two early morning T concentrations averaging <9.5 nmol/L, with neither >10.4 nmol/L [78]. The intent of the Testosterone Trials was to test whether 1 year's intervention with transdermal T would improve outcomes relating to physical function, sexual function and vitality, with sub-studies addressing cognitive function, plaque volume, bone density, and anaemia. The primary result of the T Trials showed a modest benefit of T for sexual function, and physical function when all participants were analysed, but no benefit for vitality [72]. Further results

from the T Trials showed benefits of T treatment for anaemia and bone mineral density, but not cognition [79]. In the T Trials cardiovascular sub-study, there was a greater increase in non-calcified coronary artery plaque volume in 73 T-treated men, compared to 65 men receiving placebo, with no difference for coronary calcium scores [73]. However, in that sub-study, the groups were unbalanced, with men in the placebo group having greater plaque volumes both at baseline and at 12 months, making the results challenging to interpret. The authors concluded that larger studies were needed to understand the clinical implications of that finding [73]. In a further analysis from the whole T Trials cohort, T treatment decreased total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, and slightly decreased fasting insulin and insulin resistance estimated using homeostatic model assessment [74]. The magnitude of effect was modest, with adjusted mean differences of 0.16 mmol/L for total cholesterol, 0.05 mmol/L for HDL cholesterol, and 0.06 mmol/L for LDL cholesterol [74]. T Trials were not powered for CVD events as outcomes, with no difference in cardiovascular adverse events noted between the two arms of the trial (7 men in each of the T and placebo groups died from cardiovascular causes or had an MI or stroke) [72].

The Testosterone for the Prevention of Type 2 Diabetes in Men at High-Risk (T4DM) trial randomised 1007 men aged 50–74 years, waist circumference ≥95 cm, and baseline total T ≤14 nmol/L, with impaired glucose tolerance or newly diagnosed type 2 diabetes, to 2 years of T undecanoate injections every 3 months versus placebo, with a background lifestyle intervention [75]. At 2 years, type 2 diabetes (2-h glucose ≥11.1 mmol/L on oral glucose tolerance testing) was present in 87 of 413 participants (21%) with available data in the placebo group and 55 of 443 participants (12%) in the testosterone group (relative risk 0.59, 95% CI 0.43–0.80; $p = 0.0007$). The mean change from baseline 2-h glucose was −0.95 mmol/L in the placebo group and −1.70 mmol/L in the T group (mean difference −0.75 mmol/L, 95% CI −1.10 to −0.40; $p < 0.0001$) [75]. On average, men treated with T gained 0.39 kg of muscle and lost 4.6 kg of fat, whereas men receiving placebo lost 1.3 kg of muscle and 1.9 kg of fat over the 2-year intervention period. As central adiposity and type 2 diabetes are risk factors for CVD, the action of T on top of a lifestyle program to reduce fat mass and risk of type 2 diabetes in T4DM could be postulated to have favourable cardiometabolic effects. However, cardiovascular risk was not assessed as an outcome in T4DM. In T4DM, as in T Trials, there was no signal for excess cardiovascular adverse events (17 men in the placebo group, and 12 in the T group, had a cardiovascular-related death, or had a cerebrovascular disease or ischaemic heart disease event).

Recently, a smaller 2 × 2 factorial RCT of transdermal testosterone cream combined with exercise training, testosterone alone, exercise alone, or neither over 12 weeks showed a beneficial effect of exercise on endothelium-dependent vasodilator function, whereas T alone had no effect, nor was there any additive benefit of combining T with exercise [76]. Therefore, there is a degree of equipoise over the T RCT results in terms of cardiovascular effects. The result of the TOM trial was not replicated in another RCT in a comparable population [69, 70]. While T treatment may have neutral effects on carotid intima-media thickness, coronary calcium score,

and flow-mediated dilation [71, 76], the finding from the T Trials cardiovascular sub-study of an increase in coronary artery plaque volume needs to be addressed [73]. Neither of the two largest T RCTs completed to date, T Trials ($N = 788$ men, 1-year intervention) and T4DM ($N = 1007$ men, 2-year intervention), demonstrated any signal for excess cardiovascular events. Equally, neither was ever designed nor powered for cardiovascular events as an outcome. A more definitive answer should came from the "Testosterone Replacement therapy for Assessment of long-term Vascular Events and efficacy ResponSE in hypogonadal men (TRAVERSE) study" [80]. TRAVERSE recruited men aged 45–80 years, T <10.4 nmol/L, with self-reported symptoms of decreased sexual desire, decreased spontaneous erections, low energy or fatigue, low mood or depressed mood, loss of body hair or reduced shaving, or hot flashes, who have evidence of CVD or are at increased risk for CVD. TRAVERSE recruited 5246 participants and continued until 256 primary major adverse cardiovascular events have occurred. The main aim of TRAVERSE was to determine the safety of T treatment with MACE as the pre-specified primary cardiovascular safety outcome. TRAVERSE commenced in 2018 and was completed in 2023 [81]. In TRAVERSE, for the primary composite outcome of cardiovascular death, non-fatal MI and non-fatal stroke, there were 182 events in the testosterone group (7.0%) and 190 (7.3%) in the placebo group, HR 0.96, CI 0.78–1.17 ($p < 0.01$ for noninferiority). A sensitivity analysis censoring data on events which occurred more than 365 days after the last dose of study medication was given yielded similar results (HR 1.02, CI 0.81–1.27, $p < 0.01$ for noninferiority). For the secondary composite outcome of cardiovascular death, non-fatal MI, non-fatal stroke and coronary revascularisation, there were 269 (10.4%) events in the testosterone group and 264 (10.1%) in the placebo group (HR 1.02, CI 0.86–1.21). For the outcome of death from cardiovascular causes there were 87 (3.4%) and 103 (4.0%) events respectively, HR 0.84, CI 0.63–1.12, and for the outcome of death from any cause, 144 (5.5%) and 148 (5.7%) events respectively, HR 0.98, CI 0.78–1.23 [81]. TRAVERSE is the largest T RCT conducted, designed from the outset as a cardiovascular safety trial. Its interpretation needs to consider the high drop-out rate in the study and the mean duration of treatment of 22 months. TRAVERSE indicates testosterone treatment under these conditions, does not result in excess major cardiovascular adverse events or mortality in men with or at high risk of CVD.

Meta-Analyses of Adverse Events in T RCTs

In the absence of definitive RCT data, meta-analyses of T RCTs have been undertaken to explore the association of T supplementation with cardiovascular adverse events (Table 19.4). Meta-analyses completed prior to the publication of the Basaria and Srinivas-Shankar studies did not find any significant difference in risk of cardiovascular adverse events in T compared with placebo recipients [82, 83]. One meta-analysis including both the Basaria and Srinivas-Shankar trials reported an increased risk of cardiovascular-related events reported as cardiac, cardiovascular, or vascular disorders associated with T [84]. Together with the results of the TOM trial, these and other considerations prompted the US Food and Drug Administration (FDA) to

issue a note of caution regarding prescribing T for ageing men and to mandate a label change warning of possible risks [92]. However, subsequent meta-analyses also including the Basaria and Srinivas-Shankar trials have not shown significant differences in cardiovascular adverse events related to T supplementation (Table 19.4). These include a meta-analysis focussing on larger RCTs [85], a meta-analysis including 75 trials with 5464 men and mean duration of 34 weeks [86], and a meta-analysis including 35 RCTs with 3703 men lasting ≥12 weeks [87]. In these three meta-analyses using random effects models which were more suited to inclusion of heterogeneous studies [93, 94] there was no association of T with cardiovascular adverse events (Table 19.4).

Table 19.4 Meta-analyses of cardiovascular adverse events in randomised controlled trials (RCTs) of T supplementation in men. *MI* myocardial infarction, *MACE* major adverse cardiovascular events, *OR* odds ratio, *95% CI* confidence interval, *SMD* standardised mean difference, *AMS* ageing males' symptoms. Unless otherwise specified, meta-analyses were conducted using random effects models

Study characteristics				Results	
Study author and year	N of RCTs	N active	N placebo	Adverse signal	No adverse signal
Haddad et al. (2007) [82]	30	808	834		No significant difference in odds ratio for any cardiovascular adverse event or for MI
Fernandez-Balsells et al. (2010) [83]	51	2716			No significant difference for all-cause mortality, coronary bypass surgery, or MI
Xu et al. (2013) [84]	27	2994		T associated with increased risk cardiovascular-related event (OR[a]=1.54, 95% CI = 1.09–2.18)	
Ruige et al. (2013) [85]	10 (>100 participants)	1289	848		No significant difference in cardiovascular adverse events
Corona et al. (2014) [86]	75	3016	2448		No association of T supplementation with cardiovascular risk. For MACE OR = 1.01 (95% CI 0.57–1.77)
Borst et al. (2014) [87]	35	3703			No significant risk for cardiovascular-related adverse events
Alexander et al. (2017) [88]	39	3230	2221		T treatment not associated with risk of MI (OR[a]=0.87; 95% CI 0.39–1.93), stroke (OR[a]=2.17; 95% CI 0.63–7.54). or mortality (OR[a]=0.88; 95% CI 0.55–1.41)

(continued)

Table 19.4 (continued)

Study characteristics				Results	
Study author and year	N of RCTs	N active	N placebo	Adverse signal	No adverse signal
Elliott et al. (2017) [89]	87	N/A	N/A		T improved quality of life (SMD −0.26, 95% CI −0.41 to −0.11), libido (SMD 0.33, 95% CI 0.16–0.50), depression (SMD −0.23, 95% CI −0.44 to −0.01), and erectile function (SMD 0.25, 95% CI 0.10–0.41). No significant increase in risk of cardiovascular deaths (OR = 2.15, 95% CI 0.72–6.45)
Corona et al. (2018) [90]	93	4653	3826		No clear effect of T on incidence of CVD events. For MACE OR = 0.97 (95% CI 0.64–1.46)
Diem et al. (2020) [91]	38	N/A	N/A		T treatment associated with small improvement in sexual function (SMD 0.35, 95% CI 0.23–0.46) and quality of life (SMD −0.33, 95% CI −0.50 to −0.16, AMS scale) Pooled risk for adverse cardiovascular outcomes did not differ between groups (OR[a]=1.22, 95% CI 0.66–2.23)

[a] Fixed effects model

More recent meta-analyses have shown similar results. A meta-analysis involving 39 RCTs which included 5451 men randomised to testosterone or placebo for durations ranging from 6 weeks to 3 years found no significant increase in risk of MI, stroke, or mortality [88]. Of note, only 16, 9, and 20 RCTs contributed to the analysis of MI, stroke, and mortality outcomes, respectively, and the authors concluded the low quality of evidence precluded definitive conclusions. In a meta-analysis canvassing 70 RCTs of duration ranging from 3 to 36 months, testosterone improved quality of life, depression, libido, and erectile function, with no significant increase in risk of cardiovascular deaths, albeit with low event rates limiting the robustness of those estimates [89]. Another meta-analysis drew on 97 RCTs of up to 3 years duration: 91 reported on MACE, 59 detected no events, thus the main analysis was performed on 32 RCTs finding no difference in risk between testosterone and placebo [90]. A meta-analysis of 38 RCTs, most of which had less than 12 months follow-up, with the longest follow-up being 3 years, found small improvements in sexual functioning and quality of life, but insufficient evidence to make conclusions as to long-term efficacy and safety of T treatment [91] (Table 19.4).

Thus, meta-analyses of T RCTs in general have not found T supplementation to be associated with excess cardiovascular adverse effects. However, a degree of uncertainty remains as most of the included RCTs tended to be relatively small, with limited duration of follow-up, and assessed reported cardiovascular adverse events. While the results of these meta-analyses provide some degree of reassurance, large RCTs of longer duration examining cardiovascular events as a pre-specified outcome are needed before definitive conclusions can be drawn.

Retrospective Studies of Prescribed T and Cardiovascular Adverse Events

Retrospective studies of healthcare or insurance databases have attempted to examine associations of T prescriptions with subsequent outcomes in the recipients. These have major limitations, particularly their observational nature and the absence of randomisation with the possibility of selection bias, as well as limited clinical data regarding the indications for T treatment. Furthermore, these studies have reported contrasting results (Table 19.5). Prescription of T has been associated with reduced mortality in male veterans with baseline T ≤8.7 nmol/L [95] and also reduced mortality in men with type 2 diabetes with baseline T ≤10.4 nmol/L [96]. These studies suggested a potential benefit of T supplementation, albeit care is

Table 19.5 Retrospective case-control studies of men prescribed T which examined associations of T prescriptions with cardiovascular events and mortality in middle-aged and older men. *MI* myocardial infarction, *TRT* testosterone replacement therapy, *MACE* major cardiovascular adverse event comprising death, non-fatal MI and non-fatal stroke, *HPT* hypothalamic-pituitary-testicular, *TIA* transient ischaemic attack, *CVD* cardiovascular disease

Study characteristics				Results	
Study author and year	Size (*n* of men)	Follow-up (years)	Age (years)	Favours no T	Favours T
Shores et al. (2012) [95]	1031	3.4	62.1		Male veterans with total T ≤8.7 nmol/L, T prescribed in 398. T supplementation associated with lower mortality
Muraleedharan et al. (2013) [96]	581	5.8	59.5		Men with type 2 diabetes, 238 with total T ≤10.4 nmol/L. T supplementation associated with lower mortality
Vigen et al. (2013) [97]	8709	2.3	63.4	Male veterans who had coronary angiography and total T ≤10.4 nmol/L. T prescription associated with increased risk of death, MI, or stroke	

(continued)

Table 19.5 (continued)

Study characteristics				Results	
Study author and year	Size (*n* of men)	Follow-up (years)	Age (years)	Favours no T	Favours T
Finkle et al. (2014) [98]	55,593	90 days	54.4	Men prescribed T. Higher rate of non-fatal MI in 90 days following prescription compared to preceding 1 year	
Baillargeon et al. (2014) [99]	6355; 19,065	4.1; 3.3	≥66		6355 men prescribed T vs. 19,065 matched non-users. T prescription not associated with increased risk of MI. For men with worse prognostic scores, T associated with reduced risk of MI
Sharma et al. (2015) [100]	83,010	6.2; 4.6; 4.7	66		Male veterans with low T. TRT resulting in normalisation of circulating T (n = 43,931) was associated with lower risk of death, MI, and stroke, compared to TRT without normalisation of T (n = 25,701) or no TRT (n = 13,378)
Anderson et al. (2016) [101]	4736	≥3	61.2		Men with low T. T therapy achieving normal T (n = 2241) was associated with reduced risk of MACE compared to persistent low T (n = 801). T therapy achieving either normal T or high T (n = 1694) associated with lower all-cause mortality compared to persistent low T
Wallis et al. (2016) [102]	10,311: 28,029	5.3	≥66		10,311 men newly treated with T vs. 28,029 controls. Men treated with T had lower mortality HR = 0.88 (95% CI = 0.84–0.93). Men in lowest tertile of T exposure had increased risk of mortality (HR = 1.11, 95% CI 1.03–1.20) and cardiovascular events (HR = 1.26, 95% CI 1.09–1.46), those in the highest tertile had decreased risk of mortality (HR = 0.67, 95% CI 0.62–0.73) and cardiovascular events (HR = 0.84, 95% CI 0.72–0.98)

Table 19.5 (continued)

Study characteristics				Results	
Study author and year	Size (*n* of men)	Follow-up (years)	Age (years)	Favours no T	Favours T
Cheetham et al. (2017) [103]	8808: 35,527	3.2	≥40		8808 men with diagnosed androgen deficiency and/or T <10.4 nmol/L vs. 35,527 controls. Men treated with T had lower risk of the composite cardiovascular endpoint (HR = 0.67, 95% CI 0.62–0.73). Similar results seen for stroke events (HR = 0.72; 95% CI 0.62–0.84) and cardiac events (HR = 0.66; 95% CI 0.60–0.72)
Loo et al. (2019) [104]	15,401	4.7	≥45	Men with low T and no evidence of HPT axis disease. T use was associated with an increased risk of stroke/TIA/MI composite (HR = 1.21, 95% CI 1.00–1.46). The risk was highest in the first 6 months to 2 years of T use (HR 1.35, 95% CI, 1.01–1.79). The risk of all-cause mortality was lower with current T use (HR = 0.64, 95% CI 0.52–0.78) and higher with past T use (HR = 1.72, 95% CI 1.21–2.45), compared with non-use	

(continued)

Table 19.5 (continued)

Study characteristics				Results	
Study author and year	Size (*n* of men)	Follow-up (years)	Age (years)	Favours no T	Favours T
Oni et al. (2019) [105]	1470	3.2–4.0	≥50		Male veterans with low total T. All-cause mortality was lower in men treated with T who normalised total T (*N* = 755), vs. men treated with T who did not normalise total T (*N* = 542, HR = 0.76, 95% CI 0.64–0.90), or men not treated with T (*N* = 173, HR = 0.76, 95% CI 0.60–0.98). There was no significant difference in the risk of recurrent MI between groups
Shores et al. (2021) [106]	204,857	4.3	60.9		Male veterans with low T. Current transdermal T use was not associated with risk for incident MI/ischaemic stroke/venous thromboembolism (HR = 0.89, 95% CI 0.76–1.05) in men without prevalent CVD, and in those with prevalent cardiovascular disease was associated with lower risk (HR = 0.80; 95% CI, 0.70–0.91). Current use of intramuscular T was not associated with risk for composite endpoint in men without or with prevalent CVD (HR = 0.91, 95% CI 0.80–1.04; HR = 0.98, 95% CI 0.89–1.09, respectively)

needed with their interpretation [107]. In a controversial study involving a different cohort of male veterans who underwent coronary angiography and had total T ≤10.4 nmol/L, prescription of T was associated with higher risk of adverse outcomes [97]. However, the actual observed rate of adverse outcome events in men prescribed T was half that of the men who were not prescribed T, but the direction of the results was reversed by a complex statistical model drawing critical comment [108]. A published erratum acknowledged incorrect classification of a number of men and identified 100 women who needed to be excluded [97]. Another retrospective study reported increased risk of MI within 90 days of T prescription compared to the preceding 12 months, in men aged 65 years and older, or men under the age of 65 who has a prior history of CVD, based on a relatively small number of incident events and a low absolute event rate [98]. A national sample of Medicare

beneficiaries aged ≥66 years found that T treatment was not associated with risk of MI, and in men with worse prognostic scores T treatment was associated with lower risk of MI [99].

Two more recent retrospective analyses of healthcare databases have attempted to address the issue of on-treatment T concentrations, with interesting results. Sharma et al. conducted a retrospective analysis from the Veterans Health Administration database of men who were recorded as having a T concentration below the lower limit of the normal laboratory reference range [100]. Men who were prescribed T and who had a normal T after treatment had a lower risk of death from any cause, MI and stroke compared to men who received no treatment, and also compared to men who received treatment but had subsequent low T despite this [100]. This study highlighted the possible prognostic importance of achieving a "normal" T while on supplementation. Anderson et al. conducted a retrospective analysis of men from a healthcare database who had baseline T <7.4 nmol/L, a follow-up T and at least 3 years of follow-up [101]. Compared to men whose T remained <7.4 nmol/L (82% of whom did not receive T therapy), men (all receiving T supplementation) who achieved "normal" T between 7.4 and 25.8 nmol/L had a lower rate of death, non-fatal MI, or stroke (MACE) at 3 years [101]. Men (all receiving T supplementation) who achieved a "high" T also had a lower rate of MACE at 3 years but this result was not statistically significant. Both "normal" T and "high" T groups had lower risk of all-cause mortality at 3 years compared to the "low" T group [101]. The authors commented on a non-significant trend towards higher risk of stroke in men achieving "high" T.

Wallis et al. in an analysis of men treated with T compared with controls found that T treatment was associated with lower mortality HR = 0.88 (95% CI = 0.84–0.93) and prostate cancer risk HR = 0.86 (95% CI = 0.75–0.99) [102]. In that study, shorter exposure to T treatment (2 months) was associated with increased risk of cardiovascular events and mortality, whereas longer exposure (35 months) was associated with reduced risk of these events. Therefore, a dichotomy may be present, with short term use of T carrying a different risk profile to longer duration treatment. In an analysis by Cheetham et al. of men aged ≥40 years, with a recorded diagnosis of androgen deficiency or total T <10.4 nmol/L, T treatment was associated with reduced risk of the composite of cardiovascular endpoints that included acute MI, coronary revascularization, unstable angina, stroke, transient ischemic attack (TIA), and sudden cardiac death (HR = 0.67, 95% CI = 0.62–0.73) [103]. In that study, the results were similar when cardiac endpoints and stroke events were considered separately (Table 19.5). Contrasting results were obtained by Loo et al. in an analysis using the UK Clinical Practice Research Datalink, of men with a low T concentration or a code for hypogonadism, after excluding men with diseases of the hypothalamic-pituitary-testicular (HPT) axis [104]. In these men, T use was associated with an increased risk of a composite endpoint comprising stroke/TIA/MI (HR = 1.21, 95% CI 1.00–1.46). The risk was highest in the first 6 months to 2 years of T use. Oni et al. studied a cohort of veterans with low T concentrations who had experienced an MI, finding that those who were prescribed T and normalised T concentrations had lower mortality than those who were prescribed T

without normalising T concentrations, or those who were not prescribed T [105]. Finally, Shores et al. in a recent analysis of a large cohort of US veterans found that current compared with former use of transdermal T was associated with lower risk of composite cardiovascular events (incident MI/ischaemic stroke/venous thromboembolism) in those with prevalent CVD (HR = 0.80, 95% CI, 0.70–0.91), with no increase in risk in men without prevalent CVD (HR = 0.89, 95% CI 0.76–1.05) [106]. There were no differences in risk for current compared to former intramuscular T users with or without prevalent CVD.

Thus, while some of these studies associated use of T with increased risk of cardiovascular adverse events [97, 98, 104], more reported neutral associations with these outcomes [99, 106] or lower risk of CVD events or lower mortality [95, 96, 100–103, 105]. All these retrospective case-control analyses have recognised limitations particularly the absence of randomisation and multiple potential sources of bias and confounding. The contrasting results and the limitations of such retrospective analyses illustrate the need for definitive prospective randomised controlled trials to clarify whether T supplementation in middle-aged or older men would reduce rather increase the risk of CVD. The design of such RCTs should ensure that on-treatment T concentrations are normalised but not excessive.

Mendelian Randomisation Studies

Pending definitive RCTs of T therapy, causation has been studied using the Mendelian randomisation (MR) approach [109]. If a genetic polymorphism is associated with higher circulating T concentrations, then this exposure which is effectively allocated in a randomised fashion at birth should be independent of subsequent lifestyle factors and unaffected by reverse causality. In a MR study of men and women from the CARDIoGRAMplusC4D consortium and the UK Biobank, variants in the *JMJD1C* gene region associated with higher T concentrations were associated with risk of coronary artery disease and ischaemic stroke, but *SHBG* polymorphisms also associated with higher T concentrations were not associated with these outcomes [110]. A subsequent MR analysis of the Reduction by Dutasteride of Prostate Cancer Events (REDUCE) randomised controlled trial, UK Biobank, and CARDIoGRAMplusC4D 1000 Genomes based genome-wide association study found a null association of *JMJD1C* polymorphisms with risk of MI in men [111]. The pathways by which *JMJDC1* polymorphisms influence circulating T concentrations are uncertain, and it may have other physiological actions, limiting its utility as an instrumental variable for T-related MR analyses [112, 113].

In an analysis examining multiple polymorphisms using CARDIoGRAMplusC4D 1000 Genomes and UK Biobank data, genetically predicted higher SHBG (221 single nucleotide polymorphisms [SNPs] explaining 2.6% of the variance of SHBG) or genetically predicted higher total T (133 SNPs explaining 6.9% of the variance of total T) was associated with lower risk of coronary heart disease in univariable MR [114]. However, no direct causal effect was found in multivariable MR. Using a network of MR analysis, the authors concluded that T might act as mediator in the

causal pathway from SHBG to coronary heart disease and vice versa [114]. A different approach was used in two other UK Biobank-based MR studies. Ruth et al. analysed 2571 genome-wide significant associations in women and men from UK Biobank, finding that genetically determined higher total T was associated with higher risk of type 2 diabetes in women, but a lower risk of type 2 diabetes in men [115]. The finding in men is consistent with the results of the T4DM study [75]. In an MR analysis in which multiple SNPs associated with cFT were tested against 461 outcomes in UK Biobank men, 22 a priori selected traits and other 415 diseases and 24 biomarkers, genetically predicted cFT was associated with expected traits such as higher body fat-free percentage, low body fat percentage, and heel bone mineral density mass [116]. In that study there were no significant associations of genetically predicted cFT with MI, ischaemic stroke or heart failure, or for that matter with type 2 diabetes. Therefore, the MR approach has not been as informative as hoped, as more robust instrumental variables relating to T exposure have not been clearly linked to CVD-related outcomes.

Conclusions

Lower circulating T and DHT are independent predictors for incidence of CVD, particularly stroke, and in the case of lower DHT for IHD mortality, in older men. However, neutral associations tend to be seen in younger or middle-aged men. While some longitudinal observational data are consistent with beneficial effects of T on cardiovascular risk, caution is required before inferring causality. Proving causation requires demonstration of an effect of T in RCTs to reduce cardiovascular risk and this is where there is a crucial evidence gap. One RCT which was not powered for outcomes of MI or stroke reported an association of T with cardiovascular adverse events, while other RCTs have not. The two largest T RCTs reported to date, T Trials and T4DM, showed no signal for adverse cardiovascular events. Meta-analyses of T RCTs have generally not associated T treatment with adverse cardiovascular events, noting that many T RCTs have been limited in size and of relatively short duration.

Men who are androgen deficient due to hypothalamic, pituitary, or testicular disease, who have symptoms and signs of androgen deficiency and unequivocally low early morning T concentrations confirmed by repeat measurements using accurate assays, should be considered for replacement therapy [20, 117, 118]. Additional studies are needed to clarify whether T supplementation would alter the risk of stroke or other CVD-related events in men with low-normal circulating T who do not have such pathology. This would require large-scale RCTs powered to detect effects of T on cardiovascular events. The results of TRAVERSE, a cardiovascular safety study, indicates testosterone treatment does not result in excess serious cardiovascular adverse events or mortality in men with or at high risk of CVD. Those results, together with further studies to explore mechanistic pathways by which T exerts effects on the cardiovascular system, would influence the rationale for and design of future large-scale RCTs to clarify whether or not T supplementation could

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Androgens and Women

20

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Introduction

Androgens have been long overlooked as contributors of overall and sexual health in women. In the last decades, they have emerged as essential regulators or several processes, in particular related to sexual desire. This chapter will focus on basic and clinical aspects of androgen physiology in the female gender and on the main characteristics of their pathological expressions (androgen excess and androgen deficiency). We used a practical approach, aimed at guiding students, clinicians, and researches through the most recent, evidence-based trends in diagnostic algorithms and treatments.

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Synthesis and Metabolism of Androgens Across the Life Span

Origins of Androgens

Testosterone and dihydrotestosterone (DHT) are the two biologically active C19 steroids acting as androgens on target organs. In young women, about 50% of circulating testosterone is believed of ovarian origin. The rest of testosterone production stems from the conversion of the circulating precursor androstenedione in extragonadal tissues, including the skin and adipose tissue, with a conversion rate of approximately 5%. Both the ovaries (in the thecal cells/stroma) and the adrenal glands (cortices) contribute to circulating androstenedione. With advancing age, the percentage of androgen production accounted by the adrenals increases, while that provided by the ovaries declines [1].

Upstream, DHEAS (of adrenal origin) and DHEA (of both adrenal and ovarian origin) are converted to androstenedione, thereby indirectly contributing to the formation of testosterone. DHEAS is a biologically inert steroid; its estimated production rate is up to 20 mg daily, vs. 8 mg of DHEA, 3–6 mg of androstenedione, 100–300 μg of testosterone, and 4.3–12.5 mg of DHT [2].

The adrenal production of precursors is stimulated by ACTH (adrenocorticotropic hormone); on the contrary, ovarian androgen synthesis is stimulated by LH (luteinizing hormone), with variation throughout the menstrual cycle.

Within target tissues and cells (i.e. skin fibroblasts, hair follicles, external genitalia), testosterone undergoes its transformation into the more potent androgen DHT, catalyzed by the enzyme 5α-reductase. For this reason, the androgenic action in target tissues depends in part on the levels of 5α-reductase in addition to the presence of androgen receptors (AR). DHT exerts a markedly higher androgenic activity than testosterone in terms of hair growth and virilization of external genitalia [1].

Transport and Metabolism of Androgens

Only a minor part of circulating testosterone is bioavailable, that is, able to diffuse in peripheral target tissues and exert its action. This form includes both the free fraction and the albumin-associated fraction, whereas the portion tightly bound to sex hormone binding globulin (SHBG) is not biologically active. Therefore, SHBG is one of the main regulators of the circulating amounts of bioavailable testosterone. Conditions that increase SHBG (i.e., combined oral contraceptives, tamoxifen, thyroxine therapy, and liver diseases) lead to a decrease in testosterone activity, whereas conditions that decrease SHBG (i.e. obesity and insulin resistance) result in enhanced testosterone activity. SHBG binds DHT with even greater affinity than testosterone [3].

In peripheral tissues, androstenedione may be transformed into estrone, and testosterone into estradiol, via the aromatase enzyme. Such peripheral

aromatization is directly associated with age and body mass index. DHT is metabolized to 17-ketosteroids and other products which undergo urinary excretion.

Androgens Levels Across the Life Span

Normative ranges for androgens levels in women of different ages are lacking. The physiological changes in androgen and precursors levels in women have been investigated in the last years by an Australian group. These authors have measured serum values of total testosterone, calculated free testosterone, DHEA-sulfate (DHEAS), and androstenedione in a large (n = 1423) community-based sample of women, from the early reproductive years to several years after menopause [4]. The study revealed that the levels of all measured steroids declined consistently with age, in particular across the earlier decades (Table 20.1) [4]. The physiological basis of this decline has not been clarified; the reduction in adrenal androgens production could be explained by an altered adrenal zonation, with the mass of the *zona reticularis* progressively reducing.

Noteworthy, androgen levels do not vary significantly across natural menopause. In the same study, in women aged 45–54 years, no independent effect of menopausal status on androgen levels emerged, unlike the major decrease in estrogens commonly seen at this time [4]. On the contrary, a rise in circulating DHEAS has been detected in women during the menopausal transition, as reported by longitudinal data from the Study of Women's Health Across the Nation (SWAN) [5]. Accordingly, following a midlife nadir, testosterone concentrations tend to increase during the eighth decade in some women [6].

Another important variable to consider in reproductive-aged women is the variation of androgens concentration depending on the menstrual cycle. In fact, it has been showed that testosterone and androstenedione are higher during the midcycle and luteal phase versus the early follicular phase; conversely, DHEAS does not seem to vary significantly across the cycle [7].

Table 20.1 Median [10th centile; 90th centile] for androgens by decade for the reference group. T testosterone, *DHEAS* dehydroepiandrosterone sulfate, *A* androstenedione. Data obtained from [4]

	Age groups (years)					
	18–24	25–34	35–44	45–54	55–64	65–75
Total T nmol/L	1.55 [0.86–2.47]	1.00 [0.58–1.70]	0.80 [0.50–1.40]	0.70 [0–40.1.30]	0.55 [0.20–1.25]	0.60 [0.30–1.10]
A nmol/L	7.95 [4.86–13.72]	6.30 [3.00–9.46]	4.50 [2.64–8.48]	3.80 [2.00–6.29]	2.80 [1.30–5.10]	2.80 [1.30–5.40]
DHEAS μmol/L	7.30 [4.03–10.78]	4.50 [2.20–7.90]	3.90 [1.86–7.31]	2.85 [1.30–6.20]	2.15 [0.70–5.30]	1.50 [0.50–3.10]

Signaling Mechanisms of Androgens

Like other steroids hormones, testosterone and DHT utilize nuclear receptors for signaling. Indeed, they are bound by, and thereby activate, the AR, which then acts as a transcription factor. The human AR gene is located on chromosome X.

Classically, the binding of testosterone or DHT (ligands) to AR induces a conformational change in the receptor itself, and its translocation from the cytoplasm to the nucleus of the cell. In the nucleus, AR dimerize and are bound by co-activators. Ultimately, AR bind to nucleic acid in specific regions known as androgen response elements (AREs), leading to chromatin remodeling and triggering the transcription of androgen-dependent genes, and subsequently protein synthesis.

Androgens are also able to act though rapid, non-genomic mechanisms of signal transduction, which involve the modulation of regulatory proteins localized in the cytoplasm or in the cell membrane [8].

The Role of Androgens in Women: Basic Aspects

Brain Sexual Differentiation

Since fetal life, androgens exert their effects in multiple tissues, including the central nervous system (CNS). The production of testosterone and its metabolization into DHT begins around 6th–12th week of gestation. In the second half of pregnancy, testosterone permanently organizes the developing brain, shaping the anatomy and circuits of several areas in a gender-specific manner ("organizational role"). At this stage, brain development toward the female direction stems from the lack of androgens. Later on, during puberty, the central neural pathways that have been organized in prenatal life will be functionally activated by sex steroids ("activational role") [9].

In rodents, aromatization of testosterone into estrogen plays a crucial role in brain sexual differentiation: in fact, though this may seem counterintuitive, estrogens cause the masculinization of neurons, independently of androgens' signaling through AR. In female rodents, alpha-fetoprotein binds to estrogens and protects the brain from defeminization.

Unlike rodents, aromatization does not seem to influence the masculinization of the human brain; conversely, androgens levels and their binding to ARs are probably the crucial mechanisms involved in the variability of sexual differentiation of our CNS. Sexual differentiation in the hypothalamus has been related to the development of gender identity and sexual orientation and involves a very complex interplay between androgens, genetic and epigenetic factors, endocrine disruptors, and immune response [10].

Genital Sexual Differentiation

Differentiation of the internal and external genitalia takes place in the early phases of gestation (weeks 6–12). Both the male and female embryo initially display two pairs of structures: the Wolffian ducts, located medially below the gonadal ridges, and the Müllerian ducts, parallel to their paramedial side. In females, the absence of the anti-Müllerian hormone (AMH), secreted by the Sertoli cells of the male gonad, results in the development of the mesodermal Müllerian ducts, which largely merge together and form the uterus, the upper two-thirds of the vagina and the fimbrial portions of the fallopian tubes. The lack of testosterone leads to the degeneration of the Wolffian ducts, which in males are stabilized and form the epididymis, vas deferens, and seminal vesicles. The inferior third of the vagina, the urethra, and the vestibule have a different embryogenic origin, deriving from the endodermal urogenital sinus [11].

Testosterone also elicits the development of the penis from the ambisexual genital tubercle, or of the clitoris in its absence. The neurovascular anatomy of the two organs is similar, since both the penis and clitoris have homologous *corpora cavernosa* consisting of sinusoidal erectile tissue surrounded by a thick tunica albuginea, and a homologous distal glans. The main structural difference between the penis and the clitoris is the lack of the tubular urethra within the latter [12].

Female internal and external genitalia are androgen-responsive in their anatomy, histology, and functionality since embryogenesis. Noteworthy, the AR has been detected in the mesenchymal tissue of the urogenital sinus as early as at 9 weeks of gestation [13].

Neurobiology of Androgens and Sexual Motivation

The willingness to initiate and engage in a sexual interaction, which in humans may be defined as sexual desire, origins in specific areas of the SNC. The neurobiology of these processes is still largely unknown, and a relevant part of our understanding of the topic derives from animal models. The nucleus of the excitatory system is based on dopamine neurotransmission, with the contribution of melanocortin, oxytocin, and noradrenaline. Dopaminergic pathways generally control attention and motivation; dopamine release in the incertohypothalamic and mesolimbic systems, connected to the hypothalamic nuclei, focuses attention on sexual stimuli and triggers the motor behavioral patterns related to sexual approach. Interestingly, dopamine is specifically involved in the rewarding effects of sexual activity (pleasure), and in the processes of learning/conditioning, both of which can be positively and negatively modulated by experience [14].

In ovariectomized female rodents, sex steroids have been consistently demonstrated to generate the ability to become sexually aroused and to stimulate behaviors which bring an animal into contact with the potential sex partner (“appetitive” behaviors). The medial Preoptic Area (mPOA) of the hypothalamus and its neural network with the ventral tegmental area (VTA), a hub of the mesocorticolimbic

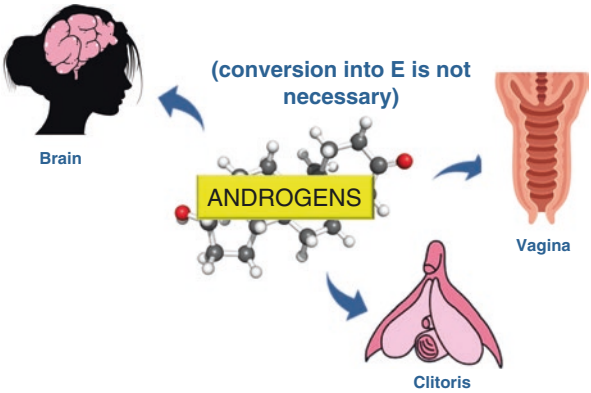

Fig. 20.1 Preclinical and clinical data suggest that androgens are essential regulators of the physiology of the main organs involved in women's sexual function: the brain, the vagina, and the clitoris. *E* estrogens

circuitry, are assumed to play a key role in dopamine-related functions such as sexual motivation and, in females, are positively regulated by ovarian hormones [15]. Estradiol increases the ability of detecting male signals within the female olfactory systems and facilitates the processing of sexual information within the amygdala, a nucleus strongly implicated in the regulation of emotions [16]. Androgens appear to act as enhancers of brain reward circuits, including those related to the sexual response, mediated by dopaminergic transmission and modulated by experience (Fig. 20.1). In this context, testosterone is involved in risk-taking behaviors, by reducing the functional coupling of the amygdala with the orbitofrontal context and by increasing the perceived value of the potentially rewarding (sexual) object [17].

At supraphysiologic concentrations, such as in case of androgen anabolic steroids use, testosterone may imbalance the neural circuits involved in the reward process, thus leading to addiction [18]. Conversely, abnormal reward processing, related to attenuated androgen signaling, may represent one of the neurobiological mechanisms by which fluctuations of androgen levels increase the risk of developing hypoactive sexual desire disorder (HSDD) risk in predisposed women [19].

In an ovariectomized rat model, after priming with estradiol, exogenous administration of testosterone, alone or combined with an aromatase inhibitor, or of DHT, a non-aromatizable androgen, was able to enhance both appetitive and receptive measures of sexual behavior, thus strongly suggesting a direct effect of androgens on the AR, independent of aromatization [20, 21].

Testosterone and the Peripheral Sexual Response

Vulvovaginal atrophy, one of the main clinical signs of menopause, has been traditionally linked to estrogen deficiency. However, the key role of androgens in maintaining of an intact and functional genital tissue has been consistently highlighted. In line with this, AR is widely expressed in the adult female genitourinary tissues (vagina, clitoris, labia, vestibule, bladder, and urethra) [22].

Several potential mechanisms by which the activation of the AR regulates vaginal function in the context of the female sexual response have been identified. These include a facilitating effect on vascular smooth muscle relaxation via the nitric oxide (NO)-cyclic GMP-phosphodiesterase type 5 (PDE5) pathway, which is essential for lubrication during sexual activity and has been demonstrated in animal models both in the clitoris and in the vagina [23, 24]. AR activation also enhances nerve fibers density and neurotransmission, with a reduction of nociception; collagen fibers thickness and compactness; thickness and contractility of the *muscularis* layer; mucin secretion in the vaginal epithelium [25]. All these processes contribute to the physiologic peripheral sexual response, ameliorating arousal, lubrication and orgasmic function, and reducing dyspareunia (Fig. 20.1). Androgens have also been reported to blunt the ability of smooth muscle cells of the human vagina to be involved in acute and chronic inflammation, reducing the local production of cytokines. This is of importance when considering that inflammation acts as a determining and precipitating factor in several conditions such as the genitourinary syndrome of menopause (GSM), genito-pelvic pain and penetration disorder, painful bladder syndrome, etc. [26].

The principle of *intracrinology* postulates that, in order to protect peripheral tissues from imbalances of circulating hormones, in specific organs, including the vagina, the cells own the enzymatic machinery necessary to produce estrogens and androgens locally [27]. This mechanism gains particular importance at time of menopause, due to the decrease in circulating sex steroids. Although the concept of *intracrinology* has been proposed several years ago, leading the way to the use of DHEA, a weak androgen precursor, as a treatment strategy for the symptoms of the GSM, only recently the vagina has been demonstrated to actively synthesize testosterone and DHT starting from DHEA. These data have been obtained in human smooth muscle vagina cells, quantifying androgen production by liquid chromatography tandem-mass spectrometry [28]. In the same study, after DHEA supplementation, estradiol was almost absent in the culture medium of the cells, thus challenging the notion of DHEAS being relevantly metabolized into estrogens in the vagina, at least in the muscle layer.

The Role of Androgens in Women: Clinical Aspects

Androgens Levels and Sexual Function

Women are exposed to relevant fluctuations in gonadal hormones across the menstrual cycle. While the female rodent is receptive only at time of ovulation, the influence of sex steroids levels on human sexual behavior is subtle, but still present. Indeed, it has been described that sexual motivation tends to be stronger during the periovulatory phase, which is also the phase in which testosterone displays its peak. Several large cross-sectional and longitudinal studies have been showing correlations between measures of sexual function and levels of specific endogenous androgens; however, the results were inconsistent [29]. A recent meta-analysis concluded

that testosterone and its derivatives (free T and FAI) have a moderate association with sexual desire; in contrast, DHEAS showed a moderate association only with global sexual function. This study included both pre- and postmenopausal women. For testosterone, the association was described as weak in studies employing the gold-standard method for measurement (LC-MS) [30] (Fig. 20.1).

Androgens and Cardiovascular Health

Sex steroids are known to influence body composition and the cardiovascular system. In men, low levels of endogenous testosterone have been convincingly related to an increased risk of cardiovascular events; nevertheless, whether there is a causality in this link, or whether male hypogonadism represents a marker of poor health, has not been clarified [31]. Conversely, the way androgen excess or deficiency may affect cardiovascular health in women is much debated. In women with polycystic ovary syndrome (PCOS), an association between androgenic activity and endothelial dysfunction has been reported in some studies [32]. However, it could be hypothesized that SHBG mediates this relationship, since low SHBG results in increased bioavailable testosterone, and low SHBG is common in case of insulin resistance, a condition strongly related to PCOS-like phenotypes [33]. On the contrary, other studies, both in pre- and postmenopausal women, suggested a detrimental effect of androgen deficiency on endothelial function. Importantly, in a prospective cohort study conducted on more than 4700 Danish women, and in a meta-analysis of 44 studies, no effect of either low or high total testosterone on stroke incidence was reported [34].

Other evidence suggests the existence of an "optimum window" of testosterone levels, which could be related with the best cardiovascular profile. A research conducted in more than 600 community dwelling women aged 50–90 years, followed up for two decades, showed that both high and low bioavailable testosterone were linked with an increased risk of incident coronary events, when compared to subjects with values in the physiologic range at baseline [35].

Androgens and Bone Mass

Testosterone is a crucial regulator of bone mass, exerting an anabolic action. Indeed, AR activation stimulates osteoblast proliferation and inhibits osteoclast activity. In addition to this direct effect, androgens are aromatized into estrogens and, by binding to ER, inhibit osteoclast proliferation and induce their apoptosis, thus negatively modulating bone reabsorption. Androgens have been reported to contribute to maintaining bone density in the presence of low estrogens, as indicated by the positive correlation between testosterone levels and body mass density in older women [36]. Accordingly, 2 years treatment with both estradiol and testosterone in patients with a history of surgical menopause was associated with a higher increase in body mass density at lumbar spine and hip, versus estradiol alone [37].

Androgens and Gynecological Conditions

The role of androgens in breast cancer is debated but clinically relevant for prognosis and response to treatment. According to preclinical evidence, the effect of androgens on the mammary epithelium appears as bidirectional: mainly proliferative, through to the aromatization into estrogens, but also anti-proliferative, because AR signaling may blunt the activity of ER. When ARs are present in breast cancer cells, they seem to activate proliferation, due to a crosstalk with the HER2 pathway [38]. Regarding ER-positive breast cancer, androgen levels have been related with increased incidence and recurrence in epidemiological studies; conversely, AR expression has been described as a positive prognostic factor [39].

As for the endometrium, androgens control important physiologic mechanisms, such as endometrial competence during the establishment of pregnancy. ARs and enzymes involved in androgen synthesis have been detected both in stromal cells of normal endometrium and in epithelial cells of endometrial cancer [40]. The anti-proliferative effects of androgens in the endometrium are demonstrated by long-term testosterone administration in transgender males, in which glandular atrophy and reduced cellular proliferation have been observed [41]. Total and free testosterone levels and aberrant expression of the enzymes involved in androgen synthesis have been positively correlated with the risk of developing endometrial cancer [42]. A dysregulated androgen signaling has also been described in endometriosis lesions [43].

Furthermore, androgens are involved in reproductive function in women, including stimulation of oocytes and granulosa cells and facilitation and regulation of the different phases of follicular development. Low androgens are associated with low functional ovarian reserve, defined as an abnormally low number of small growing follicles. Controlled ovarian stimulation protocols using adjuvant treatment with DHEA showed good clinical outcomes in terms of achieving pregnancy in poor ovarian responders undergoing in vitro fertilization [44].

Finally, an important role for androgens has been also described for female bladder physiology [22, 45].

Androgen Excess

Androgen Excess in Premenopausal Women

Androgen excess in women can be expressed by the presence of clinical and/or biochemical evidence of hyperandrogenism (i.e., hyperandrogenemia). The polycystic ovary syndrome (PCOS) is the most frequent cause of androgen excess in premenopausal women, affecting between 5% and 10% of the female population; however, a diagnosis of PCOS should be performed only after having ruled out other less common causes of hyperandrogenism [46].

Clinical presentation of hyperandrogenism may include hirsutism, acne, and/or male-pattern hair loss, while more severe signs, like deepening of the voice and clitoromegaly, are defined as virilization and could be of neoplastic origin [46].

Hyperandrogenemia is characterized by elevated serum androgen concentrations; specifically, serum total testosterone should be evaluated in all women with clinical signs of hyperandrogenism. A cut-off value for serum total testosterone in women has not been universally established; however, measurement by liquid chromatography-tandem mass spectroscopy (LC-MS/MS) is considered as the gold standard, and the upper limit of the normal range with this method is 45–60 ng/dL (1.6–2.1 nmol/L) [46]. A serum total testosterone >150 ng/dL may suggest the presence of an androgen-secreting tumor and requires further evaluation [46]. Routine measurement of free testosterone by equilibrium dialysis or calculated from total testosterone and SHBG is not recommended [46], while the determination of the free androgen index (FAI) could be useful in women with PCOS [47].

The routine measurement of DHEAS is not recommended in the management of PCOS, while it is suggested in case of virilization of recent onset and rapid progression, because a serum DHEAS >700 to 800 μg/dL (18.9–21.7 μmol/L) is strongly suggestive for an adrenal secreting tumor [48]. The role of serum androstenedione in the evaluation of hyperandrogenism is debated, but it has been demonstrated that a serum concentration >5 nM correlates with PCOS [49].

Androgen-secreting tumors of ovarian origin, which represent 5% among all ovarian tumors, are usually Sertoli-Leydig cell tumors, granulosa-theca cell (stroma) tumors, and hilus cell tumors. They commonly secrete testosterone and can be often identified by transvaginal ultrasonography [48].

Androgen excess is rarely caused by adrenal tumors: rarely the source of testosterone is an adrenal adenoma, while elevated serum DHEAS concentrations are suggestive for an adrenal carcinoma, which often produces not only androgens (mostly DHEA and DHEAS), but also cortisol [48].

Other uncommon causes of hyperandrogenism in premenopausal women are 21-hydroxylase deficiency, severe insulin-resistance syndrome, Cushing's disease, acromegaly, and some medications, such as valproate, an anti-epileptic drug.

Pathophysiology and Causes of Hirsutism

Hirsutism may be a clinical manifestation of hyperandrogenism, together with acne and androgenetic alopecia. It is clinically characterized by the presence of excess terminal hair growth in androgen-dependent areas (i.e., upper lip, chin, midsternum, upper abdomen, back, and buttocks) [49].

Hair follicles in humans are about five million; among them, 80,000–150,000 are on the scalp [48]. Androgens can affect the follicle size and type of hair, while the number of follicles remains the same over lifetime. Vellus hair is thin, soft, and not pigmented, while terminal hair is long, coarse, and pigmented [48].

The hair growth cycle involves three phases: *anagen*, the growth phase, which lasts differently depending on body area (about 4 months for facial hair); *catagen*,

the involutional phase, which takes 2–3 weeks of time; *telogen*, the resting phase, which lasts 3–4 months and ends with loss of hair from the follicle [48].

The differentiation of pilosebaceous units (PSUs) depends on androgens: before puberty, the hair is vellus-like, and the sebaceous glands are small in androgen-sensitive areas, while at puberty the increasing levels of androgens cause the switch from vellus to terminal hair in sexual hair areas and the growth of sebaceous follicles in sebaceous areas [48].

Hirsutism may be a clinical sign of circulating androgen excess, which is associated with increased hair growth in androgen-sensitive sites and loss of hair in the scalp region, due to the reduced duration of the anagen phase. Even if biochemical hyperandrogenism is quite common in cases of hirsutism, serum androgen concentration only modestly affects the entity of hair growth, which mostly depends upon local factors, end-organ sensitivity to serum androgen concentration, and local conversion of testosterone to DHT [48].

Several androgens may be secreted in excess (testosterone, DHEAS, androstenedione, and DHT). When the production rate of testosterone is increased, this may not result in serum total testosterone above the normal range, because increased androgen production suppresses SHBG [48].

The prevalence of hirsutism in women of reproductive age is 5–10%. In most cases it is a clinical manifestation of PCOS, together with menstrual irregularity and other clinical signs of hyperandrogenism (acne or male-pattern balding) and/or hyperandrogenemia (Table 20.2) [48].

A common feature of PCOS is an excessive secretion of testosterone, which becomes evident at puberty and adrenarche, when ovarian and adrenal androgen

Table 20.2 Main conditions presenting with clinical hyperandrogenism. PCOS=Polycystic ovary syndrome. *CCAH* classic congenital adrenal hyperplasia, *NCCAH* non-classical congenital adrenal hyperplasia

Condition	Incidence or prevalence	Age at presentation	Time of onset/ progression of symptoms	Virilization
PCOS	10% of women of reproductive age	Peripubertal	Years	Rare
CCAH	1:15,000	Congenital	Birth or infancy (ambiguous genitalia)	±
NCCAH	1:200	Congenital	Adolescence/adult	Rare
Androgen-secreting ovarian tumors	<1% of ovarian tumors (300,000 cases/year)	Third decade or later (usually postmenopausal)	Weeks/months, rapidly progressive	+
Androgen-secreting adrenal tumors	1–2 cases per million/year	Any age	Weeks/months, rapidly progressive	+
Cushing's disease	1–6 cases per million/year	Any age	Months/years	±
Ovarian hyperthecosis	Unknown, rare	Third decade or later (usually postmenopausal)	Months/years, slow onset	+

production increases, respectively. Less often the predominant androgen is androstenedione [48].

PCOS should be distinguished from idiopathic hirsutism, which affects women without hyperandrogenemia, menstrual irregularity, and identifiable causes of excessive hair growth, and is sometimes due to steroidogenic abnormalities [48].

Another condition presenting with hirsutism is non-classical congenital adrenal hyperplasia (NCCAH), which affects 1–15% of hirsute women (Table 20.2). The onset is usually around puberty with excessive hair growth, menstrual irregularity, or primary amenorrhea; it is characterized by an increased production of 17-hydroxyprogesterone and androstenedione due, in most cases, to 21-hydroxylase (P450c21) deficiency. Differently from the classic form (CAH), cortisol production is intact [48].

When hirsutism occurs together with virilization of recent onset and rapid progression and/or is associated with serum total testosterone or DHEAS way above the normal range, the presence of an androgen-secreting tumor (ovarian or adrenal) should be carefully ruled out (Table 20.2). Other uncommon causes of hirsutism are endocrine disorders, such as Cushing's disease, hyperprolactinemia, acromegaly, hypothyroidism, severe insulin resistance, or medications (valproate, minoxidil, phenytoin, and cyclosporine) [48].

Hirsutism must be distinguished by generalized hair growth, which involves androgen-independent hair and hypertrichosis: in the first case, soft vellus unpigmented hair (called *lanugo* in infants) covers the entire body, while in the second case there is a diffusely increased total body hair growth, usually due to a drug (phenytoin, penicillamine, diazoxide, minoxidil, cyclosporine) or to a systemic illness (hypothyroidism, anorexia nervosa, malnutrition, porphyria, dermatomyositis, paraneoplastic syndromes) [48].

The most commonly used objective evaluation method for hirsutism is the modified scale of Ferriman and Gallwey (F-G), which grades nine androgen-sensitive body areas from 0 to 4 [50]. The assessment should be made at baseline and at follow-up visits in order to check response to therapy. The cut-off to identify hirsutism using the F-G score should consider race and ethnicity, which are associated with different expression of hair growth. Hirsutism is identified by scores $\geq$8 in Black and White women, $\geq$9–10 in Mediterranean, Hispanic and Middle Eastern women, and $\geq$2–3 in East Asian and Native American women [48].

Noteworthy, the assessment of hirsutism also involves its impact on quality of life. In fact, even if the F-G is below the abovementioned cut-offs, we should treat any woman with "patient-important" hirsutism, defined by the Endocrine Society guidelines as "unwanted sexual hair growth of any degree that causes sufficient distress for woman to seek additional treatment" [51].

Diagnosis and Management of Polycystic Ovary Syndrome in Adults

PCOS is one of the most common endocrine disorders in women, with a prevalence of 5–10%, depending upon the population studied. The diagnosis should be suspected in any women of reproductive age with irregular menses and

hyperandrogenism, often presenting together with overweight or obesity. A particular focus should be put on hyperandrogenism, since PCOS is the most frequent cause of hirsutism. Early diagnosis and treatment are important, because this condition may impair fertility and is often associated with cardiovascular risk factors, including obesity, glucose intolerance, dyslipidemia, nonalcoholic fatty liver disease (NAFLD), and obstructive sleep apnea. Nevertheless, diagnosis is often delayed [48].

According to Rotterdam criteria, the diagnosis of PCOS requires two out of the three following: oligomenorrhea, hyperandrogenism, and polycystic ovaries detected at ultrasound [52]. Most importantly, the diagnosis can be established only once other conditions with features similar to PCOS have been ruled out, including NCCAH, thyroid disease, and hyperprolactinemia ("diagnosis of exclusion") [52].

Menstrual alterations usually occur, starting around puberty: menarche may be delayed, and there is often oligomenorrhea (infrequent menstruation, namely bleeding that occurs less frequently than every 35 days or $\leq$ 10 periods in 1 year), while amenorrhea (absence of period for at least 3 consecutive months) is less common. Menstrual cycles show a tendency to become regular after a pregnancy or after 40 years of age [48].

In any woman with oligomenorrhea/oligoovulation, other causes of irregular menses should be ruled out, by testing human chorionic gonadotropin (HCG), prolactin, thyroid-stimulating hormone (TSH), and follicle stimulating hormone (FSH). Luteinizing hormone (LH) may be relevant to help exclude hypogonadotropic and hypergonadotropic causes of oligoovulation; furthermore, a LH:FSH ratio $\geq$2 is often found in PCOS but is not considered a criterium for diagnosis [48]. Similarly, there are no established thresholds for serum anti-Müllerian hormone (AMH) concentrations suggestive for PCOS; however, AMH is usually high or in the upper range of normal levels in PCOS women compared with normal ovulatory subjects [53]. This polypeptide plays an inhibitory role in the recruitment of primordial follicles and on FSH action, likely contributing to ovulatory disturbances and hyperandrogenism [54]. In the absence of international standard ranges for AMH assays, it has been demonstrated that a serum AMH $\geq$5 ng/mL is highly predictive of PCOS [53].

Hyperandrogenism in PCOS may be clinical (hirsutism, acne, male-pattern hair loss) and/or biochemical, and in most cases both manifestations occur. In the presence of clinical signs of hyperandrogenism, a measurement of serum total testosterone is recommended, ideally by the LC-MS/MS gold-standard method; further androgen evaluation is based on clinical signs. If the patient is already on pharmacotherapy for hirsutism (i.e. oral combined contraception, metformin, or spironolactone), serum androgens should be evaluated 4–6 weeks after stopping it [50].

If other causes have been excluded, and the patient exhibits both oligomenorrhea and hyperandrogenism, the diagnosis for PCOS can be performed without adding the transvaginal ultrasound (TVUS) criterium; conversely, if the patient does not meet both criteria (oligomenorrhea and hyperandrogenism, clinical and/or biochemical), TVUS is necessary to identify a polycystic ovarian morphology (PCOM) [48]. According to the Rotterdam criteria, PCOM at ultrasound is defined by the

presence of 12 or more follicles measuring 2–9 mm in diameter at least in one ovary, and/or ovarian volume >10 mL [52]. Following the progresses in US technology, in 2018 an international evidence-based medicine group recommended a threshold of ≥20 follicles in each ovary [55]. However, it has been recognized that strong evidence on the cut-off of relevant US parameters of healthy subjects is still lacking in literature [56].

Once the diagnosis of PCOS is confirmed, associated risk factors should be evaluated. Cardiometabolic risk assessment is suggested, since obesity and insulin resistance represent a common comorbidity, having also an etiopathogenetic role in PCOS. PCOS patients have an increased risk of developing type 2 diabetes mellitus (T2DM), dyslipidemia, and coronary heart disease (CHD) [57]. At diagnosis, evaluation should include blood pressure, body mass index (BMI), waist circumference, fasting lipid profile, a 2-h oral glucose tolerance test (OGTT) or, if it is not feasible, a fasting glucose together with glycated hemoglobin (HbA1c). The screening for T2DM should be repeated every 2 years in patients with normal glucose tolerance (or more frequently in presence of additional risk factors), and annually in patients with impaired glucose tolerance (IGT), while routine assessment of insulin resistance is not recommended [58].

Furthermore, signs and symptoms of sleep apnea syndrome should be investigated (snoring, excessive daytime sleepiness, morning headache) [58].

Since anovulation and infertility are common, TVUS has a role in monitoring follicular growth and number (FNPO, follicle number per ovary) in women undergoing ovulation induction, while it is not useful as a screening method for endometrial hyperplasia or cancer in premenopausal women. TVUS may be used also to determine ovulation in women with oligomenorrhea looking for pregnancy [48].

An increased risk for nonalcoholic fatty liver disease (NAFLD) has been observed in PCOS. A universally recognized algorithm for diagnostic screening of this condition and its treatment are lacking; therefore, the focus for therapy is on lifestyle intervention. A number of diagnostic tools have been proposed, including the Fatty Liver Index (FLI) and the NAFLD Liver Fat Score, validated in the general population or in severe obese populations, Low SHBG (<33 nmol/L) has been described as a simple and reliable surrogate marker able to identify PCOS women with a high likelihood of NAFLD [33].

Regarding psychological health, a higher prevalence of mood disorders (depression and anxiety), eating disorders (i.e. binge eating), and body image concerns have been reported in PCOS than in the general population. For this reason, brief, validated questionnaires such as the Patient Health Questionnaire (PHQ)-9 for depression and the Generalized Anxiety Disorder (GAD-7) can be administered to investigate these aspects [48].

The management of PCOS should be targeted according to the patient's wish for pregnancy [59]. In general, the aim is to treat the different individual manifestations of the syndrome, including hyperandrogenism and ovulatory disfunction, reduce cardiometabolic risk, and prevent endometrial hyperplasia and carcinoma [59].

In overweight and obese women, the first-line therapy is weight loss through lifestyle intervention (calorie-restricted diets and exercise), since it has been

demonstrated that a weight loss of 5–10% is enough to improve metabolism, hyperandrogenism, and ovulation [59]. If lifestyle changes are not effective, they can be followed by pharmacotherapy and, if necessary, bariatric surgery [59].

The first-line therapy for hyperandrogenism are combined oral contraceptives (COCs). Before prescribing a COC, risk factors for venous thromboembolism (VTE), including obesity, patient age, smoking, hypertension, and family and personal history for VTE should be carefully assessed, according to the Medical Eligibility Criteria for Contraceptive Use [60]. Special attention should be paid to obesity, which is common in this population [61]. If expected benefits outweigh possible risks, it is suggested to start with 20 μg of ethynyl estradiol (EE) combined with progestins with low androgenicity, but similar VTE risk compared with levonorgestrel, such as norethindrone or norethindrone acetate [59]. Desogestrel, cyproterone acetate, drospirenone, and norgestimate are other progestins with low androgenicity: among them, only norgestimate has been correlated with a low VTE risk, similar to norethindrone and levonorgestrel, but currently there are no COCs containing this progestin combined with 20 μg of EE [59]. Transdermal or vaginal ring preparations has the same VTE risk as COCs containing levonorgestrel, but at present there is no evidence for their use in the management of hirsutism [59].

When hirsutism does not show a relevant improvement after 6 months treatment with COCs, add-on therapy is indicated, based on antiandrogens such as spironolactone, 50–100 mg twice daily [51]. In some cases, if clinical signs of hyperandrogenism are severe, COCs and antiandrogens can be started together, waiting at least a month of COC only; in these cases, higher doses of EE (30–35 μg) can also be chosen [51]. If the patient has contraindications to COCs, spironolactone may be prescribed alone, with an alternative form of contraception, since it is associated with developmental anomalies of external genitalia in a male fetus and menstrual irregularities [51]. In this regard, women with oligomenorrhea should perform a pregnancy test before starting COCs or spironolactone.

Other available antiandrogens to be used with contraception include 5-alpha-reductase inhibitors finasteride and dutasteride (which inhibit 5-alpha-reductase type 2 and both types, respectively, the enzymes converting testosterone to DHT), cyproterone acetate and flutamide. Flutamide has been recently advised against for its hepatotoxicity risks [51, 59].

In addition to contraception and their effect on signs related to clinical hyperandrogenism, COCs also prevent endometrial hyperplasia through daily progestin antagonizing the endometrial proliferative effect of estrogen. If COCs are contraindicated or unwanted, cyclic progestin therapy, continuous progestin therapy, or a progestin-releasing intrauterine device (IUD) for the treatment of menstrual dysfunction may be suggested [60, 61]. Medroxyprogesterone acetate (5–10 mg) for 10–14 days every 1–2 months can be prescribed for cyclic progestin therapy; an alternative is micronized progesterone 200 mg with the same posology. With both regimens, the patient should be advised about the lack of effect on hyperandrogenism and contraception. Conversely, both contraception and endometrial protection can be provided by continuous progestin therapy or levonorgestrel-releasing IUDs.

Gonadotropin-releasing hormone (GnRH) agonists represent another strategy effective in the suppression of ovarian androgens, but too expensive and complex. Metformin alone in the management of hirsutism is not recommended because it is less effective compared to COCs and antiandrogens; it improves ovulation in 30–50% of women with PCOS, but there are not sufficient data on endometrial protection [59]. Hirsutism may be managed also by mechanical treatments such as shaving, waxing, depilatories, electrolysis for blond hair, or laser treatment for dark hair; a topic alternative for small body areas such as chin and cheeks is eflornithine hydrochloride cream (13.9%), which inhibits hair growth by targeting ornithine decarboxylase, an enzyme involved in hair follicle development, but only during its use [51].

Thiazolidinediones have been reported to reduce ovarian androgen production and restore regular menses, but they are not recommended in women with PCOS without DM [59]. Another alternative drug is liraglutide: it is approved for obesity and the few available data in PCOS shows that it is associated with greater weight loss than placebo [59].

For women who are trying to conceive, first-line therapy is weight loss, too: it has been demonstrated that ovulation and live birth rates are higher in overweight/obese women who lose weight before starting ovulation induction therapy [59]. The strategies for weight loss are the same as for the general population.

First-line therapy for ovulation induction is letrozole, a nonsteroidal aromatase inhibitor which appears to be more effective than clomiphene citrate alone or combined with metformin, which had represented the recommended treatments for oligo-anovulation for many years [62]. However, letrozole is not approved with this indication by the U.S. Food and Drug Administration (FDA) nor the European Medical Agency (EMA), and risks, benefits, and available alternatives should be discussed with the patient before acquiring an informed consent. Metformin monotherapy is less effective than clomiphene or letrozole monotherapy on live birth rates; therefore, its routine use is not the best choice in obese women with PCOS looking for a pregnancy, unless in the presence of glucose intolerance and failure of lifestyle interventions [62].

Another alternative for ovulation induction is exogenous gonadotropins, but their administration in women with PCOS is associated with a higher risk of ovarian hyperstimulation syndrome (OHS): it has been demonstrated that a serum AMH >3.6–5 ng/mL is predictive of OHS [63]. This approach is complex and expensive and an assessment of fallopian tube patency is necessary before starting these therapies.

There are inconclusive data about the effect of other treatments on oligo-anovulation, such as thiazolidinediones, pulsatile GnRH, and acupuncture, whereas a recognized second-line treatment for women not responsive to either letrozole or clomiphene citrate is laparoscopic ovarian drilling, which shows lower risk of high order multiple gestations or OHSS compared to exogenous gonadotropins, even if it is associated with surgical risk and potential adhesion formation [59].

If these approaches are unsuccessful, in vitro fertilization (IVF) may be required: metformin administration before or during IVF cycles and transfer of frozen rather

than fresh embryos are possible strategies to reduce the risk of hyperstimulation and multiple gestation, which is increased in women with PCOS [62].

Other comorbidities like T2DM, dyslipidemia, obstructive sleep apnea, NAFLD, and depression/anxiety require specific treatment.

Diagnosis and Management of Polycystic Ovary Syndrome in Adolescents

Diagnostic criteria of PCOS in adolescents are similar to those identified for premenopausal women. However, once differential diagnoses have been ruled out, a diagnosis of PCOS should be avoided in those adolescents who display transient hyperandrogenemia without clinical signs of hyperandrogenism, because it may represent a physiological consequence of anovulation in the first menstrual cycles [64]. A reassessment is advised if hyperandrogenism and irregular menses persist for at least 2 years, and PCOS diagnosis in mid-adolescence is allowed when hirsutism, hyperandrogenemia, and menstrual abnormalities occur all together [65]. In the presence of clinical manifestations of PCOS that do not fulfill the diagnostic criteria, PCOS should not be ruled out until menstrual cycles and serum androgen concentrations normalize [65].

In this population, US plays a role in differential diagnosis in order to exclude ovarian androgen-secreting tumors and other pelvic conditions, but should not be used to identify PCOM, since about one-quarter to one-half of normal adolescents between 14 and 17 years of age appear to meet Rotterdam adult criteria for PCOM as a result of a transient stage of normal ovarian development [65].

Adrenal Hyperandrogenism

DHEA and DHEA-sulfate (DHEAS) are the primary adrenal androgens, secreted under the stimulation of ACTH; in fact, the ovaries produce less than 10% of these precursors. Adrenal hyperandrogenism is characterized by an excess of their secretion; however, due to their weak androgenic activity, hirsutism and virilization are actually caused by their conversion to more potent androgens like androstenedione and testosterone in both adrenal glands and peripheral tissues (hair follicles, sebaceous glands, external genitalia, adipose tissue) [66].

A little amount of DHEA and DHEAS is secreted during infancy and early childhood, while their production increases at adrenarche, a phenomenon occurring in the prepubertal phase, characterized by the emergence of pubic and/or axillary hair in parallel with the development of the zona reticularis of the adrenal cortex, but independent from the secretion of ACTH and cortisol. In women, androstenedione and testosterone derived directly from the adrenals or peripherally from DHEA are a consistent part of total androgen production, with changes throughout the menstrual cycle: for example, in the follicular phase 65% of testosterone is produced by adrenals, versus only 4% in the midcycle, due to a rise in ovarian production. DHEA

and DHEAS secretion physiologically increases from puberty to the third decade of life and then shows a progressive decline [2, 3].

In case of excess of adrenal androgens, clinical manifestations depend upon the age at onset: prepubertal girls may develop signs of heterosexual precocious puberty (hirsutism, acne, clitoromegaly) together with premature epiphyseal fusion leading to short adult height, while pubertal girls may experience virilization, primary or secondary amenorrhea, increased skeletal maturation and, in case of concurrent hypercortisolism, gonadal suppression and stunt linear growth; adult women may develop clinical hyperandrogenism (hirsutism, acne, male-pattern baldness) or frank virilization, oligomenorrhea, and infertility [66].

Adrenal hyperandrogenism can be primary adrenal- or ACTH-dependent. Primary adrenal causes include premature adrenarche and adrenal tumors. Premature adrenarche is the isolated development of pubic or axillary hair without other signs of puberty before the age of 8 in girls; it is not progressive and treatment is not usually necessary, because puberty usually begins at the expected time [67]. Conversely, adrenal tumors include benign, malignant, or bilateral macronodular adrenal hyperplasia, producing mainly or only androgens. Adrenal adenomas are usually not-secreting, while rarely they produce androgens or testosterone only that are not suppressed by dexamethasone, resulting in mild clinical signs of hyperandrogenism [68]. Androgen-secreting adrenal carcinomas are extremely rare malignancies and typically secrete not only androgens, but also other steroid precursors and cortisol; their clinical presentation is more severe, and a palpable abdominal mass or a metastatic disease is possible at diagnosis, associated with increased serum concentration of DHEA, DHEAS, and often cortisol not suppressed by high-dose dexamethasone and high urinary 17-ketosteroid and cortisol excretion [66]. Androgen-secreting adrenal carcinomas account for a tiny proportion of women presenting with signs of androgen excess.

The clinical picture of bilateral macronodular adrenal hyperplasia is mostly characterized by subclinical or overt Cushing's syndrome, but sometimes cortisol may be co-secreted with other steroids, including androgens.

In women with hirsutism or virilization of unknown cause the measurement of serum DHEA or DHEAS concentrations is recommended, and the results should be interpreted according to age: a serum DHEAS >500 μg/dL (13.6 μmol/L) in a young woman is strongly suspicious for the presence of an adrenal tumor, even if a higher cut-off of 700 μg/dL (19 μmol/L) has been proposed [66]. Computed tomography (CT) and MRI (magnetic resonance imaging) are performed to detect adrenal masses: typically, adenomas have low signal intensity on T1- and T2-weighted MRI images and are smaller than 4 cm, while carcinomas have low signal intensity on T1-weighted images and enhanced activity on T2-weighted images, and in most cases are larger than 5 cm and extended to the capsule or to the surrounded tissues. For both, surgery is the treatment of choice when feasible.

ACTH-dependent causes of adrenal hyperandrogenism include ACTH-dependent Cushing's syndrome, congenital enzyme deficiencies (congenital adrenal

hyperplasia, hexose-6-fosfate-dehydrogenase deficiency, placental aromatase deficiency, and P450 oxidoreductase deficiency), and primary glucocorticoid resistance syndrome and require specific treatment if necessary [69].

Evaluation and Management of Postmenopausal Hyperandrogenism

As already discussed, hyperandrogenism affects about 10% of women, but its onset in menopause is rare [70]. Normal serum androgen concentration in postmenopausal women depends upon the dosage method and the laboratory, but generally the following cut-off is accepted: total testosterone 20–70 ng/dL (0.5–2.8 nmol/L), androstenedione 0.5–2.8 ng/mL (1.5–12 nmol/L), DHEAS 18–185 μg/dL (0.5–5 μmol/L) [71].

Hirsutism and male-pattern hair loss are the most common signs of hyperandrogenism in menopause, while virilization usually occurs only when serum androgen concentrations are very high. Virilization signs (i.e. clitoromegaly) should always direct toward the suspicion of an androgen-secreting adrenal or ovarian tumor, of ovarian hyperthecosis, even if symptom progression is not rapid. A detailed history is helpful in identifying non-oncological causes of hyperandrogenism, such as PCOS and NCCAH (functional hyperandrogenism), exogenous administration, or drugs [72].

The first laboratory test to assess is serum total testosterone, together with serum DHEAS concentration in order to identify a possible adrenal source of androgens. If these laboratory tests result within the normal range, urinary 17-ketosteroid excretion could be useful in the diagnosis of an adrenal carcinoma. Other laboratory tests include prolactin, 17-hydroxyprogesterone, and dynamic tests for Cushing's syndrome or acromegaly in case of a specific clinical suspect or these endocrinopathies. If 17-hydroxyprogesterone serum concentrations are high, it is suggested to rule out an androgen-secreting tumor before confirming the diagnosis of CAH, especially in the presence of new onset virilization and suppressed serum gonadotropin concentrations [73]. It has been also proposed to measure inhibin serum concentrations in order to identify an ovarian androgen tumor [74].

Functional hyperandrogenism usually manifests clinically before menopause. If symptoms and signs of hyperandrogenism worsen after menopause, are associated with mild/moderate biochemical hyperandrogenism, display a low progression and virilization is absent, the picture is consistent with the pre-existing or unrecognized forms of functional hyperandrogenism aggravated by menopause. In other cases (new onset of hirsutism and/or male-pattern hair loss after menopause, severe biochemical hyperandrogenism, rapid progression, virilization present) ovarian TVUS/MRI and/or adrenal CT/MRI should be performed [73]. Severe biochemical hyperandrogenism is defined as total testosterone >150 ng/dL or DHEAS >700–800 ng/dL [71].

Small ovarian tumors identified through pelvic RM or fluorodeoxyglucose-positron emission tomography (FDG-PET) have been described, but limited data

are available. Chemical shift MRI can reveal intracytoplasmic crystal of Reinke, which is histologically suggestive but not diagnostic of Leydig cell tumor. If this diagnostic assessment is inconclusive, an ovarian and adrenal sampling is rarely performed in menopause. In this case the most frequent source of androgens is an ovarian tumor, which is usually too small to be identified by imaging. A low-dose dexamethasone suppression test could be helpful in differentiating neoplastic and non-neoplastic origin [71, 73].

The most frequent ovarian virilizing tumors are Sertoli-Leydig cell and Leydig cell tumors, which represent less than <0.5% of all ovarian tumors and occur after menopause in a quarter of cases. These tumors are usually confined to ovary at diagnosis and are rarely malignant [75].

Ovarian hyperthecosis is a functional postmenopausal pathology, characterized by slowly progressive virilization. In these cases, TVUS usually reveals ovaries of an increased volume compared with healthy postmenopausal women; the diagnosis should be confirmed histologically [73].

Surgery is the treatment of choice for androgen-secreting tumors and ovarian hyperthecosis, while GnRH agonist therapy is an effective alternative for patients who are not good surgical candidate or do not want surgery [73].

The management of the other forms of hyperandrogenism is the treatment of the underlying disease or the removal of the trigger cause.

Androgen Deficiency

Diagnostic Controversies

Female androgen deficiency is a controversial concept. In the early 2000s, it was described as a clinical entity characterized by low libido, fatigue, loss of bone density with no other apparent etiology, reduced muscle strength, mood changes, reduced general well-being, hair thinning, and perceived alteration in cognition and memory. However, the most recent guidelines on the topic do not support the diagnosis of an "androgen deficiency syndrome" in otherwise healthy women, given the lack of specific signs and symptoms and of age-specific reference ranges for androgen concentrations; moreover, serum androgen levels do not reliably correlate with a clinically defined syndrome [76]. These shortcomings also prevent to establish a cut-off for testosterone, which would allow the diagnosis of "low testosterone"-related female sexual dysfunction. This is different from men, in which the diagnosis of functional hypogonadism is recommended in case of clinical symptoms (especially sexual symptoms: low libido, reduced morning erections, and erectile dysfunction) and serum testosterone levels repeatedly below the normal range, measured in the morning with a well-validated assay [77].

Other diagnostic pitfalls for androgen deficiency in women are relative to the fact that circulating levels of testosterone and its derivatives are claimed to not reflect the real exposure of peripheral tissues to androgens, due to dramatic genetic differences in the distribution and sensitivity of the AR and in the distribution and activity of

5α-reductase and aromatase in peripheral tissues. For these reasons, the decision to start a therapy with testosterone in women with hypoactive sexual desire disorder (HSDD) should not be taken according to the serum levels of testosterone or other androgens, although these should be measured at baseline, to avoid treating a patient with clinical hyperandrogenism (see *systemic testosterone therapy and female sexual dysfunction*).

Causes

The most common cause of androgen insufficiency in women is represented by the physiologic decline with age from mid-to-late reproductive years, substantiated by the decline in production by the ovaries and adrenal glands.

Other conditions or physiological states include [78]:

- hypogonadotropic hypogonadism, which may result from alterations of the hypothalamic-pituitary-gonadal axis (i.e. pituitary secreting and non-secreting adenomas, functional hypothalamic amenorrhea in underweight women, anorexia nervosa, athletes, or in chronic stressful conditions);
- primary ovarian insufficiency secondary to surgery, chemotherapy or radiation therapy, autoimmune oophoritis, or genetic defects (mutations of FMR1, Turner syndrome);
- loss of adrenal production of pre-androgens, due to central mechanisms (panhypopituitarism of ACTH deficiency) or adrenal insufficiency (Addison's disease, chronic systemic glucocorticosteroid therapy);
- hyperprolactinemia (prolactin-secreting tumors or functional hyperprolactinemia, i.e. in women taking antipsychotic agents) causing suppression of pituitary gonadotropins;
- reduced bioavailable testosterone secondary to increased SHBG: treatment with COCs, phenobarbital, phenytoin, carbamazepine, and thyroxine; chronic liver diseases; HIV infections; hyperthyroidism,
- pregnancy, puerperium, and lactation.

A clinical picture suggestive of hypoandrogenism can also be found in the absence of the abovementioned conditions, in premenopausal women who menstruate regularly; at laboratory investigation, they may present idiopathically low levels of total and free testosterone and other androgens.

Among women presenting iatrogenic causes of androgen insufficiency, those undergoing surgical menopause experience the most abrupt and significant reduction of circulating androgens levels. Indeed, testosterone and androstenedione decline by approximately 50% after oophorectomy. This may contribute to the high prevalence of female sexual dysfunction in this population; however, evidence supporting the stronger negative impact of surgical versus natural menopause on female sexuality is not conclusive. Although in a cross-sectional survey of women aged 20–70 years surgical menopause was associated with a clinically relevant and

significantly higher risk of HSDD than natural menopause [79], in another study oophorectomized women showed a lower risk of dyspareunia than those with a history of natural menopause, despite having lower testosterone levels [80].

Regarding women with hypopituitarism, in a Swedish study, hypoandrogenism—defined as subnormal levels of DHEAS and/or androstenedione—was demonstrated in 61% of the total sample and in 92% of those with ACTH deficiency [81].

Systemic Testosterone Therapy and Female Sexual Dysfunction: The Evidence

Since 2019, the use of testosterone therapy for women should follow the Global Consensus Position Statement, endorsed by the main international societies involved in women's sexual health [82]. Several RCTs and a meta-analysis on 36 RCTs and 8480 participants [83] have established the short-term (24 months) efficacy and safety of testosterone in naturally and surgically postmenopausal women, combined or not with estrogen replacement therapy, in doses that approximate physiological concentrations for premenopausal women (Table 20.3). The therapeutic effect was judged as moderate. Conversely, evidence is lacking to support the use of testosterone in premenopausal women [82]. The only evidence-based indication is hypoactive sexual desire disorder (HSDD) [82, 83], although sexual symptoms relative to other domains of sexual function, such as poor lubrication or orgasm ability, which are often comorbid with low libido, may benefit from treatment. In fact, meta-analytic data showed a significant improvement on secondary outcomes including arousal, responsiveness, self-image and sexual-related concerns and distress with testosterone versus placebo or a comparator [84].

In a recent retrospective study on 81 women with sexual complaints, 6 months systemic testosterone administration, alone or combined with local estrogens, was correlated with a positive effect on clitoral blood flow, namely an increase in clitoral artery peak systolic velocity evaluated at Doppler ultrasound [85]. If these data are confirmed in larger, prospective, and controlled studies, testosterone could be considered also for the treatment of female genital arousal disorders.

Systemic Testosterone Therapy and Female Sexual Dysfunction: A Practical Guide

Recently, the International Society for the Study of Women's Sexual Health (ISSWSH) endorsed a clinical practice guideline for the use of systemic testosterone for HSDD in women [86]. According to this document, the candidates for therapy are postmenopausal (natural or surgical) women with a diagnosis of HSDD, with or without concomitant estrogen replacement therapy. It is important to note that HSDD should be carefully diagnosed according to the biopsychosocial model of care as a state of "reduced or absent spontaneous desire (sexual thoughts or fantasies), and/or reduced or absent responsive desire to erotic cues and stimulation,

Table 20.3 Available and off-label local and systemic androgen-based therapies for the treatment of vulvovaginal atrophy/genitourinary syndrome of menopause and related sexual dysfunction. Modified and reproduced with permission from [25]

Compound	Formulation	Status	Known/proposed benefits on vaginal function	Supposed mechanisms of action in the vagina	Quality of evidence
Local DHEA (prasterone)	Daily 6.5 mg intravaginal insert 0.50%	Approved in the USA and Europe for moderate-severe dyspareunia in menopausal women	Beneficial effects on dyspareunia, vaginal dryness, VMI, and pH	Converted into E and A within the vagina (intracrine metabolism)	Placebo-controlled RCTs
			Improvement in desire/interest, arousal, and orgasm	Aromatization-independent effects to be clarified	
			Potential application for GSM-related UTIs		
Systemic DHEA	Oral 25–100 mg daily	Available over-the-counter in USA as a food supplement, off-label in Europe[a]	No clear improvement in sexual function in menopausal women with normal adrenal function	Converted peripherally into E and A	Meta-analysis of RCTs
Local testosterone	Testosterone vaginal cream	Off-label	Improvement in self-reported vaginal dryness, dyspareunia, and sexual function	Increase in local blood flow, density of nerve fibers, mucous secretions, and formation of collagen	Overall low quality (open label designs and lack of control arms)
			Improvement in objective clinical outcomes (vaginal trophism)	Studies on women taking AIs support an aromatization-independent effect	
Systemic testosterone	Different compounds	Off-label in most countries (1% cream available in Australia)	In postmenopausal women T treatment at high physiologic doses, with or w/o HRT, improves not only desire, but also arousal, orgasmic function, pleasure, and sexual responsiveness	Direct vaginal effects of systemic therapy have to be clarified	Meta-analysis of RCTs

A androgens, *AI* aromatase inhibitors, *DHEA* dehydroepiandrosterone, *E* estrogens, *GSM* genitourinary syndrome of menopause, *HRT* hormonal replacement therapy, *RCT* randomized clinical trials, *T* testosterone, *UTI* urinary tract infections, *VMI* vaginal maturation index

[a] DHEA-based galenic preparation for systemic treatment have been recently prohibited in Italy due to its potential use as an illicit doping agent

and/or inability to sustain desire or interest in sexual activity once initiated" [87]. These symptoms should be persistent, generalized, and associated with clinically significant distress, and not accounted for by potentially modifiable factors such as psychiatric diseases, use of medications or couple issues [87]. According to the biopsychosocial model of care, testosterone treatment may be considered after and/or during appropriate management of other psychological, biological, and socio-relational conditions that may contribute to low desire [19].

When prescribing testosterone therapy, oral preparations are not recommended due to the potential adverse events on lipid profile, while pellets or intramuscular injections may lead to supraphysiologic dosing and should be avoided [82].

Testosterone is not approved for women by most regulatory agencies, including FDA and EMA, whereas a 1% (10 mg/mL) cream is available in Australia (Australian Register of Therapeutics Goods) for the treatment of symptoms associated with androgen deficiency, with a suggested starting dose of 0.5 mL dose (=5 mg of testosterone/day, bioavailability around 10%). In 2006, a 300-μg/24-h testosterone patch was licensed in Europe for HSDD in women with surgical menopause receiving estrogen replacement therapy but was removed from the market after some years for commercial reasons. Since compounded products lack evidence for efficacy and safety, it is reasonable to prescribe off-label transdermal approved male formulations at approximately 1/10 of the male dose, in order to mimic the physiological production rate of 0.3 mg/day (300 μg/day) in women. Dosing should be targeted to achieve testosterone concentrations in the physiologic premenopausal range [82].

On average, efficacy of treatment becomes evident after 6–8 weeks and maximal at about 12 weeks. It is recommended to discontinue testosterone after 6 months if no improvement on sexual desire is acknowledged by the patient. Noteworthy, no safety data are available beyond 24 months, and the decision to continue the treatment should be carefully discussed with every patient, examining potential risks in the context of her clinical history and expectations [82].

There is no serum testosterone level to aim at as a treatment goal. Total testosterone levels should be measured before starting therapy, in order to exclude women with midrange to high baseline concentrations, 3–6 weeks after initiating therapy and after 6 weeks if dosing is increased to exclude over-dosing, and finally every 4–6 months once stable levels are achieved [82].

Side effects of systemic testosterone treatment include increased risk of developing acne and hair growth. Patients should be assessed for these and other signs of androgen excess and virilization, such as alopecia, clitoromegaly, or voice deepening; however, these adverse events are usually dose-related and have not been described with transdermal formulations and premenopausal physiologic levels [76, 84].

Regarding androgen and estrogen-sensitive tumors, adequately powered RCTs, specifically designed with the primary outcome of breast cancer, endometrium and ovarian cancer incidence, are needed to provide data on these aspects of safety. At the moment, testosterone should not be prescribed in women with a history of, or at

high risk, for these malignancies. In several small trials, no change in mammographic breast density has been detected and no increased risk of breast cancer, endometrial hyperplasia, or cancer in prior testosterone users has been highlighted up to 24 months [88]. Noteworthy, testosterone therapy in postmenopausal women was not associated with a significant increase in estradiol levels in a meta-analytic study, suggesting that aromatization is not clinically relevant, at least at systemic level [89]. Clinical surveillance with pelvic examination and mammography is recommended in women treated with testosterone in accordance with age and contemporary country-specific guidelines [86].

Systemic Testosterone Therapy: Non-sexual Effects

Systemic testosterone therapy did not result in significant improvement in terms of body composition (lean body mass, total body fat), musculoskeletal parameters (bone mineral density, fracture risk, and muscle strength), or cognitive performance in a recent meta-analysis of RCTs; however, it should be noted that the number of subjects included in these sub-analyses was scarce [84]. In women with hypopituitarism, androgen deficiency has been postulated to contribute to reduced bone mass and increased mortality and morbidity. Accordingly, it has been shown that these patients may benefit from short-term testosterone therapy for various outcomes, including hip body mass density, muscle mass, and mood [90], although these data should be confirmed in larger studies.

Transdermal testosterone has not been associated with a worsening of lipid profile, differently from oral testosterone, which tends to increase low density lipoprotein (LDL) cholesterol levels and should therefore be avoided [84]. No increase in blood pressure, fasting glucose, or insulin levels has emerged in trials up to 24 months, independently of the route of administration [84].

Regarding the effect of testosterone on cognition, conclusive evidence is lacking, highlighting the need for adequately powered trials with this as a primary outcome. In 50 surgically menopausal women, combined therapy with oral testosterone undecanoate and estrogen valerate was associated with a deterioration of immediate verbal memory versus estrogen combined with placebo [91]. On the contrary, in a study on 89 postmenopausal women not on estrogen therapy, transdermal testosterone improved verbal learning and memory compared with placebo [92].

It is well known that hypogonadal men are at risk for mood disorders, and that testosterone-replacement therapy carries a mitigating effect on depressive symptoms in males; in line with this view, depression is more prevalent in women and clinical and preclinical data support a protective effect of endogenous androgens on mood. Large, controlled studies addressing the efficacy of exogenous testosterone on these outcomes in women with or without depression are missing. Available evidence of RCTs conducted in patients with sexual dysfunction, hypopituitarism, bilateral oophorectomy, or anorexia nervosa suggest a modest improvement in mood as a secondary endpoint [93].

Other Systemic Preparations

Systemic DHEA, in galenic preparations or as a food supplement, has been prescribed in the last decades with the aim of improving general well-being and sexual function in peri- and postmenopausal women; however, its effectiveness and safety have been debated (Table 20.3). In 2015, a Cochrane Database Systematic Review on controlled trials concluded that there was no evidence that DHEA improved overall quality of life; uncertain findings emerged on menopausal symptoms and sexual function [94]. In the recent Global Consensus Position Statement, systemic DHEA is not recommended for the treatment of postmenopausal women with normal adrenal function and HSDD, due to the lack of convincing evidence regarding its benefits on desire or sexual function [86].

Local Androgen Therapy

Intravaginal DHEA (prasterone) is currently approved both in the USA and in Europe for the treatment of moderate to severe vulvovaginal atrophy in menopausal women (Table 20.3). In a phase III, placebo-controlled clinical trial, daily intravaginal 0.50% prasterone 6.5 mg for 12 weeks resulted in a statistically significant improvement of dyspareunia, vaginal pH and VMI (vaginal maturation index), used as an objective assessment of the estrogen status of the vaginal epithelium [95]. The majority of studies does not indicate a relevant increase in circulating sex steroids levels with intravaginal DHEA.

Recent data, obtained using in vitro supplementation of DHEA in human vaginal smooth muscle cells and LC-MS for steroid measurement, have demonstrated that the beneficial effects of this pro-hormone are mediated not so much by its conversion to estrogens, as by its transformation into active androgens, such as androstenedione, testosterone, and especially DHT [28].

Further research on the efficacy and safety of intravaginal DHEA is warranted in women with a history of hormone dependent cancers.

Testosterone-based preparations for intravaginal use are off-label (Table 20.3). In 2018, a review on the topic highlighted that, in six available clinical trials, the overall findings showed an overall positive effect, with a reduction in lower vaginal pH, an increase in the proportion of vaginal lactobacilli, and an improvement in the VMI (vaginal maturation index) [96]. The VMI is often used in trials as an objective assessment of the estrogen status of the vaginal epithelium. Data in sexual outcomes (i.e. dyspareunia) with intravaginal testosterone are encouraging, although of low quality.

Interestingly, studies on women taking aromatase inhibitors (AIs) for breast cancer and experiencing an improvement on genitourinary and sexual discomfort with intravaginal androgens support an aromatization-independent effect [97].

Conclusions

Androgens play a pivotal role in women across the life span. Over the last years, there have been some advances in our understanding of the involvement of testosterone and its precursors both in the normal physiology and in pathologic conditions. For example, hyperandrogenism is implicated in the etiopathology and clinical manifestations of the polycystic ovary syndrome, the most common endocrinopathy in women of reproductive age, carrying major consequences on fertility, cardiometabolic risk, and well-being. However, clinicians should be able to manage hyperandrogenism also in the postmenopausal phase since it can be the expression of life-threatening disorders such as androgen-secreting tumors. Finally, female androgen deficiency is a much-debated issue: current evidence is against a diagnosis, as the syndrome is not well defined, but supports short-term systemic testosterone therapy in postmenopausal women with primary, generalized hypoactive sexual desire disorder, identified following a full clinical evaluation.

Major pitfalls and research gaps remain: the limited use of accurate methods for the measurement of androgens levels, the lack of age-specific cut-offs, and a superficial knowledge of the effects of endogenous and exogenous androgens on countless areas, including but not limited to: sexual function, mood, cognition, musculoskeletal health, cardiovascular health, hormone-dependent cancers, and fertility.

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Transgender Adult Males and Testosterone Hormone Therapy

21

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Introduction

The term "transgender" is generally used to describe a diverse group of individuals. "Transgender" must be used as an adjective ("transgender people") and not as a noun ("transgender") [1, 2]. The new guidelines have replaced the term "transsexual" with gender dysphoria or gender incongruence and thus advise against using the term transsexual in clinical practice [1, 2].

This chapter will use the term "transgender males" in the broadest sense to include any male with incongruity between gender identity and external sexual anatomy at birth. This chapter will discuss the care and therapy of adult transgender males and will not focus on the assessment and management of adolescents or transgender female.

Gender identity is conceptualized as an individual's innate sense of feeling masculine, feminine, neither, nor some combination of both. Transgender males (female-to-male, FTM) have a male gender identity but were assigned the female sex at birth [1, 3, 4].

We call gender expression the way gender is presented to the outside world (e.g. female, male, androgynous). It does not necessarily correlate with sex or gender identity assigned at birth. Gender dysphoria or incongruity, however, arises from the

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anguish or discomfort that can occur in the individual when the gender identity and the sex assigned at birth are not completely congruent [1, 3, 4].

Many people confuse "sexual orientation" and "gender identity." Gender identity refers to an individual's innate sense of feeling masculine, feminine, neither, nor some combination of both. However, sexual orientation relates to an individual's pattern of physical and emotional arousal (including fantasies, activities, and behaviors) and the gender(s) of the people they are physically or sexually attracted to (gay/lesbian, heterosexual, bisexual) [1, 2].

More recently there has been an increase in the number of people identifying with non-binary gender. They include individuals of any sex assigned at birth who have a gender identity that is neither male nor female, is a combination of the two, or is fluid. Other terms that can be used for non-binary gender identity include: genderqueer, gender creative, gender independent, bigender, noncisgender, agender, two-spirit, third sex, and gender blender [2, 4].

Healthcare professionals, along with their caregivers, should be familiar with the most used terms and understand that there is a diversity of identities within the transgender community.

Long-term prospective studies for most health problems in transgender individuals are lacking, resulting in variable recommendations for preventive care based primarily on observational studies and experts' opinions [1, 5]. A study of a historical cohort from the Netherlands involved 966 transgender female and 365 transgender male patients, with hormone use of at least 1 year and an average follow-up of 18.5 years [6]. Mortality of transgender males was like that of women in the general population. Another retrospective study of Veterans Health Administration, including 5117 transgender patients, identified that the suicide rate among transgender males was higher than that of the general population, but similar to the suicide rate for veterans with severe mental illness (e.g., depression or schizophrenia) [7].

Diagnostic Criteria

Current criteria for gender incongruity include: (1) persistent incongruity between gender identity and external sexual anatomy at birth; (2) absence of a mental disorder or other associated abnormality that could confuse the diagnosis [1, 3, 4].

The diagnosis of gender incongruity must be made before considering hormone therapy or surgical therapy for gender affirmation and must include evaluation with a multidisciplinary team of professionals. It is essential to identify any medical and/or psychiatric diagnoses that may require treatment before considering hormone therapy [1, 4].

The diagnosis of gender dysphoria should preferably be made by a mental health professional or by a health professional who has experience and appropriate training. Current diagnostic criteria for gender dysphoria follow the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) [8]. Central components of the DSM-5 diagnosis of gender dysphoria include longstanding discomfort with the incongruity between gender identity and external sexual anatomy at birth,

along with interference with social life, school activities, or other areas of function [8].

Recently the new International Classification of Diseases (ICD-11) came into force. The World Health Organization (WHO) removed from ICD-11 the so-called gender identity disorder from the mental illness section and included "gender incongruity" in the section "conditions related to sexual health" (HA60).

Clinical Evaluation

The clinical evaluation of global health is like that of non-transgender patients and must include respect, acceptance, and confidentiality from the first evaluation. Health professionals must ask the transgender patient about their preferred name and pronoun, and these should be respected [1, 3, 4, 9].

During the clinical history, there are some topics that must be addressed, such as: possible hormonal and surgical interventions previously related (or their desire to perform them in the future); personal and family history (including risk of thromboembolism and hormone-sensitive neoplasms); reproductive history (gynecological and obstetric history, preventive tests according to specific guidelines and desire for future fertility); social history; sexual history with evaluation and guidance regarding the risk of sexually transmitted diseases and sexual function; psychiatric history (should be evaluated for mood disorders, especially depression and anxiety, risk or history of suicide attempt, substance use and abuse, and post-traumatic stress disorder, including history of physical, sexual, or emotional trauma or abuse) [1, 3, 4, 9].

The physical examination should be based on the organs present and the symptoms presented by the patient. However, it is pointed out that many transgender males feel uncomfortable with the exposure of their bodies and physical examination is recommended after creating a relationship of trust and respect [1, 3, 4].

Screening and prevention of chronic diseases in transgender males is the same as in the general population. However, possible health conditions that may be associated with risks or that may be aggravated by hormone therapy must be evaluated (Table 21.1) [1].

It is noteworthy that the prevalence of smoking is higher among transgender patients compared to the general population. Similar to non-transgender patients,

Table 21.1 Medical risks associated with sex hormone therapy in transgender males

Moderate risk of adverse outcomes
• Breast or uterine cancer
• Cerebrovascular disease
• Coronary artery disease
• Hypertension
• Severe liver dysfunction (transaminases, threefold upper limit of normal)
Very high risk of adverse outcomes
• Erythrocytosis (hematocrit > 50%)

Modified from: Hembree WC et al. J Clin Endocrinol Metab. 2017 Nov 1;102(11):3869-3903 [1]

smoking cessation should be encouraged and resources to make it happen must be provided [4].

Transgender males, including those with risk factors for diabetes mellitus (DM), including family history, obesity, metabolic syndrome, hypertension, acanthosis *nigricans*, history of gestational diabetes, and the presence of polycystic ovary syndrome (PCOS), should be screened for DM according to specific guidelines [10–13].

The recommendations for screening for sexually transmitted infections are the same as the ones for non-transgender patients. It is particularly important to have a good sexual history, as screening should be based on behaviors and number of sexual partners. Screening should also consider the current anatomy of the patient [9, 14, 15].

Hormone Therapy

The two main goals of hormone therapy are: (1) to reduce the levels of endogenous sex hormones and thereby reduce the secondary sexual characteristics of the birth gender and (2) to induce hormone levels consistent (in the physiological range) with the sex of identity using the principles of hormone replacement treatment of patients with hypogonadism [1, 3, 4, 16–19].

The main medical societies recommend in their guidelines that before starting hormone therapy the diagnostic criteria for gender dysphoria/gender incongruity must be confirmed mainly by an experienced physician [1, 3, 4].

The physical changes induced by sexual hormone transition are usually accompanied by an improvement in mental well-being [20, 21] and although mental health problems are more prevalent in transgender people compared to cisgender people, fewer psychological difficulties and greater life satisfaction occur with gender-affirming hormone treatment [22]. However, we must emphasize that the beginning of hormone therapy should follow protocols and it should not be started if there are doubts about the diagnosis. The physician must evaluate the patient's decision-making capacity and the use of hormone therapy must be fully informed and consented to (preferably from a written and free informed consent containing all risks and benefits of gender-affirming hormone therapy). If there are associated mental health conditions, these must be controlled. In case of doubt, therapy must be postponed until sufficient safety data are available for its prescription [1, 3, 4, 17, 18].

Similarly, it is recommended that medical conditions that may be associated with gender-affirming hormone treatment be evaluated and addressed, as described in Table 21.1 [1].

Historically, some transgender males have self-medicated with hormones for a variety of reasons, including fear of rejection by health professionals, delays in starting hormone therapy, and the cost of treatment. Therefore, there must be a careful evaluation of self-medication, both past and current, [1, 4] and guidance on the health risks of this practice must be provided.

After the start of hormone therapy, it is advised that hormone levels are evaluated during treatment to ensure physiological levels of the desired sex hormones and the suppression of sex hormones of the birth sex [1].

In transgender males, the initial goal of hormone treatment is to stop menstruation and induce virilism, including a male pattern of facial, body, and sexual hair, thickening of the voice [23, 24], and male physical contours. To this end, the main hormone treatment is a preparation containing testosterone [1, 4].

Similar to androgen therapy in men with hypogonadism, testosterone treatment in transgender males results in increased muscle mass and decreased fat mass [25, 26], breast reduction [27], increased sexual desire [28–31], increased skin oiliness and acne [32, 33], male pattern baldness in those genetically predisposed individuals [32, 33], clitoromegaly and temporary or permanent decrease in fertility [1]. Transgender males' height and hip configuration do not change with testosterone treatment [34].

The cessation of menstruation can occur within a few months with testosterone treatment alone. However, if uterine bleeding continues, the addition of progestins or endometrial ablation may be considered [35, 36]. Another option that may be considered is the pretreatment administration of GnRH analogues or depot medroxyprogesterone to stop menstruation before the start of testosterone treatment [1, 3, 4].

There are some references in the medical literature to indicate hysterectomy to avoid the risk of endometrial cancer by exposure to androgen. However, there is no evidence of an excessive risk of endometrial cancer in transgender males receiving androgen therapy. In a study of 35 transgender males receiving testosterone undecanoate 1000 mg every 12 weeks for 1 year, the average endometrial thickness (by pelvic ultrasound) decreased from 9.9 to 5.7 mm [30]. In a second study, histological analysis found inactive and atrophic endometrium in transgender males on long-term testosterone therapy, like that observed in postmenopausal biological women not on estrogen therapy [37].

Many of the physical changes that are expected during hormone therapy occur within the first 6 months. However, changes such as thickening of the voice [23, 38], clitoromegaly, and male pattern hair loss [26, 39, 40] may occur only at the end of the first year. The effects of virilism found in the process of gender-affirming hormone therapy and their expected average times are showed in Table 21.2.

Table 21.2 Masculinizing effects in transgender males

Effect	Onset	Maximum
Clitoral enlargement	1–6 months	1–2 years
Skin oiliness/acne	1–6 months	1–2 years
Vaginal atrophy	1–6 months	1–2 years
Fat redistribution	1–6 months	2–5 years
Cessation of menses	1–6 months	[a]
Deepening of voice	6–12 months	1–2 years
Increased muscle mass/strength	6–12 months	2–5 years
Facial/body hair growth	6–12 months	4–5 years
Scalp hair loss	6–12 months	[b]

Modified from: Hembree WC et al. J Clin Endocrinol Metab. 2017 Nov 1;102(11):3869-3903 [1]

[a]Menorrhagia requires diagnosis and treatment by a gynecologist

[b]Prevention and treatment as recommended for biological men

Spontaneous thickening of the voice occurs during testosterone treatment of transgender males; however, voice therapy with a speech therapist may be an additional option to assist in the transformation of speech patterns to the desired sex [1].

Among transgender males, testosterone therapy can increase libido. Sexual function (libido, arousal, pain during sex, and orgasm) varies after sex reassignment surgery and must be evaluated on an individual basis [29].

Studies have also shown the effect of testosterone replacement use on structural changes in the brain detected by quantitative magnetic resonance. Evidence suggests that there is an induction of increased cortical volume and thickness and subcortical structural volume likely due to anabolic effects. Animal models, developed specifically to test the anabolic hypothesis, suggest that testosterone and estradiol, its aromatized metabolite, participate in the control of astrocyte water traffic, thereby controlling brain volume. The clinical significance of these brain modifications remains uncertain to date [41, 42].

Clinical studies have demonstrated the effectiveness of various androgenic steroid preparations to induce virilism in transgender males [16, 24, 39, 43]. Treatment regimens follow the same principles as hormone replacement treatment of male hypogonadism of cisgender males [19, 44] (Table 21.3).

The most used preparations are parenteral or transdermal, always aiming to achieve testosterone levels in the normal male range according to the laboratory test used. Other testosterone formulations can also be evaluated on an individual basis but are often difficult to access in some countries. The use of formulations containing oral 17-alkylated testosterone is not indicated due to its high hepatic toxicity [1, 19].

It is suggested to start preferably with the use of testosterone esters administered intramuscularly or the use of testosterone gels, depending on the patient's preference. However, higher testosterone levels are more easily achieved with parenteral therapy [1, 3, 4, 19].

It is common to carry out treatment with 50– 100 mg of testosterone enanthate or testosterone cypionate weekly or 200 mg every 2 weeks. However, it is recommended to start treatment with lower doses of testosterone and gradually increase its levels according to the physical and emotional responses of each patient, like that performed in the induction of puberty of cisgender males with hypogonadism [19].

Table 21.3 Hormone regimens in transgender male

Parenteral testosterone
• Testosterone enanthate or cypionate 100–200 mg SQ (IM) every 2 weeks or SQ (SC) 50% per week
• Testosterone undecanoate ([a]) 1000 mg every 12 weeks
Transdermal testosterone
• Testosterone gel 1.6% ([b]) 50–100 mg/day
• Testosterone transdermal patch 2.5–7.5 mg/day

IM intramuscularly, *SQ* sequentially, *SC* subcutaneously. (Modified from: Hembree WC et al. J Clin Endocrinol Metab. 2017 Nov 1;102(11):3869-3903 [1])

[a] One thousand milligrams initially followed by an injection at 6-week then at 12-week intervals

[b] Avoid cutaneous transfer to other individuals

With the use of parenteral therapy, serum total testosterone is normally measured midway between injections. However, in specific cases, serum levels can be evaluated in the first few days after the injection or immediately before the subsequent injection [1, 3, 4].

Testosterone gels (1% or 1.6%, 2.5 to 10 g/day) may also be used, but virilism may be slower when compared to parenteral formulations. Some physicians choose to switch to some gel as soon as the initial virilism is complete, trying to help avoid supraphysiological concentrations of testosterone or as an option early in the process, due to the caution with the rapid rise in serum testosterone levels and its emotional effects [1, 3, 4].

Monitoring

Hormone therapy for transgender males confers many of the same risks associated with hormone replacement therapy for cisgender males. Risks arise and are aggravated by the inadvertent or intentional use of supraphysiological doses of sex hormones [1, 19, 24, 25, 44].

Thus, it is suggested to perform regular clinical evaluation in all transgender males receiving hormone therapy in search of potential adverse changes and to perform laboratory tests to monitor total testosterone levels every 3 months during the first year of therapy and then once or twice a year [1].

Serum concentrations of total testosterone must be maintained in the physiological range for men (approximately 400– 700 ng/dL). The goals for individuals using testosterone gel are similar, but serum testosterone levels tend to be at the lower end of the normal range. Serum estradiol can be monitored during the first 6 months of testosterone treatment or until there is no uterine bleeding for 6 months. Estradiol levels must be below 50 pg/mL (184 pmol/L) [1].

Also, routine clinical health evaluations must be performed on all patients including monitoring of weight, blood pressure, evaluation of tobacco use, symptoms of depression, and risk of events such as deep venous thrombosis/pulmonary embolism [45] and other adverse effects of sex steroids [1, 46].

In Table 21.4, there is a standard monitoring plan for transgender males on testosterone therapy. The main concerns involve polycythemia, sleep apnea, arterial hypertension, excessive weight gain, salt retention, lipid changes, and skin changes such as oiliness and acne. The risk of liver toxicity with the use of parenteral or transdermal formulations at physiological levels is low [1].

Therapy Care

Androgen therapy has been shown to be safe for most patients; however, long-term safety data on mortality rates, oncological risk, and cardiovascular, cerebrovascular, and thromboembolic events are lacking [14]. Among the best-known potential effects, we highlight:

Table 21.4 Monitoring of transgender male on gender-affirming hormone therapy

1. Evaluate patient every 3 months in the first year and then one to two times per year to monitor for appropriate signs of virilization and for development of adverse reactions
2. Measure serum testosterone every 3 months until levels are in the normal physiologic male range:
   (a) For testosterone enanthate/cypionate injections, the testosterone level should be measured midway between injections. The target level is 400–700 ng/dL to 400 ng/dL. Alternatively, measure peak and trough levels to ensure levels remain in the normal male range
   (b) For parenteral testosterone undecanoate, testosterone should be measured just before the following injection. If the level is < 400 ng/dL, adjust dosing interval
   (c) For transdermal testosterone, the testosterone level can be measured no sooner than after 1 week of daily application (at least 2 h after application)
3. Measure hematocrit or hemoglobin at baseline and every 3 months for the first year and then one to two times a year. Monitor weight, blood pressure, and lipids at regular intervals
4. Screening for osteoporosis should be conducted in those who stop testosterone treatment, are not compliant with hormone therapy, or who develop risks for bone loss
5. If cervical tissue is present, monitoring as recommended by the American College of Obstetricians and Gynecologists and other international medical societies
6. Ovariectomy can be considered after completion of hormone transition
7. Conduct sub- and periareolar annual breast examinations if mastectomy performed. If mastectomy is not performed, then consider mammograms as recommended by the American Cancer Society and other international medical societies

Modified from: Hembree WC et al. J Clin Endocrinol Metab. 2017 Nov 1;102(11):3869-3903

- *Polycythemia*: It is the most observed consequence of androgen therapy. It is suggested to keep hematocrit levels below 50% in transgender males. If there is an increase, the change in testosterone dose, the formulation for use or the indication for phlebotomy should be evaluated [1].
- *Metabolic Effects*: Testosterone administration to transgender males increases lipid parameters associated with increased cardiovascular risk, but studies have shown no negative effects on other risk factors such as blood pressure [1, 10, 11, 33, 47]. Studies of testosterone's effect on insulin sensitivity have mixed results [10]. Long-term studies from the Netherlands found no increased risk of cardiovascular mortality [6, 48–50]. Meta-analysis of 19 randomized studies in non-transgender males on testosterone replacement showed no increased incidence of cardiovascular events [51], although some retrospective studies show 1.2–3.7 times higher rates of myocardial infarction in transgender males when compared to cisgender females [52]. It is recommended to follow the same recommendations for screening and treatment of diabetes mellitus as in the general population. Testosterone therapy may increase visceral fat with effect on insulin resistance [10, 11, 25]. A Dutch observational study observed an increased prevalence of type 2 diabetes mellitus among transgender males compared to same-age and non-transgender male groups [48].
- *Effects on the Breast*: Transgender males who have undergone mastectomy do not require routine mammograms as a form of screening [53]. Testosterone does not seem to increase breast cancer risk in transgender males [48, 53]. However,

breast self-examination may still be important for adequate monitoring since there are reports of breast cancer cases among transgender males on hormone therapy, even after thoracic surgery [54, 55]. The best evidence comes from a retrospective Dutch study of 795 transgender males treated with testosterone and mastectomy for an average of 20.1 years [53]. If mastectomy is not performed, mammography should be performed following guidelines specific to cisgender female.

– *Effects on Endometrium*: Menstruation usually ceases within a few months after the start of testosterone [39]. However, in some individuals, bleeding may continue for a longer period. Individually evaluate the possibility of endometrial changes if there is no complete cessation of bleeding with the measures suggested above. Although the aromatization of testosterone to estradiol in transgender males has been suggested as a risk factor for endometrial cancer [56], no cases have been reported. Given the discomfort transgender males experience when accessing gynecological care, evaluation of total hysterectomy and oophorectomy on an individual basis is recommended. In transgender males with an intact cervix, the Papanicolaou should be performed, especially when there is sexual activity with vaginal penetration. There is no evidence that testosterone increases or reduces the risk of uterine cervical cancer. However, testosterone may affect the performance of the Papanicolaou test. A study of 233 transgender males found a higher rate of unsatisfactory or inadequate Papanicolaou tests compared to non-transgender female in the same clinic, associated with the length of testosterone therapy [57]. Since testosterone therapy may result in atrophic dysplasia-like changes in the cervical epithelium, the pathologist should be informed about the hormonal status of the patient [58].
– *Effects on Fertility*: Transgender male taking hormone therapy may limit their fertility potential. Individuals who undergo oophorectomy completely lose their reproductive potential. Thus, before starting any treatment, patients should be oriented about their desire for fertility and encouraged to consider issues related to cryopreservation of oocytes or embryos [59, 60]. Recent review on the subject encourages a comprehensive and individualized approach to fertility preservation advice and to defining the best time for its performance [61].
– *Effects on Bone Mass*: Proper testosterone dosage is important for maintaining bone mass in transgender males [62, 63]. The protective effect of testosterone can be mediated by peripheral conversion to estradiol, both systemically and locally in the bone. There are no long-term studies on fracture risk, especially in a transgender population of older adults. Loss of bone density is more likely after castration in those patients with other risk factors. Transgender males start with an average of 10–12% less bone density than cisgender males, before any hormonal or surgical intervention [64, 65]. Studies suggest that uninterrupted testosterone therapy maintains or increases bone density in transgender individuals [66, 67].

Attention should be paid to the adequate intake of calcium and vitamin D, the adequate practice of physical activity, in addition to the existence of other risk

factors associated with bone mass loss. There are still discussions about the best way to evaluate dual energy X-ray absorptiometry (DXA) from existing databases since there are no well-established parameters for the transgender population. It is suggested that bone density measurements for transgender males should be compared with male standards [68–70].

To date, there are no specific guidelines for monitoring bone mineral density in the population of transgender males. It is recommended that from the age of 65 all patients must have their bone mineral density evaluated. Between the ages of 50 and 64 years, evaluation must be performed in those patients with established risk factors for osteoporosis [71–73]. Including individuals with evidence of prolonged hypogonadal status and, regardless of age, transgender males undergoing castration and with a history of at least 5 years without hormone replacement must be screened.

Surgical Treatment

For many transgender adult males, gender-affirmation surgeries may be the important step toward achieving their goal of successfully living in their desired gender. However, before the indication of any surgical procedure, patients must be guided and advised on the limitations of the methods and on the expectations of the results. Also, from the appropriate multidisciplinary evaluation, patients in whom the social transition has not been satisfactory, if the person is not satisfied or is ambivalent about the effects of treatment with sex hormones, or if the person is ambivalent about surgery, then the individual must not be referred for surgery [74].

Surgical treatments can be divided into those that directly affect fertility (such as removing the uterus and ovaries) and those that do not interfere (such as removing the breasts). Surgeries that affect fertility are often governed by the legal system of the state or country in which they are performed [1].

The most desired surgery by transgender males is thoracic reconstruction surgery (removal of the breasts). It is suggested that the timing of this surgery should be determined based on the physical and mental health status of the individual. There is not enough evidence to recommend a specific age requirement. For some patients, oophorectomy, hysterectomy, and/or vaginectomy may be considered, but it is not always the end goal of treatment. These surgeries must be performed by experienced surgeons and must always be evaluated on an individual basis [1, 3].

Gender-affirmation surgery is usually the last step in the process and not all transgender males want to go through it. They are usually done after a few years (at least 1 year) of androgen therapy and living in the desired gender role (unless hormone therapy is not desired or medically contraindicated), but many individuals can and do live in their preferred gender role without genital surgery [1].

Genital reconstruction procedures (neophallus creation) must be performed in specialized centers with experienced physicians, but most transgender males are unable to access external genital surgery due to the small number of these professionals, the high surgical cost and the potential complications associated [1, 75]. Surgical techniques are varied and can use free flaps removed from arms or legs and

penile and testicular prostheses. However, due to surgical limitations, the creation of a neophallus has often been unsatisfactory. Recently, penis transplants are being proposed [1].

An alternative to neophallus surgery is metoidioplasty. With this technique, the urethra is lengthened through an anterior vaginal wall flap until it reaches the tip of the phallic glans, and the clitoris is partially released and stretched by resection of the ventral cords. From the labia majora, a scrotum can be built into which testicular prostheses can be implanted [76]. This surgical intervention allows the patient to urinate while standing.

An endocrinologist or an experienced physician must monitor transgender males after surgery since those who have undergone castration will need long-term hormone replacement therapy and surveillance to prevent the adverse effects of chronic hormone deficiency [1].

Conclusion

In recent years, there has been an increasing demand for medical care from transgender males aimed at gender-affirming hormone treatment. Androgen therapy with the use of testosterone is the basis of sex-affirming hormone treatment in transgender males aimed at increasing testosterone levels, suppressing estrogen levels, and treating gender dysphoria. Testosterone is widely used for male hypogonadism but is comparatively little investigated in transgender people. More studies are needed to define the safety of its long-term use, especially on mortality rates, oncological risks, and cardiovascular, cerebrovascular, and thromboembolic events. Regarding surgical procedures, there has been an increase in knowledge of surgical techniques, but the rate of transgender males who have access to their performance remains low due to the costs, the reduced number of trained centers and related morbidity.

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Influence of Endocrine Disruptors on Male Reproductive Tract

22

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Introduction

The concern about the preservation of the environment has not sufficiently taken into consideration the accelerated global development in various fields, such as industry, agriculture and animal husbandry. Especially, after the expansion of the industrial revolution, several chemicals have been released into the environment; they are present in the air, soil, water, food and consumer products raising concern about their effects. These substances are able to accumulate in adipose tissue for several years; it is now possible to assess the long-term effects on the health of humans and animals, as well as on subsequent generations. The data obtained so far, mainly observed in the contamination of animals, confirm the hypothesis that many of these compounds can alter the normal balance of the endocrine system, and this group of chemicals has been given the name endocrine disruptors [1].

An endocrine-disrupting compound was defined by the U.S. Environmental Protection Agency (EPA) as "an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of our natural hormones responsible for homeostasis, reproduction and developmental process". Many of the endocrine disruptors are persistent, lipophilic and have low vapor pressures facilitating their widespread dispersal. Endocrine-disrupting chemicals (EDCs) can profoundly disturb organ differentiation because they can act as hormone agonists or antagonists. Organs at particular risk for developmental abnormalities are those with receptors for steroid hormones: external genitalia, mammary

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glands, fallopian tubes, uterus, cervix, vagina, prostate, seminal vesicles, epididymis, testes, brain, skeleton, thyroid, liver, kidney and immune system [1].

In addition to the binding of EDCs on steroid hormone receptors, recent evidence suggests that they could disrupt the endocrine system through different pathways. The EDCs can act through the recruitment of coactivators or corepressors, in the enzymatic activities altering hormone synthesis and/or metabolism or act directly on gene expression through epigenetic modifications. Epigenetic changes have greater importance, since they produce effects in the exposed individual, as well as in subsequent generations (a transgenerational effect) [1–3].

Some chemicals considered to be endocrine disruptors are important in the manufacturing industry and there has been controversy about whether low, environmentally doses can produce deleterious effects in humans. For many years toxicologists believed that the effects of toxic substances were directly proportional to the doses, thus, larger doses could result in more side effects than smaller ones, based on a monotonic dose–response curve. However, several studies of EDCs contradict this concept, similar to the hormones, EDCs could present a biphasic dose–response curve, U-shaped or inverted U (nonmonotonic dose–response curve); consequently, very small amounts could have significant effects on cell proliferation or development [1]. It is worth noting that the period of development in which exposure occurs is another important factor that should be taken into account when determining the lowest dose that could cause adverse health effects.

Although the EDC exposure to adult populations is a great concern, the exposure of fetuses and infants is more worrisome since their susceptibility to the EDC adverse effects is much greater. These adverse effects during development can occur with smaller doses than those considered deleterious for adults [1]. The reasons for this increased sensitivity in fetuses and infants are due to the absence of the protective mechanisms normally present in adults, such as: DNA repair mechanisms, efficient detoxifying enzymes and liver maturity allowing adequate plasma clearance. Fetal EDC exposure could take part of the fetal programming of diseases in adulthood; especially those involving the reproductive tract.

It is generally assumed that chronic and low-level of EDC exposure after maturity does not permanently alter the functioning of hormone-responsive tissues; however, the possibility does exist. This hypothesis is reinforced by the finding of an increase in the incidence of breast, prostate and testicular cancers, hypospadia and cryptorchidism and a decline in the quantity/quality of sperm in the US and several European countries [4]. It is now suspected that the increased incidence of these disorders could be related to exposure to pesticides and other endocrine-disrupting chemicals.

In this chapter, we reviewed data from the literature on a subset of topics for which the translational evidence is strongest for most common EDCs, which interfere in the testosterone synthesis or action, and in the development of male reproductive system (Table 22.1).

Table 22.1 History, route of exposure, sources, half-life, and effects of common EDCs

EDC	Introduction date	Banned/ restricted date	Route of exposure	Sources	Half-life	Effects
BPA	1960	Restricted 2012	Ingestion, inhalation, dermal absorption	Polycarbonate plastics, epoxy resins, plastic toys and bottles, lining of food cans, thermal papers	4–5 h	Estrogenic, obesogenic, neurological effects, adverse thyroid hormone action, reproductive and developmental effects
DDT	1940	Banned 1972	Ingestion, inhalation, dermal absorption	Contaminated water, soil crops, fish	6–10 year	Carcinogen, central nervous system, kidney, liver, and peripheral nervous system effects
Dioxins (TCDD)	1872		Ingestion, inhalation	By-product of chlorinated herbicide production, smelting, chlorine bleaching of paper	7–11 year	Liver damage, weightloss, atrophy of thymus gland, immunosuppression, reproductive effects, and cancer
MXC	1948	US 2003 banned use as pesticide	Ingestion, inhalation, dermal absorption	Contaminated soil, water, and food	Aerobic soil ~100 days	Central nervous system depression, damage to liver and kidney, developmental and reproductive effects in animals, transgenerational kidney and ovary disease, obesogen
PCBs	1927	Banned 1979	Ingestion, inhalation, dermal absorption	Contaminated air and food, skin contact with old electrical equipment	12 days to 16 years	Carcinogen, chloracne, stomach and liver damage, reproductive and nervous system effects and thyroid injury
PFOA	1940s	US, 2015 voluntary production restriction	Ingestion, inhalation	Contaminated food and water, dust, floor waxes, fire fighting foam, electrical wiring, lining of food wrappers, stain resistant carpeting	2–4 years	Liver, and mammary gland developmental, and immune system toxicant, carcinogen
Phthalates	1920s	Restricted 2009	Ingestion, inhalation, dermal absorption	Contaminated food, PVC plastics and flooring, personal care products, medical devices and tubing	~12 h	Carcinogen, liver damage, reproductive and developmental effects, asthma, obesogen
Vinclozolin	1981		Ingestion, inhalation, dermal absorption	Diet and occupational	Aerobic soil 28 days, plasma 20 h	Antiandrogenic activity, male reproductive and neurological effects, transgenerational reproductive effects, potential carcinogen

BPA bisphenol A, *DDT* p,p'-Dichlorodiphenyltrichloroethane, *DES* diethylstilbestrol, *EE2* ethinyl estradiol, *MXC* methoxychlor, *PVC* polyvinylchloride, *TCDD* tetrachlorodibenzo-p-dioxin, *PCBs* polychlorinated biphenyl, *PFOA* perfluorooctanoic acid

EDCs Affecting Testosterone Synthesis and/or Action

A wide variety of chemical compounds with EDC activity have been recognized for environmental control agencies worldwide, including among them pesticides, pollutants, substances used in the production of plastics. The EDCs can be classified according to their use, for example, pesticides, or its structural property, like dioxins, steroids and polyaromatic compounds. All these compounds are widely found in the environment and can even be transported over long distances [1].

Industrial Materials

Bisphenol A

Bisphenol A (BPA) is a monomer used in the manufacture of thermal paper, polycarbonate plastics and epoxy resins. It has been shown to leach from these materials due to incomplete polymerization and to degradation of the polymers caused by exposure to high temperatures that occurs under normal conditions of use. BPA is one of the most commonly produced and used chemicals in the world; it is used in the production of various products including water-pipes, food containers, toys, baby bottles, medical equipment, dental products, electronic devices and CD/DVD discs. Thermal paper is produced in massive quantities because it is used in receipts, faxes and labels and it is also utilized (after recycling) to produce brochures, tickets, mailing envelopes, newspapers, kitchen rolls, toilet paper and food cartons. Populations around the word are exposed to BPA mainly through food and drinking water and by dust inhalation and dermal contact, although food is the most important source of BPA exposure [3–5].

BPA is one of the most ubiquitous endocrine disruptors in human fluids and has been identified in several biological fluids, such as: serum of adults, maternal and fetal plasma, placental tissue, milk of nursing mothers, amniotic fluid and in urine samples [1, 4, 5]. BPA binds to several receptors including estrogen and androgen receptors as well as to aryl hydrocarbon receptor and peroxisome proliferator-activated receptor [6, 7].

It has been proven that BPA behaved similarly to natural estrogen 17-β estradiol, inducing estrogen receptors, but in the concentrations about one thousand higher (10^{-6} to 10^{-4} M) than estradiol [8]. Initially, BPA was described as a weak environmental estrogen, whose activity toward classical nuclear estrogen receptor (ER) α and β was over 1000–10,000 times lower in comparison to 17β-estradiol. Nevertheless, further investigations showed that BPA even in very low concentrations (pico/nanomolar) exerted multidirectional effects on physiological functions of cells and tissues by binding with receptors present out of the nucleus [9].

Estrogenic activity of BPA has been well documented in animals' studies [1]. Male mice exposed to BPA during gestational days 16–18 showed increased anogenital distance and prostatic size and decreased epididymal weight; these changes persisted during adulthood and decreased sperm production was also observed [10–13]. The prostate enlargement is mediated through the ERs present in the stroma,

and this effect was blocked by antiestrogens [10]; BPA also increases the expression of androgen receptors in the prostate stroma of mice [14]. Other study also identified an increase in the number and size of dorsolateral prostate ducts and overall increase in prostate duct volume in mouse fetuses due to an altered proliferation of basal epithelial cells [11]. Taken together, these data indicated that prenatal BPA exposure results in permanent alterations of the morphology, histoarchitecture and cell proliferation in the prostate and other androgen-target tissues, which could predispose to diseases in adult life.

BPA is able to bind and activate both ERα and ERβ and is shown to suppress androgen production by rat Leydig cells, in vivo and in vitro [15]. BPA exposure of male rats with a high fat diet impaired antioxidant capacity in the testis [16]. Exposure of Swiss albino mice to a range BPA concentration of 0.5, 50 and 100 μg/kg body weight/day, intraperitoneally for 60 days, increased the nitrite and malondialdehyde levels and the testicular injury scores, whereas the sperm count, serum testosterone levels and catalase activity were reduced. These results suggest that BPA induces oxidative stress by altering the expression of inducible nitric oxide synthase (iNOS), which consequently leads to downregulation of steroidogenic acute regulatory protein (StAR) expression in the testis [17].

BPA exposure at 10^{-8} M, 10^{-7} M and 10^{-5} M concentrations for 72 h in vitro inhibited testosterone production in both, rat and human fetal testis. Due to the current uncertainty regarding the effects of BPA fetal exposure on human testis, and due to the insufficient number of epidemiological studies on BPA endocrine disruptive effects, caution should be taken in extrapolating these results to human reproductive health [18].

Besides sex hormones, BPA also disrupts the function of several hormones including leptin, insulin and thyroxin; moreover, hepatotoxic, immunotoxic, mutagenic and carcinogenic effects have been observed. Recent data has suggested that human BPA exposure increased the risk for obesity, diabetes and heart disease [19].

Taking into consideration the possible toxicity of this compound, some countries ceased the production of baby bottles made with BPA polymers, minimizing the infants' exposure, a population under greater risk of adverse EDC effects. In 2008, Canada was the first country to ban BPA in baby bottles, in 2011, The European Commission has restricted the use of BPA in plastic infant feeding bottles. In 2013, Food and Drug Administration banned the use of BPA in baby formula packaging.

Bisphenol A has been replaced by structural analogues such as: bisphenol S (BPS), bisphenol F (BPF), bisphenol AF (BPAF) and tetrabromobisphenol A (TBBPA). However, these analogues can exert similar adverse effects, especially on the reproductive system, and their toxicological data are still limited [20].

Dioxins

There are more than 400 types of dioxin-related compounds, about 30 of which are significantly toxic to human health, with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) being the most toxic. TCDD is an organochlorine that is a product of industrial processes, such as paper bleaching, smelting and the manufacture of herbicides and pesticides. TCDD is lipophilic and has a long half-life, of approximately

7–11 years, predisposing to its bio-accumulation in humans, animals and in the environment [1].

Many adverse effects on the male reproductive system, such as abnormal testicular morphology, decreased spermatogenesis, impairment of testicular steroidogenesis and adverse effects on reproductive performance have been demonstrated when overtly toxic doses of TCDD were administered to post-pubescent mouse. However, it was reported that even TCDD doses as low as 0.16 pg/kg in pregnant mouse resulted in significantly reduction in serum testosterone levels shortly after birth, as well as in significant delay in testicular descent, decreased seminal vesicle and ventral prostate weights, which are androgen-dependent parameters. These results demonstrated that perinatal TCDD exposure could affect androgenic status without causing overt toxicity. In mouse, the male reproductive system appears to be more sensitive to the toxic effects of in utero and lactational TCDD exposure than any other organ [21, 22].

Polychorinated Byphenyls

Polychlorinated biphenyls (PCBs) are a class of industrial chemicals that were mass-produced globally from the late 1920s until they were banned in 1979. These compounds were used in many applications, including plasticizers in rubber and resins, carbonless copy paper, adhesives, and paints and inks. Because of their stability and persistence, PCBs remain ubiquitous contaminants in the environment and in human population even today [23]. The general population is exposed primarily through ingestion of contaminated foods (e.g., fish, meat and dairy products), because PCBs can bio-accumulate up the food chain [1].

Many PCBs have estrogenic or antiandrogenic activity and as such may affect the prostate gland. In vitro analysis of the effects of many PCBs in human prostate cancer cell line LNCaP found that some compounds reduced cell proliferation, PSA secretion and 5α-reductase activity, whereas others (PCB153 e 118) presented biphasic effects on proliferation and PSA secretion at low concentrations. Significant associations have been demonstrated between PCBs and increased prostate cancer risk and/or mortality in man occupationally exposed to PCBs [24].

Studies in fish demonstrated effects of PCBs on the GnRH system, decreasing preoptic-hypothalamic GnRH content, pituitary GnRH receptors and the LH response to GnRH stimulus. This effect was mimicked by an inhibitor of serotonin synthesis, suggesting the possible mediation of PCBs effects on the serotonergic pathway [25].

Gestational PCB exposure of rats reduced testosterone levels in males and resulted in changes related to sexually dimorphic brain regions in females, such as masculinization of anteroventral periventricular nucleus through the modification of normal developmental apoptosis. Gene expression of brain derived neurotrophic factor, GABAβ receptors 1 and 2, IGF-1, kisspeptin receptor, NMDA receptor subunits (NR2b and NR2c), prodynorphin and TGFα, known to play important roles in differentiation and migration of hypothalamic neurons, were also changed by PCB exposure [26].

Epidemiological studies indicated that semen quality in men might be adversely affected by PCB exposure even in adulthood. A review of representative studies assessing the potential effects of PCBs during adulthood on male reproductive health indicated some association between PCB and lower sperm motility and to some extent, decreased sperm DNA chromatin integrity and lower levels of free testosterone [27].

Phthalates

Phthalates (phthalic acid esters) are a family of chemicals commonly used as plasticizers, and their presence in a large number of consumer products results in their widespread distribution in the environment. Phthalates and phthalate esters are used as liquid plasticizers in a wide range of products including plastics, coatings, cosmetics and medical tubing. These compounds were first introduced as additives in the production of plastic in the 1920s and resulted in the rapid widespread use of polyvinylchloride plastic in the 1930s and later. Because they are not chemically bound to the plastic, phthalates can leach into the environment. Moreover, a variety of consumer products contain phthalates, including personal care products, medical tubing, vinyl flooring materials and toys. In fact, phthalates are detectable in human fluids, such as urine, serum and milk [1].

These esters have been shown to induce several testicular effects in rodents. The target for the postnatal testicular toxicity of phthalates is the Sertoli cells, but effects on Leydig cell structure and function have been also reported in pubertal and adult animals. Their effects exhibit an age dependency for the induction of testicular toxicity with neonatal animals being more sensitive than those pubertal ones, which are in turn more sensitive than their adult counterparts for a given dose of an active ester. “Phthalates syndrome” is characterized by malformations of the epididymis, vas deferens, seminal vesicles, prostate, external genitalia (hypospadias), cryptorchidism and testicular injury together with permanent changes (feminization) in the retention of nipples/areolae (sexually dimorphic structures in rodents) and reduced anogenital distance [28].

Among all phthalates, the one of greatest concern is DEHP (diethylhexylphthalate), whose annual production exceeds two million tons. Studies with low doses of DEHP in mice identified toxicity to the reproductive system, being associated with the presence of testicular dysgenesis. In humans, epidemiological studies indicate a correlation between phthalate concentrations in umbilical cord blood and lower gestational age at delivery. Antiandrogenic activities are also described in humans, with a direct correlation between maternal urinary concentrations and the prevalence of cryptorchidism in newborns and an inverse correlation with the anogenital distance, which is an indicator of prenatal androgenic activity [3].

Perfluorinated Compounds

Perfluorinated compounds (PFC) are synthetic chemicals with lipophobic and hydrophobic properties. These compounds are widely used for industrial purposes (lubricants, surfactants) and consumer products such as non-stick cookware,

clothing, carpets and paper. Exposure of adult male rats to perfluoro-n-octanoic acid (PFOA) reduced serum testosterone levels and increased estradiol levels [29].

Perfluorooctane sulfonate (PFOS) is a neurotoxic agent. The effects of PFOS exposure on the hypothalamic-pituitary-testicular axis (HPT) were evaluated in adult male mice and an increase was observed in the noradrenaline concentration in the anterior hypothalamus and in the median eminence; additionally, it was observed that GnRH gene expression decreased and LH and FSH gene expressions also increased. Thus, PFOS exposure in adult male mice can modify the physiological activity of the reproductive system, which could be explained, at least in part, by structural alterations in the hypothalamus and gonadotrophic cells [30].

Insecticides, Pesticides, Fungicides

P,P′-Dichlorodiphenyltrichloroethane

The p,p′-dichlorodiphenyltrichloroethane (DDT) is a synthetic industrial and household insecticide with a long half-life, extensive use and lipophilic nature that have made it a major environmental contaminant. The United States banned DDT in 1972 due to its effects on the environment and potential humahn health effects. DDT and its metabolites bind and transactivate ERα, ERβ and induce estrogenic effects. They have been associated with endocrine-related diseases such as testicular tumors, endometrial cancer, type 2 diabetes mellitus and breast cancer [1].

The effects of DDT on hepatic testosterone metabolism and testosterone hydroxylase activity ratios were tested in male and female Wistar rats. DDT increased testosterone biotransformation and modified the profile of metabolites produced in a sex-dependent manner. Males produced relatively less 2α-hydroxytestosterone (OHT), and 16α-OHT, whereas treated females produced less 7α-OHT but higher 6α-OHT than their respective controls. In both sexes, DDT decreased the relative proportion of androstenedione and increased that of 6β-OHT, suggesting that the androgenic pathway was affected. The testosterone 6α−/15α-OHT ratio, a proposed indicator of demasculinization, was increased in treated males, supporting the suspected demasculinizing ability of DDT. Interestingly, this ratio was reduced in treated females indicating that DDT shifted testosterone hydroxylations toward a more masculine pattern. Thus, DDT altered the hepatic sexual dimorphism in testosterone metabolism and decreased the metabolic differences between male and female rats [31].

In males, relationships of DDT and its metabolite DDE with endogenous hormones have been variable. The cross-sectional findings from the 1999–2004 National Health and Nutrition Examination Survey did not find that DDE is significantly related to testosterone [32]. One study of males living in a highly contaminated rural area observed an inverse association of testosterone with o,p'-DDT [33]. A cross-sectional study of 50 South African malaria control workers observed positive associations of p,p′-DDT with both estradiol and testosterone levels, but no association with p,p′-DDE [34]. Relationships of DDT and its metabolite DDE with endogenous hormones in males have been variable.

Methoxychlor

The organochlorine pesticide methoxychlor (MXC) was introduced in 1948 and widely used as replacement for DDT; in 2003, its use was banned as a pesticide in the USA [1]. MXC and its major metabolite 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl) ethane (HPTE) present endocrine disruptor effects, both have estrogenic activities via binding to estrogen receptors and also have antiandrogen mediated through inhibition of testosterone biosynthetic enzymes. They directly impair testosterone production in rat Leydig cells via inhibiting CYP11A1 activity. In a study using purified pig CYP11A1, [^{14}C] MXC was found to irreversibly bind to CYP11A1 abolishing the enzyme activity, this indicated that MXC is a non-competitive inhibitor of CYP11A1. MXC also inhibits human and rat testicular 3ß-hydroxysteroid dehydrogenase (HSD3B) activity in a non-competitive model [35].

Organotins

Organotins have been widely used as antifouling biocides for fishing nets and ships, agricultural fungicides and rodent repellents. Organotins tributyltin and triphenyltin have been shown to have antiandrogen action (Fig. 22.1). Organotins are known to induce disorders of sex development in marine neogastropods and are suggested to act as specific endocrine disruptors, inhibiting the enzyme-mediated conversion of steroid hormones. Studies in vertebrate and invertebrate animals have shown that they interact with steroid synthesis and clearance, inhibiting testosterone biosynthetic and metabolizing enzymes [36].

Tributyltin (TBT) and triphenyltin (TPT) inhibit pig CYP17A1 activity with a half maximal inhibitory concentration (IC50s) of about 117 μM, and TBT inhibits rat CYP17A1 with less than half concentration. TBT is a primarily competitive inhibitor of rat testicular HSD3B activity and both organotins inhibit the 17β-hydroxysteroid dehydrogenase type 3 (HSD17B3) activity in pig Leydig cells [37]. The in vitro effects of TPT on human testosterone biosynthetic and metabolizing enzymes include alterations in the he HSD3B type 2, HSD17B3 and 5α-reductase type 2 (SRD5A2) activities. The inhibition of SRD5A2 activity may be mediated by the interaction of TPT with critical cysteine residues of the enzymes [36]. The testosterone metabolism is also affected by TBT, which inhibits human 5α-reductase type 1 (SRD5A1) and SRD5A2, the inhibition of TBT on SRD5A1 is competitive while that on SRD5A2 activity is irreversible [38].

Another study investigated the effects of organotins on testosterone conjugation activities, microssomal acyltransferases and cytosolic sulfotransferases in different invertebrate phyla (molluscs, crustaceans an echinoderms). It was observed that organotins compounds altered both testosterone esterification and sulfation, with significant differences among species [39].

Vinclozolin

Vinclozolin (VIN) is a fungicide that is commonly used for turfgrass, ornamental plants, grapes and other fruits and vegetables. VIN and its major metabolites (enanilide and butenoic acid) act as antiandrogens by inhibiting androgen receptor (AR) activity. AR activity is required for male reproductive development, and expression

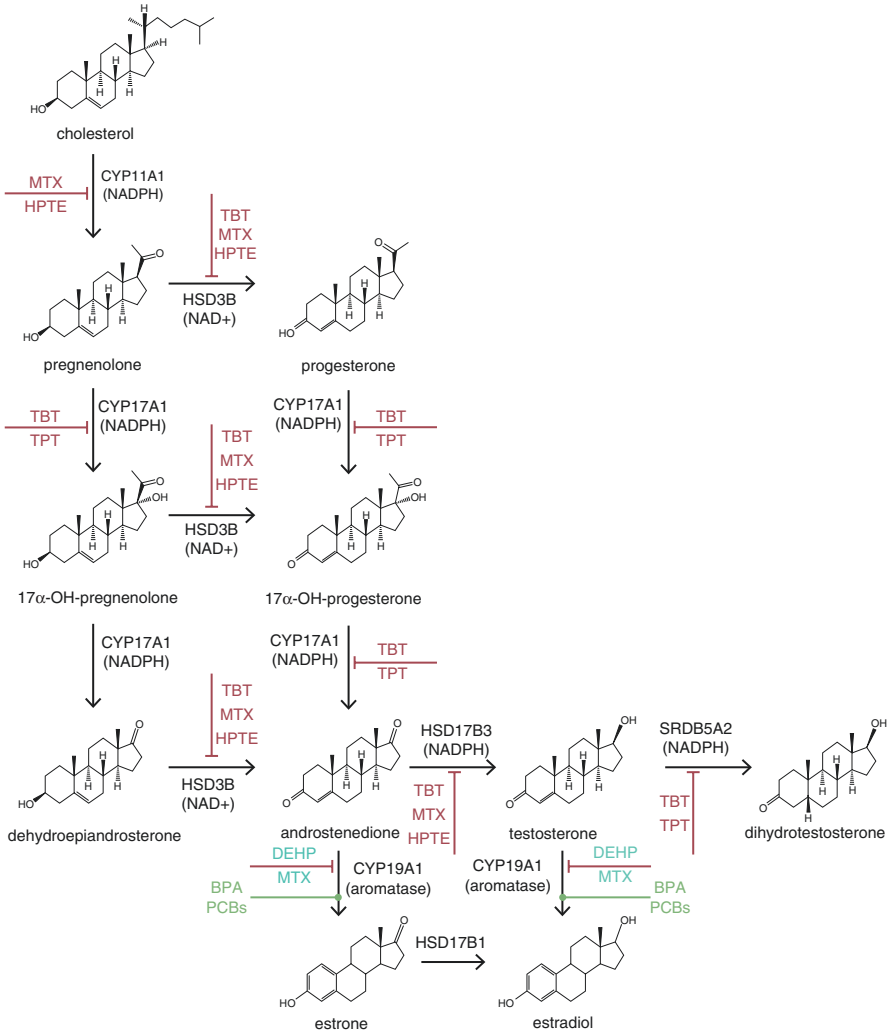

Fig. 22.1 Action of endocrine disruptors on steroid biosynthetic and metabolic pathways. *BPA* bisphenol A, *PCBs* polychlorinated biphenyl, *DEHP* diethyl phthalate, *MTX* methoxychlor, *TBT* tributyltin, *TPT* triphenyltin, *HPTE* 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl)ethane. (Adapted from: Ye L et al., Molecules 2011 [35])

of AR influences sexual differentiation, gonadal formation and reproductive functions [40].

Transient intrauterine exposure to VIN, during embryonic gonadal sex determination in male rats, induced decreased spermatogenic capacity and increased incidence of male infertility in adults from the F1 generation. These effects were transferred through the male germ line to nearly all males of all subsequent generations examined (F1 to F4). The effects on reproduction correlated with altered DNA

methylation patterns in the germ line [41]. In another study, the direct effects of in utero VIN exposure were investigated on the F1 generation, analyzing rat testis transcriptome. A total of 576 differentially expressed genes were identified, mainly related to vascular development, cellular apoptosis, transcription and signaling by calcium, insulin, *Wnt* and androgen receptor [40].

Although the EDCs may disrupt the endocrine system as a whole, many of the effects are due to changes in estrogen signaling, one of the most conserved pathways in evolution of species. Other well-known activities of EDCs are antiandrogenic ones, which may lead to changes in the reproductive system, sexual differentiation and puberty. Details regarding the effects of endocrine disruptors on male gonads will be described.

Human Disorders Related to EDC Exposure: Evidence from Association Studies

Humans are exposed to at least hundreds of environmental chemicals and a major limitation of epidemiological studies is that usually they measured the human exposure to a single EDC or, at best, to a set of isomers within the same EDC family. A broader understanding of the potential risks to the male gonads requires the study of complex mixtures, to which humans are generally exposed.

Male sexual differentiation is androgen-dependent and therefore it is expected that different diseases could be observed in males as a result of fetal EDC exposure, whereby the period of development in which it occurred will have greater influence.

Development of Genital and Sexual Dimorphic Brain Regions

Growing number of evidence indicates that estrogens can play an important role in the regulation of Leydig cell function and steroidogenesis at different stages of development. Environmental chemicals with estrogenic activity can also bind to ERα, mimic the action of estradiol and suppress androgen production in Leydig cells at different stages of development and thereby have a negative effect on the proper formation of reproductive organs and reproductive potential [15].

Anogenital distance (AGD) has been measured either as anoscrotal distance, i.e., the distance between anus and perineoscrotal junction, or as an anopenile distance, i.e., the distance between anus and cephalad insertion of the penis [42]. Anogenital distance is sexually dysmorphic and in males is typically twice that of females and is a sensitive marker of prenatal androgen exposure. In humans, the prenatal male programming window (MPW) is presumed to occur between 8 and 14 gestational weeks (GW) [43]. Prenatal exposure to antiandrogenic EDCs has been associated with short AGD in male rats [44]. Several human studies have evaluated associations between prenatal EDC exposure and anogenital distance in infant and young boys. Many studies suggested negative associations between anoscrotal or anopenile distance and phthalate, BPA/phenol, pesticides, PCB or PBDE exposure levels

in maternal urine samples collected during pregnancy. For some chemical groups, only a few human studies have been published so far and it is difficult to draw any conclusions. Differences in results of the studies may be explained by variation in exposure levels, in timing of the sample collection, in matrices analyzed, in the age of the males at examination, in other factors included in the statistical analysis (e.g., stress) and in metabolites/chemicals analyzed [45]. It also has been suggested that human-rodent differences in results concerning associations between prenatal EDC exposure and anogenital distance could be due to species differences in regulation of fetal androgen production [46].

Congenital cryptorchidism is one of the most common congenital malformations in male newborns presenting prevalence between 1 and 8% in full term newborns in prospective cohort studies [47]. The first phase of testicular descent is dependent on insulin-like peptide 3 (INSL3), a hormone produced by Leydig cells. Estrogens have shown to downregulate the expression of INSL3 gene. Furthermore, androgen action is important especially for the last phase of testicular descent, the inguinoscrotal phase [48]. Therefore, fetal exposure to EDCs with antiandrogenic and estrogenic properties might be associated with cryptorchidism. Bonde et al. studied associations between in utero or infant exposure to environmental EDCs and cryptorchidism in a meta-analysis. The analysis included studies based on chemical measurements of different biological matrices. No significant association was observed between exposure to environmental EDCs and cryptorchidism in the analysis of 8 studies [49].

Hypospadias is due to failed fusion of penile urethra folds during sixth to 11th embryonic weeks. In hypospadias, the opening of urethra is situated on the ventral side of the penis, or in the scrotum or perineum. Penile development is dependent on androgens and both genes and environment are thought to have a role in the etiology of hypospadias. Animal studies have showed that hypospadia is a common outcome in male pups that have been exposed in utero to antiandrogens. In the meta-analysis by Bonde et al., associations between exposure to environmental EDCs and hypospadias were also studied. Based on 18 risk estimates no significant association was found [49]. No significant link was either found when studying association of hypospadias with specific exposures to DDE (degradation product of pesticide DDT) and PCBs. Differences in severity of cases, in exposure levels, in timing of the sample collection, in matrices and statistical analyses may also explain differences in results of the studies [45].

The testicular dysgenesis syndrome (TDS) is a disorder beginning during fetal life. Cryptorchidism, hypospadia, testis cancer and poor semen quality are all manifestations of TDS, which has become more frequent in late decades. Geographical differences in the incidence rates of some symptoms have been reported, but migrant study argues against the possibility that genetic differences may account for all the observed geographical differences. Epidemiological data suggested that lifestyle and environmental factors are important contributors to the increasing incidence of the TDS symptoms, and genetic factors may be important for the individual susceptibility to endocrine disrupters [50].

"Idiopathic" partial androgen insensitivity syndrome, PAIS-like phenotype, may in some cases be related to EDC contamination during fetal life. Gaspari et al. demonstrated that 11/28 (39.3%) 46,XY newborn/infant males with differences of sex development (DSD) presented with normal androgen production, and no identified AR, SRD5A2 and steroidogenic factor 1 (SF1) gene mutations. Parental fetal exposure to EDCs before/during the patients' fetal life could be involved. The mean estrogenic bioactivity, analyzed by ultrasensitive bioassay, in these 11 patients with fetal EDC exposure was significantly increased (6.65 ± 8.07 pg/mL) in comparison to those remaining 17 cases (1.27 ± 0.34 pg/mL) and to controls (1.06 ± 0.44 pg/mL; $P < 0.05$) [51].

Impaired androgen production, associated with EDC exposure, was observed in a study with a significant sample size. In this cross-sectional study, serum and semen samples of 247 healthy men were evaluated to investigate if PFC exposure influences testicular function. Serum testosterone, E2, sex hormone-binding globulin, LH, FSH and inhibin-B levels and 14 PFCs, including PFOS, were measured. PFOS levels were negatively associated with testosterone levels, calculated free testosterone (FT), free androgen index (FAI) and ratios of T/LH, FAI/LH and FT/LH [29].

In an interesting study, Hauser et al. examined the probability of EDCs causing male reproductive disorders and quantified the potential burden of disease and costs. The panel focused on four exposure-outcome relationships: phthalates versus infertility, polybrominated diphenyl ethers (PBDEs) versus testicular cancer, PBDEs versus cryptorchidism, and phthalates versus reduced serum testosterone levels. They identified strong toxicological and low epidemiological evidence for male infertility, lower testosterone levels in relation to phthalate exposure; and low epidemiological and weak toxicological evidence for testicular cancer and prenatal PBDE exposure. The authors concluded that EDCs may contribute to male reproductive disorders, which may result in an annual treatment cost of €15 billion in the European Union [52].

Research on animal models has demonstrated that sex differences in brain and behavior are induced by steroid hormones during specific, hormone sensitive, developmental periods. It was shown that typical male neural and behavioral characteristics develop under the influence of testosterone, mostly acting during perinatal development. By contrast, typical female neural and behavioral characteristics may actually develop under the influence of estradiol during a specific prepubertal period [53]. In primates, male sexual brain differentiation is thought to result from a combination of estrogen receptor and androgen receptor-mediated processes [54]. Low estrogen levels are required for development of the female brain phenotype and effects on developing brain could therefore be expected from estrogenic, as well as antiandrogenic EDCs [55].

Many neuroimaging studies have focused on sex differences in overall or regional gray mass (GM) volumes derived from structural magnetic resonance image scans [56]. Overall sex differences have been found in total brain size, with the male brain being on average 11% larger than the female brain. Sex differences have been found in the amygdala, hippocampus, insula and parts of the frontal cortex, among many

other regions. In a recent neuroimaging study, [53] cortical thickness and subcortical GM volumes were compared between 16 women with complete androgen insensitivity syndrome (CAIS) and groups of control men and women ($n = 32$ per sex). A female typical pattern similar to control women was found in CAIS in some brain regions (i.e., they had thicker parietal and occipital cortices and a thinner left temporal cortex, and larger volumes of the hippocampus than control men). These findings suggest that these sex differences are established under the influence of androgens. However, CAIS women also showed a male pattern in some brain regions, such as a significantly thinner cortex in the precentral and postcentral gyrus and smaller volume of the caudate nucleus, compared to control women and thus were similar to control men. Furthermore, CAIS had larger overall brain volumes compared to control women, which might be related to their height because they are generally taller than control women. Taken together, CAIS women showed a mixed male and female pattern in brain structure, suggesting direct effects of sex chromosome genes in addition to steroid hormone effects [57]. Neuroimaging studies on brain function in congenital adrenal hyperplasia (CAH) have primarily focused on amygdala function in response to emotional pictures and predominantly in adolescents. In one such study, it was found that girls with CAH showed a similar pattern of activation of the amygdala when viewing negative faces as control boys (i.e., increased activation of the amygdala) [58]. By contrast, CAH boys did not differ from control boys. These results thus suggest a potential role of prenatal androgens in emotional processing by the amygdala.

At present, strong evidence exists for a predominant role of steroid hormones in the sexual differentiation of the human brain. Data obtained from both CAH and CAIS point to an important role of (prenatal) androgens in inducing typical male neural and psychosexual characteristics in humans. Whether estrogens play a similar masculinizing role in humans as has been shown in rodent species appears to be less clear, at least when studying DSDs and focusing on those domains showing strong sex differences (e.g., toy preferences, mental rotation). However, studies in which steroid hormone receptor polymorphisms were investigated in relation to gender incongruence have suggested a specific role for the beta estradiol receptor in some aspects of brain masculinization [59]. Specific allele combinations for steroid receptors were identified for transgenders (i.e., an inverse allele interaction between beta estradiol receptor and androgen receptor is characteristic of gender incongruence in natal males, whereas both estradiol receptors are associated with gender incongruence in natal females). However, at present, the mechanisms of how such steroid receptor polymorphisms will potentially lead to altered steroid hormone actions during brain development and, consequently, to gender incongruence, remain unknown.

In addition to a potential role in brain masculinization, estrogens might be involved in active brain feminization as has been suggested by some rodent studies, although the current evidence is rather limited to some observations made in Turner syndrome women. Thus, sex differences in the human brain appear to reflect a combination of steroid hormones, genetic factors and, last but not least, socialization-related effects and the relative contribution of each factor might depend on the brain

area and/or function [53]. In sum, more research is clearly needed to better identify the different players and the potential role of EDCs in the sexual differentiation of the human brain.

Puberty and Gynecomastia

Puberty is an essential parameter of reproductive health, marking the sexual maturation of the hypothalamic-pituitary-gonadal (HPG) axis that culminates in adult hormonal profiles and physical changes essential for reproductive fitness. An earlier onset of thelarche and pubarche in females has been consistently observed in recent decades, while the tendency in male pubertal timing is less clear. Some studies report an earlier age of onset for pubic hair and testicular development in males, whereas others contest such temporal change. An important contribution to the secular trends in female pubertal timing is the significant improvement in overall public health over the past century. Several reports, however, also emphasized the potential role of environmental chemicals, specifically EDCs on the puberty development [1].

For instance, the exposure of developing rodents to high doses of estrogenic EDCs advances puberty and alters their reproductive function. Low environmental doses of these compounds may also affect development in humans. Effects have become apparent in humans over the past half century that are consistent with those seen in EDC highly exposed animals, such as an increase in incidence of genital abnormality in boys and earlier sexual maturation in girls [60].

Although most compounds with weak estrogenic activity cause earlier onset and/or more rapid progression of puberty in females, a spectrum of effects may be observed in male exposed to compounds with estrogenic activity, ranging from no noticeable effect to pubertal delay. Low doses of diethylhexylphthalate (DEHP) were associated with an earlier onset of male puberty and increased serum testosterone levels in rats. Higher doses (750 mg/kg) paradoxically caused a 5-day delay in pubertal onset and inhibited testosterone concentrations. The authors also showed that the rise in serum testosterone was not accompanied by any changes in pituitary LH mRNA expression, suggesting that the DEHP effects were probably mediated directly on the testosterone biosynthetic pathway rather than via the HPG axis. This dose-dependent stimulatory effect of DEHP, causing earlier pubertal onset, has not been reported in males exposed to other EDCs [61].

In 2006, Den Hond and Schoeters reported an overview of the literature about the effect of potential EDCs on the onset of puberty. They selected epidemiological research in boys and in girls accidentally exposed to EDCs. In boys, the exposure to PCBs, polychlorinated dibenzofurans (PCDFs), or pesticides was associated with delayed puberty and/or decreased penile length. In girls, earlier age at menarche was reported after exposure to PCBs, polybrominated biphenyl (PBBs), DDT and phthalate esters. Nevertheless, a delaying effect of dioxin-like compounds on breast development was related in the same population and no effect on age at menarche [62].

In a recent large review study, Cargnelutti et al. surveyed the existing literature on the impact of EDCs on the development of testicular, andrological parameters, testicular cancer, and particularly on pubertal age, providing the most current information available. Reports based on human evidence were given precedence over experimental results in animals and in vitro. The authors found only a few original papers on the effects of EDCs on male puberty. Nevertheless, they concluded that EDCs should cause harm and adverse consequences on male puberty and andrological health, with reduced testosterone levels and increased SHBG and aromatase, leading to delayed puberty and adrenarche and also delayed sexual maturation (Fig. 22.2) [13].

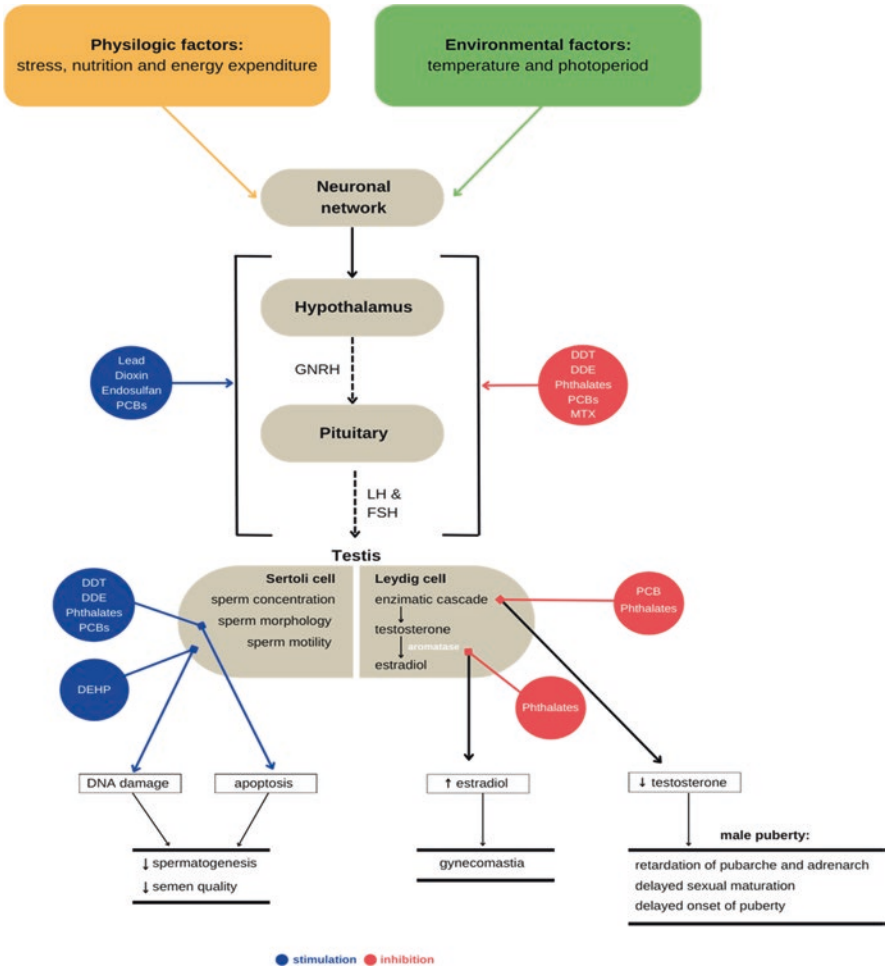

Fig. 22.2 Endocrine disruptors and actions on the male reproductive system. (Adapted from Cargnelutti et al. [13])

Pubertal gynecomastia is usually caused by an imbalance between estrogens and androgens, which may be attributed to excessive estrogen activity, deficient androgen activity, increased aromatase activity or a combination of these effects on breast tissue. DEHP and its main metabolite, mono- (2-ethylhexyl) phthalate (MEHP), have an antagonist effect on androgen receptors [1]. It was observed that plasma levels of DEHP and MEHP were significantly higher in pubertal patients with gynecomastia when compared to controls [63]. However, this association was not confirmed in a large cohort comprising 555 healthy boys [64]. These data evidence the need to perform longitudinal studies in order to verify the role of DEHP modulation in pubertal gynecomastia.

Fertility

The decline in semen quality throughout men's lifetime has been the subject of an international debate, some studies suggest that the quality of human semen decreases before 50 years of age, while others do not report a decline [54].

Despite the relevance of EDC exposure, especially PCBs, pesticides and phthalates, the epidemiological evidence of its relationship with the semen quality is still discussed in adults. A large study in male partners of subfertile couples from an infertility clinic in Massachusetts found an association between exposure to monobutyl phthalate (MBP, the hydrolytic metabolite of dibutyl phthalate) with low sperm motility and sperm concentration [55]. In contrast, a Swedish study found no association of MBP or monobenzyl phthalate with any of the semen parameters [65]. Possible reasons to explain these discordant results include the differences in age and fertility of populations evaluated. Epidemiologic evidence indicates an inverse correlation between serum concentrations of PCBs and semen quality, specifically with decreased sperm motility. These relationships have been consistently observed in different countries like India, the Netherlands, Taiwan, Sweden and the USA. In these series, serum PCB levels ranged from low to high values and were related to the consumption of fish or contaminated rice oil [66, 67]. Regarding exposure to dioxins, it is suggested that the exposure period can also play a critical role in semen quality [68]. The explosion of a chemical plant in 1976 in Seveso, Italy, led to environmental contamination with high levels of TCDD. The exposed men were evaluated in 1998 and it was observed that exposure during the prepubertal period had an inverse relationship between serum concentrations of TCDD and the quality of semen, while exposure during adolescence had a positive association with semen quality, which was explained by a stimulatory effect. Interestingly, exposed men between 18 and 26 years of age had no association with serum TCDD levels and semen quality.

Additionally, in another study, it was observed that men exposed to PCBs under the age of 20 years were found to have a lower chance of fathering a male offspring compared to non-exposed age-matched men. The sperm of men born to those women exposed to PCBs had abnormal morphology, reduced motility and strength [63]. These results confirm the hypothesis that the exposure period is of fundamental importance in the phenotype caused by EDC exposure.

Testicular Germ Cell Tumors

The frequency of testicular germ cell tumors (TGCT), which comprises more than 95% of all testicular cancers, increased significantly in the period between 1973 and 2002, far beyond the expected growth of the populations. Currently, about 1% of Danish and Norwegian young people will be diagnosed with testicular cancer during their lifetime. This marked increase in TGCT is much lower than that observed in the changes in semen quality. However, it is important to note that data from several non-Western countries should be interpreted with caution due to the lack of longitudinal data, as well as the lack of cancer registries across the country [13, 69].

It is observed that there is geographic variability in the increases of the TGCT incidence, while the increase of the incidence occurred in a relatively short period, these data indicated that genetic factors alone cannot explain this phenomenon. Therefore, environmental factors and lifestyle may play a causative role in this process. These hypotheses are supported by migration studies in which the first generation of immigrants have incidence rates similar to their country of origin, but their descendants have tumor rates similar to those of the country in which they reside [70].

So far, the evidence on the relationship between EDCs and the risk of TGCTs are limited. Interestingly, in a case-control study, no association was observed between serum concentrations of organochlorines in patients with TGCT and controls; however, there was an association with serum organochlorine levels in their mothers in the prenatal period, this being a predictor of increased risk of TGCT in adulthood [71]. The measured organochlorine included hexachlorobenzene, PCBs, pp′-DDE (a DDT metabolite); the data reinforced the theory that EDCs may be part of the fetal program of diseases in adult life, including tumors.

Regarding the studies of EDC effects on male reproductive system, much of the results found in populations are in accordance with experimental studies in animals, but there are some pitfalls. A mixture of different compounds with antagonistic effects (estrogenic, antiestrogenic, antiandrogenic) is present in the environment. Another important problem is the limited knowledge about the time lag between exposure and effect. For most effects, the critical window of exposure has not yet been identified, so that it is not always clear whether to look for in utero, perinatal, pubertal or life-long exposure. Additionally, epidemiological research in general may be influenced by many factors such as selection of the study area, sample size, gender, age, body fat, so that adjustment for confounders has to be done before interpretation.

Conclusion

The true impact of endocrine disruptors on human health is difficult to assess because specific end points may be differentially affected at different ages. Another limitation inherent in epidemiological studies is that humans are not exposed exclusively to the chemical being investigated, but instead to a mixture of chemicals, some of them acting through common pathways. In addition, no single compound

can act as a surrogate or marker for the others because the contaminant profile varies among individuals.

In 2009 and 2012, the Endocrine Society published position statements about EDCs and principles for public health protection [72]. Recently, these statements were revised, summarized and released [1]. The expansion of data reviewed in the later statement strengthens the hypothesis that EDCs are contributing to the increased prevalence of chronic diseases, including obesity, diabetes mellitus, reproduction, thyroid cancers and neuroendocrine and neurodevelopmental functions. The EDCs are an international problem and the population, the media, politicians and governmental agencies should be educated on ways to avoid EDC exposure and to protect developing children, in particular.

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Testosterone Misuse and Abuse

23

Rakesh Iyer and David J. Handelsman

Background

The pivotal role of the testis as the source of virility and fertility has been known since antiquity. Castration of men and animals was practised in ancient times to generate obedient slaves or harem guardians, as punishment, religious self-mutilation or revenge for sexual misdemeanour as well as to render domesticated animal species more docile. The Chinese eunuch system, a traditional practice dating from the imperial period, persisted into the turn of the twentieth century [1] as did the European practice of castration of boys to preserve their high pitched voices combined with large adult lung capacity for opera singing [2]. Furthermore, since ancient times, building on this vague perceptions of testicular function together with the decline of virility with age, the desire for rejuvenation fostered attempts to revive youthfulness and virility by manipulation of the testes to restore their youthful functions. This led to the development of organotherapy as a means for rejuvenation. Outbursts of rejuvenation fads have erupted whenever there has been sufficient affluence to afford indulgence in that health hobby. Episodes included the sixteenth century expeditions of Juan Ponce de Leon to the Caribbean in a search for the fabled Fountain of Youth and other imagined schemes for life extension [3]. But, undoubtedly the greatest flowering of rejuvenation quackery occurred at the turn of the twentieth century associated with the names of Brown-Sequard, Steinach and Voronoff [4]. Organotherapy had garnered scientific credence in the late nineteenth century when Berthold demonstrated experimentally the androgen dependence of male secondary sexual characteristics by finding that castration-induced changes in the capon of roosters were reversed by implanting testes into the abdominal cavity of the castrated roosters [4]. It gained mainstream attention after Charles Edouard

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Brown-Sequard, a genuine pioneer of experimental endocrinology during his working life, claimed at a meeting after his retirement that self-injection of crude animal testicular extracts restored his vitality, heightened his intellectual capacity and sexual potency for prolonged periods. This was derided by contemporary peers as fantasy [5] and subsequently proven to be a placebo effect because his well-documented aqueous extraction procedure obtained only trivial amounts of hydrophobic testosterone [6]. Nevertheless, treatment by the Brown-Sequard's method of organotherapy was enormously popular among the affluent turn-of-the century European and North American public by offering the façade of scientific respectability to the wishful concept of rejuvenation by administering extracts of testes. Concurrently, the Austrian surgeon Steinach promoted a vas ligation procedure as an alternative rejuvenation procedure, which was reportedly performed on 100 University professors in Vienna including Freud as well as the great Irish writer WB Yeats. The third alternative was developed by Serge Voronoff who grafted slices of various non-human species testis onto the capsule of the human testis. These popular mass delusions only subsided in the 1930s with the coincidence of the Great Depression, which withdrew the disposable cash to spend on frivolous pursuits, as well as the scientific discovery of testosterone as the major male hormone secreted by the testis.

In 1935, testosterone was fully characterised as the principal circulating androgen produced by the human testis [7] in a discovery first published by a Dutch group headed by Ernst Lacquer, who also coined the name testosterone, and soon confirmed by others (Butenandt, Ruzicka). In another anomaly of Nobel history, only the latter two went on to share the 1939 Nobel Prize for Chemistry. This discovery was quickly followed by the first reported clinical use of testosterone in 1937 [8]. After the hiatus of World War II, the usage of testosterone proliferated in the post-war decades and it remains one of the oldest marketed drugs still in regular clinical use. Yet despite decades in clinical use, the proper use of testosterone for hormone replacement therapy in pathological disorders of the reproductive system remains clouded by various wider claims asserting unproven and/or unlikely benefits, mostly the unrecognised reincarnation of wishful thinking about rejuvenation. Here we address appropriate indications for testosterone use as well as the misuse of testosterone, defined as prescribing without valid clinical indications, and abuse of testosterone and related synthetic androgens (androgen abuse), defined as the non-prescription use of androgens without medical indications, such as by athletes, bodybuilders and others for recreational, cosmetic or occupational reasons.

Testosterone Use

The testis has two distinct but inter-related physiological functions: steroidogenesis (synthesis and secretion of testosterone) and spermatogenesis. Pathological hypogonadism results from a wide variety of disorders of the testis (primary hypogonadism) or of the hypothalamus and/or pituitary (secondary hypogonadism). Disorders of the testis result in damage to Leydig cells, thereby reducing their ability for LH-dependent testosterone secretion whereas defects of the hypothalamus-pituitary unit reduce pituitary LH secretion which impairs the LH drive to Leydig cell

testosterone secretion. Physiological testosterone replacement remains the appropriate treatment for men with pathological hypothalamo-pituitary or primary testicular disorders which are usually lifelong and irreversible. The one variation is that gonadotrophin deficient men (i.e. with secondary hypogonadism due to hypothalamo-pituitary disorders) seeking fertility can have spermatogenesis induced as well as testosterone production maintained by administration of hCG and FSH treatment [9].

Any hormone deficiency state resulting from a pathological disorder of the endocrine organ and/or its neuroendocrine or other regulation warrants replacement of the deficient hormone aiming to rectify the deficiency by restoring physiological exposure to the deficient hormone. In that process it is aimed to recreate in androgen deficient men the health, efficacy and safety experience of people with normal functions of that endocrine organ. Nevertheless, any hormone is likely to have pharmacological effects, which may be exploited medically in non-endocrine conditions, subject to rigorous proof of efficacy, safety and cost-effectiveness as for any other xenobiotic drugs.

Hence testosterone can be used in two distinct clinical modes. In one, testosterone is used for physiological androgen replacement therapy (ART) for men with androgen deficiency due to pathological disorders of their reproductive system. The medical and scientific rationale for such hormone replacement therapy is analogous to that of hormone replacement for other hormone deficiency states or, analogously, replacement of non-endocrine organs by transplantation for organ failure. The other mode of testosterone use is as part of pharmacological androgen therapy (PAT) whereby testosterone, or more usually a synthetic androgen analog based on testosterone structure, is used aiming to improve the natural history of an underlying non-endocrine systemic disorder rather than rectify a hormonal deficiency state. The synthetic androgens have the advantage of enhanced potency and other pharmacological properties (e.g. oral bioavailability, prolonged duration of action) so as to be more useful therapeutically in non-endocrine disorders. One key difference between physiological and pharmacological applications is that in replacement therapy, only testosterone is used because it has a distinctive full spectrum of effects, which also depend on its two bioactive metabolites, dihydrotestosterone, a more potent androgen, and estradiol, the sole potent oestrogen. By contrast, pharmacological androgen therapy has no restrictions on the chemical structure of androgen analogs employed, most of which may be non-aromatizable and/or inactivated by 5α reduction in contrast to testosterone. In PAT the dose and duration of treatment are dictated by evidence of its efficacy, safety and cost-effectiveness in the various non-endocrine applications regardless in the first instance of androgen status.

Androgen Replacement Therapy (ART)

Androgen deficiency (AD) due to pathological hypogonadism remains the only unequivocal therapeutic indication for testosterone treatment in men [10]. Exogenous testosterone, when administered in physiological doses, emulates the effects of endogenous testosterone resulting in induction and maintenance of

secondary sexual characteristics, and positive impact on target tissues such as bone and muscle and rectification of somatic symptoms of androgen deficiency although it cannot substitute for gonadotrophins required to induce spermatogenesis. The prevalence of classical AD necessitating replacement therapy is 1 in 200 men, which makes it the most common hormonal deficiency among men [10]. Due to its variable and often subtle clinical features AD remains under diagnosed as exemplified by Klinefelter's syndrome, the most frequent single cause of classical AD, in which only ~25% are diagnosed during their lifetime [11] even in countries with national health system which ensure access to medical care regardless of financial limitation. Classical AD is a clinical diagnosis with a pathological basis and confirmed by hormonal assays. However, its clinical features differ depending on chronicity, disease severity and age at diagnosis. The resultant clinical features can range from disruption of male sex differentiation and somatic development during foetal life, to incomplete sexual maturation during adolescence and to regression of virilisation and non-specific symptoms when the presentation is in the adult. The non-specific symptoms, such as lethargy, fatigability, tiredness, decreased libido and depression are common to virtually all hormone deficiency states as well as most chronic diseases. Hence while such non-specific symptoms are typical of classical AD, occurring highly consistently and at reproducible serum testosterone concentrations within individuals [12] but varying greatly between individuals. As a result individual non-specific symptoms would have extremely low positive predictive value for pathological hypogonadism when applied to men without known disorders of the reproductive system. Interpreting non-specific clinical symptoms in conjunction with blood testosterone and gonadotropin levels may potentially reduce misattribution of non-specific symptoms to AD but remains highly likely to over-diagnose apparent AD.

A sound knowledge of testosterone physiology and influences on it is required to interpret serum testosterone measurements appropriately. As serum testosterone shows diurnal and intra-individual variation [13], multiple morning blood samples to measure serum testosterone together with serum LH, FSH and SHBG are required to confirm the clinico-pathological diagnosis of AD. Despite testosterone deficiency causing considerable morbidity and suboptimal quality of life, life expectancy is minimally shortened among men with lifelong classical AD [10] or castration in early life [14]. As a result, improvements in life expectancy from testosterone replacement therapy are implausible. However, testosterone replacement effectively reduces morbidity by virtue of its action on androgen sensitive tissues leading to normalisation of physical and somatic symptoms and by maintaining its positive effects on somatic tissues.

Pharmacological Androgen Therapy (PAT)

Use of testosterone, or more often its synthetic androgen analogs, as treatment for non-reproductive disease is pharmacological androgen therapy (PAT). The primary purpose of PAT is to exploit the beneficial somatic (non-reproductive) effects of

androgen in chronic disease regardless of background endogenous androgen status. PAT improves the morbidity or quality of life in certain conditions by modifying the natural history of the underlying disease. For example, PAT has undergone clinical trials and has some therapeutic role in aplastic anaemia and anaemia due to renal failure, AIDS wasting, respiratory and cardiac failure and prevention of hereditary angioedema with varied symptomatic benefits [15]. However many of these traditional indications for PAT have been consigned to second tier therapeutic status by newer more expensive, disease-specific treatments although androgens often remaining a cost-effective, affordable alternative.

Testosterone Misuse

Misuse of testosterone is defined as prescribing of testosterone without valid medical indications. This includes prescribing androgens for male sexual dysfunction (in the absence of proven AD), male infertility and as a tonic for non-specific symptoms associated with ageing, especially sexual dysfunction and reduced vitality. Despite absence of any valid new indications, off-label testosterone prescribing has increased markedly in more than 40 countries especially over first decade of the new century especially over its second half [16], most dramatically in Canada where per capita testosterone sales increased by 40-fold. Over this decade in the USA, testosterone sales increased ten-fold [16], whereas prescriptions among health-insured men 40 years or older increased by only three-fold [17] indicating that the greatest increases in testosterone prescribing was among non-insured men [18]. Yet, only 20% of new testosterone users, predominantly men without pathological hypogonadism, received treatment for more than 30 days [17] suggesting lack of valid indications or sustained benefits of testosterone treatment in these men. In a review of testosterone prescribing practice in one insured population, only 3.1% of new prescriptions met the hormonal assay criteria required to confirm the diagnosis with 32% failing to record crucial baseline safety parameters and 25% had no testosterone levels measured in the previous 12 months [19].

This epidemic of testosterone over-use/misuse seems primarily driven by direct-to-consumer marketing and single-issue clinics promoting testosterone as a tonic to combat perceptions of middle and older age health problems [18]. In addition to rising trends in testosterone prescription across the globe there are also notable regional and differences between countries [20–23] with the most dramatic escalation in Canada [16] where many internet pharmacies are physically based and not subjected to FDA regulatory controls. In addition to the direct-to-consumer advertising in North America, and single-issue and anti-ageing clinics, the remarkable coordinated global increase in testosterone prescribing is also notably due to the permissive US [24] and European-based [25] clinical guidelines, republished in 2005–2006 virtually unchanged [26, 27]. The European recommendations appeared in 11 different peer-reviewed journals and were widely cited (>1000 times, Web of Science, Dec 2015).

A major additional driver complicit in this greatly expanded "market" for testosterone has been professional societies that promulgated prescribing guidelines which abolished the fundamental distinction between pathological hypogonadism and functional disorders associated with low circulating testosterone. Such recommendations were enthusiastically adopted and broadcast by single-issue or anti-ageing clinics marketing new more convenient testosterone products. In particular the rising proportion of transdermal products confirms that testosterone was being prescribed primarily for older men as younger men with androgen deficiency due to pathological hypogonadism facing lifelong treatment mostly prefer long acting rather than daily administration products for long-term therapy [28]. Although newer testosterone products were licenced for the stated indication of pathological hypogonadism thereby avoiding any need to prove safety and efficacy, after licencing they were marketed mainly for the off-label use in age-related states associated with a low serum testosterone, a loophole which allowed bypassing the need for proof of safety and efficacy which should have been required for use for other than pathological hypogonadism.

Testosterone and Male Ageing

Presently, the most prominent misuse of testosterone is as an unproven treatment for male ageing. The search for means of an ageless existence remains a resilient social fantasy which recurs whenever sufficient social stability and affluence allow for indulgence in the wishful fantasy of eternal youth or its revival contra ageing. In that setting the prospect of ameliorating male ageing with testosterone has attracted wide interest in the public and among professionals. After the collapse of organotherapy in the 1930s, rejuvenation fell into a state of hibernation until the last two decades when such wishful thinking re-emerged to regain public and professional attention as neo-rejuvenation therapy for middle-aged and older men [18, 29]. In this revival it is alleged to alleviate symptoms that accompany ageing using testosterone to treat newly minted conditions under various misnomers such as "andropause", "LowT" and "late-onset hypogonadism".

Modern medicine aims not only to prolong life but also to improve its quality and foster healthy ageing in the process. Increasing life expectancy alone brings more evident age-related co-morbidities based on the physical and mental changes as part of diminishing capacities and organ functions with ageing. Broadly, endocrine organs are also subject to changes that accompany ageing with declining serum testosterone like that of thyroxine or IGF-I. However, there is growing evidence that the gradual, inconsistent and modest decline in circulating testosterone associated with progressive ageing may be due to the co-morbidities of ageing rather than ageing itself. Hence, the extension of testosterone treatment to men with partial, subclinical and/or compensated androgen deficiency states remains not only unproven but also increasingly implausible in the face of the evidence that the decline in testosterone is the consequence of co-morbidities of ageing rather than their cause or a deficiency state.

Among unselected men in the community, serum testosterone levels consistently decline with ageing [30, 31]. From population-based observational studies the annual rate of decline in serum testosterone has been estimated as 0.5–2% per year [32–34] with more recent, representative studies show lower rates [31, 35] or no decline at all among men who remain in excellent health, free from major co-morbidities [36]. Similarly, reproductive function is also maintained even after sixth decade in sexually active men [37, 38] with well-known examples of men in their eighties or older fathering children leading to the maxim that natural male fertility only ends with death, in contrast to natural female fertility. Nevertheless, the modest and inconsistent decline in serum testosterone observed in male ageing has been misleadingly equated with menopause or pathological hypogonadism. However the functional decline in circulating testosterone accompanying male ageing or its co-morbidities cannot be equated with the abrupt and virtually complete cessation of estradiol secretion in menopause nor with the inability to secrete testosterone in pathological hypogonadism. Other than flushing, which is rare and usually due to acute androgen deprivation (e.g. castration for advanced prostate cancer), all symptoms associated with androgen deficiency are non-specific. Such non-specific symptoms are common to virtually all hormonal deficiency states as well as chronic diseases so that, not surprisingly, they are increasingly prevalent as various disorders accumulate with ageing. Hence, characterising "andropause" based on arbitrary blood testosterone thresholds levels together with non-specific symptoms [26, 27] remains fundamentally misguided. Such non-specific symptoms have minimal positive predictive value for authentic androgen deficiency when applied to unselected middle-aged or older men. Coupling such non-specific symptoms with a coincident low serum testosterone only selects for men who have co-morbidities of ageing. Hence, in essence, this "definition" of andropause is merely a surrogate for ageing and its co-morbidities, not a novel medical condition in itself.

It is increasingly recognised from studies that age itself does not determine androgen status in ageing men but that acquired co-morbidities that accumulate during ageing determine the perceived impact of ageing on circulating testosterone levels which can be mistakenly attributed to age itself. In particular obesity, incident chronic illness, increased use of medications, smoking, physical deconditioning, depression and stressful changes in social circumstances accelerate the decline in circulating testosterone as men age [34, 39, 40]. One post-hoc definition of ill health, defined by the presence of obesity, excessive alcohol and/or chronic illness, adds 10–15% to the annual decline in serum testosterone [34], which can be equated the impact of an extra decade of ageing [39]. Meta-analysis confirms that weight loss in obese men increases serum testosterone levels [41]; however, these changes provide no symptomatic benefit [42]. This suggests that somatic symptoms experienced by older men are more likely related to age-associated co-morbidities and not the accompanying mild reduction in serum testosterone so that testosterone treatment of older men without pathological hypogonadism is unlikely to provide any clinically meaningful benefit.

Potential Benefits of Testosterone Treatment in Older Men Without Pathological Hypogonadism

At any age genuine, pathologically based androgen deficiency remains an unequivocal indication for testosterone replacement based on effective improvement of signs and symptoms of androgen deficiency [43]. On the contrary, however, among men without pathological hypogonadism but experiencing functional states associated with lower serum testosterone and symptoms and/or sexual dysfunction, there is minimal evidence of improvement of somatic symptoms with testosterone treatment [44–46].

Testosterone treatment produces small (2–4 kg), dose-dependent decreases in fat mass and corresponding increases in muscle (lean) mass and strength regardless of androgen status with similar effects whether men are healthy, eugonadal, genuinely hypogonadal or have functional low testosterone states [47–52]. Hence such responses are not evidence of a prior testosterone deficiency state.

Similarly although testosterone treatment improves bone mineral density among men with pathological hypogonadism [43], it remains unclear whether this is also true of men without pathological hypogonadism. Although some studies showed positive results [53, 54], a meta-analysis concluded that older men without pathological hypogonadism treated with testosterone showed increase in lumbar mineral density but no increase in femoral bone density leaving only weak evidence for testosterone treatment to improve bone density in older men without pathological hypogonadism [55].

Male sexual function involves a complex interplay of central and local mechanisms. While sexual desire and arousability are centrally mediated, penile tumescence, orgasm and ejaculation are locally mediated. The major recognised impact of testosterone is on sexual interest and motivation (libido). It is well known that testosterone replacement therapy in young men with pathological hypogonadism results in improved libido, erections and sexual function [43]. However, among men presenting with erectile dysfunction (ED), genuine androgen deficiency is a rare cause [56]. This is because the common basis for ED is a neurovascular disorders, which is a sentinel feature of underlying cardiovascular disease not a hormonal deficiency state. Hence as men presenting with ED rarely have underlying pathological hypogonadism, testosterone treatment is not expected to improve erectile function nor does it [57, 58]. A confusing feature is that recent evidence shows that sexual activity maintains serum testosterone. Hence among men with sexual inactivity due to ED, some mild reduction in serum testosterone is expected and not to be confused with testosterone deficiency [59–62]. PDE inhibitors improve erectile function; however, testosterone when given alone or in conjunction with a PDE 5 inhibitor does not improve erectile function in men with ED [44, 61, 63]. A large meta-analysis concluded that testosterone treatment increases libido but provides no significant benefit for erectile function [58], a disjunction between desire and performance that Shakespeare's Macbeth considered an adverse outcome of alcohol ("*it provokes the desire, but it takes away the performance*"). Short-term studies have suggested that testosterone treatment may improve verbal and spatial memory

in healthy older men without pathological hypogonadism [64] but not among those with mild cognitive loss and low normal testosterone levels [65].

In conclusion, there is no definite evidence that modest decline in circulating testosterone associated with male ageing is due to ageing itself rather than the co-morbidities that accumulate during ageing. As such it represents functional hypothalamic-mediated reaction to chronic illness rather than a rectifiable deficiency state with potential to improve any somatic features of male ageing. However, definitive proof requires adequately powered, placebo-controlled clinical studies which should rather be directed at the co-morbidities of ageing rather than age itself. In the interim, the massive increases in testosterone prescribing over the recent decades is driven more by disease mongering and marketing to wishful thinking about rejuvenation.

Androgen Abuse

Androgen abuse is defined as the use of testosterone or another synthetic androgen for non-medical purposes, without any medical indication. Androgen abuse aims to exploit the muscular and motivational effects of high dose androgens and has been practised as a variant of social drug abuse for decades.

The original motivation for androgen abuse was for its effects on muscle mass and strength. Androgens are particularly effective ergogenic drugs for power sports such as lifting, throwing, sprinting or fighting [66]. These ergogenic benefits arise mainly from increases in muscle mass and strength but are also aided by an increase in erythropoiesis leading to increased circulating haemoglobin. In concert, these effects produce major competitive advantage and represent the most potent ergogenic class of drugs known in power sports. In the wider community, androgens are abused for cosmetic and recreational purposes such as bodybuilding, the body-beautiful subculture and for developing an intimidating physique in security-related professions.

Androgen abuse was initially restricted to marketed synthetic androgens as the only available androgens. However, in recent times, the growing market demand has fostered the development of never-marketed, so-called designer and nutraceutical androgens. These are synthetic androgens with the distinction between these designations based simply on the ephemeral circumstances in which they are marketed and identified. These synthetic androgens are based on the largely forgotten, expired patent literature of the 1960s and 1970s when thousands of synthetic androgens were either developed or foreshadowed by expansive coverage of ambitious patents. By definition, all androgens combine intrinsic anabolic and androgenic properties, which have never been meaningfully separated [67], manifest via the androgen receptor, a protein encoded by a single copy gene. Hence the singularity of androgen action means that the terms “anabolic steroid” or “androgenic-anabolic steroids” remain an obsolete terminology making a distinction between androgenic and anabolic effects where there is no real difference [67]. This obsolete yet widely used terminology represents a vestige of the unsuccessful quest by the

pharmaceutical industry to dissociate the virilising from anabolic properties and remains in the public mind mainly as a media piñata. Androgen abuse, a more appropriate term which encompasses illicit use of all available androgens will be used in this chapter.

Origins of Androgen Abuse: From Epidemic to Endemic

Androgen abuse began in the 1950s as a form of cold war competition whereby Eastern European countries used surreptitious systematic national doping schemes to demonstrate their social superiority to Western capitalist countries. This challenge was rapidly reciprocated by some Western sporting teams, and androgen abuse has eventually spilled over to the general community beyond elite sports driven by their ability to create iconic uber-masculine body image. Originally, androgen doping was embraced by elite athletes and coaches from competing nations, reaching epidemic proportions in certain sports particularly power sports where the increases in muscle mass and strength and haemoglobin gave athletes competitive advantage [68]. With the fall of Berlin Wall state sponsored systematic androgen doping by East Germany was unearthed [68] and other comparable Eastern European national doping programs likely remain undisclosed. Towards the end of the Cold War among countries with sustainable illicit drug subculture networks, androgen abuse spread from elite power sports to the larger market of recreational user for cosmetic and image enhancing effects. By the 1990s this had transformed from an epidemic focused on elite power sports into a new, endemic dimension of illicit urban drug consumption easily grafted onto body-beautiful and image enhancement subcultures. The spread into the community was fuelled by entrepreneurial drug dealers fostering an underground folklore, epitomised by the infamous Underground Steroid Handbook of the early 1980s, culminating in the present situation where presently the majority of current androgen abusers now aim to achieve cosmetic goals such as bodybuilding and/or image sculpting for recreational or occupational reasons rather than sports doping [69, 70]. In the recent times the alarming practice of systematic state sponsored doping as exemplified by the East German state sponsored doping program [68] may have resurfaced involving bribery, corruption and high-level suppression of doping tests and results involving the Russian field athletes and the international athletic federation [71].

Prior to the mid 1990s, the ergogenic effect of androgen doping was discounted by medical scientists who attributed any benefits to placebo responses [72, 73]. In 1996, it was proven that supra-physiological doses of testosterone increased muscle mass and strength providing an unequivocal ergogenic advantage. Subsequent well-controlled clinical studies have shown that the effects of testosterone (vs. placebo) on muscle are dose-dependent from below to well above the normal range, do not plateau at even 6 times standard doses, and produce equivalent effects in older as in younger men [47]. From the 1950s during the quest for the pure anabolic steroid, thousands of synthetic androgens were synthesised and patented; however, only a minority of those covered by such patents were ever marketed. Most synthetic

androgens were 17-α alkylated, which made them more potent and orally active but created a class-specific hepatotoxicity comprising risks of hepatic adenoma, carcinoma, peliosis, cholestasis and hepatitis. These problems have led to the progressive withdrawal of such 17α alkylated androgens from the legitimate clinical market due to their toxicity as safer androgens were available. Nevertheless a handful of the notorious synthetic 17α alkylated androgens such as stanozolol, methandienone and oxandrolone are still widely available on the Internet exclusively for illicit androgen abuse [74–79]. These compounds with well-known chemical structures are readily detected by mass spectrometry-based detection methods [80], deterring androgen doping using these compounds as they are highly likely to get detected, a fact widely publicised by the 1988 disqualification of Ben Johnson's Olympic gold medal winning 100 m sprint performance for use of stanozolol. Consequently athletes intent on gaining illicit ergogenic advantage from androgens have developed several alternatives means to exploit androgens for their ergogenic effects but trying to evade detection. For example, novel never-marketed synthetic androgens (e.g. designer androgens) have been developed to evade detection. One approach was to review the literature of expired patents to identify never-marketed synthetic androgens whose unfamiliar and undisclosed chemical structures make them impossible to detect with mass spectrometry-based urine detection tests until their structures and metabolites are known [77, 81, 82]. The first such designer androgen identified in an athlete's urine was norbolethone, a 17-α alkylated androgen originally synthesised in 1960 but never marketed [83]. Soon after, tetrahydrogestrinone (THG), a previously unknown androgen produced illicitly by a one-step chemical reduction of a marketed alkylated progestin (gestrinone) was identified structurally [84] and then as a potent androgen by an in vitro androgen bioassay [85]. Subsequently, desoxymethyltestosterone (Madol), another never-marketed androgen patented in 1960s was identified [86]. A recent review notes at least six designer androgens available over the Internet [79]. Nevertheless once identified, these designer androgens have never again been detected in regular doping tests reflecting effective deterrence. More broadly, similar never-marketed androgens are also commonly found in unregulated, over-the-counter and Internet marketed food supplements, which often do not identify steroids on the label but are promoted as purportedly legal bodybuilding alternatives to androgens [77, 87].

Despite intensive research over decades in the post-war Golden Age of steroid pharmacology during which oral contraceptives and synthetic glucocorticoids were developed, the pharmaceutical industry's goal to synthesise an androgen with purely anabolic but devoid of virilising properties so it could be effectively used in women and children remains a remote and increasingly implausible dream [67]. Nevertheless in a triumph of hope over experience, the quest for a selective androgen has been revived under the guise of new class of drugs called specific androgen receptor modulator (SARM) [88, 89], directly analogous to the class of oestrogen mixed partial agonists/antagonists specific known under the marketing term, specific oestrogen receptor modulators (SERM). In theory, SARMs are tissue selective AR ligands synthesised with an aim to improve tissue selectivity, extend the clinical utility beyond the primary indications of testosterone or other androgen therapy

while negating their undesirable side effects. The first non-steroidal androgen was invented in 1998 [90] but the efficacy and safety profile of any non-steroidal androgen (or SARM) has not yet been fully evaluated [91]. Although no SARM has been approved for clinical use to date, SARMs in development and in pre-marketing phase have become available over the Internet with one such compound Andarine, prohibited in sport since 2008, has been identified in the urine of athletes [92].

Epidemiology of Androgen Abuse

Androgens have been prohibited in elite sports since 1970s and WADA (established in 1999) regularly releases updates on anti-doping statistics that provide an insight into the extent of doping detected in elite sports. As the most potent doping agents in elite power sports due to their myotrophic effects, androgens provide the majority of positive doping tests recorded in WADA's 32 approved national anti-doping labs. For example, in 2014, among the total of 283,304 samples analysed, 1.36% had either adverse analytical findings (AAF, presumptive anti-doping rule violations, 1.1%) or atypical findings (ATF, abnormal test results not necessarily an anti-doping rule doping violation, 0.25%), with nearly half of all positive tests (including AAF and ATF Table 23.1) due to androgen doping, three times higher than the next category of prohibited drugs [93].

However, recent allegations of widespread doping and related corrupt practices among Russian Olympic field athletes and African long distance runners have again raised the spectre of systematic doping on an organisational scale, not reported since the revelations of the East German national doping program [68]. An independent panel commissioned by WADA concluded that there was firm evidence of collusion by state authorities, national anti-doping and sporting authorities involving high profile athletes and coaches to evade doping detection by corruption of detection testing as well as interference and intimidation of laboratory processes leading

Table 23.1 2014 WADA statistics on adverse analytical findings and atypical findings for androgen doping

2014—Type of Androgen Doping	AAF	ATF
	Number (%[a])	Number (%[a])
Direct		
Natural and synthetic androgens	1199 (38.0)	333 (46.7)
SARM (non-steroidal)	15 (0.5)	–
Indirect		
hCG	17 (0.5)	92 (12.9)
Anti-oestrogen (SERM)	79 (2.5)	–
Aromatase inhibitor	38 (1.2)	–
LH	–	6 (0.8)
TOTAL	3153	713

AAF adverse analytical findings, an anti-doping rule violation, *ATF* atypical analytical findings, abnormal findings but that are not an AAF

[a]percent of all AAF or all ATF

to sanctions on the Russian athletics team, national anti-doping laboratory and agency.

In recent decades, androgen abuse has become an established endemic in affluent countries. It has spread from elite athletics into high school sports programs as well as gym and fitness centres. The endemic androgen abuse has been fostered by shifts in cultural norms with an increasing focus on uber-masculine body image which forms part of the micro-culture in many gyms as well as among drug suppliers with commercial interests as a means to attain otherwise unattainable hyper-muscular physique. Other driving factors for androgen abuse in the community include psychological well-being to boost self-esteem, confidence and concentration, securing sports scholarship and family and/or peer influence [94].

A recent meta-analysis of 187 studies estimated the global lifetime prevalence of androgen abuse in general population as 3.3% with the prevalence 4 times higher in men (6.4%) than women (1.6%) [95]. The prevalence of community-based androgen abuse varies according to geographical location, with highest prevalence reported in Middle East and South America, as well as among immigrants and minorities within a country [96], findings suggesting the influence of psychosocial and economic factors [95]. These are striking findings despite the known limitations of questionnaire-based surveys such as inflated prevalence estimates due to ambiguity in self-report of "steroid" use confusing it with non-androgens (e.g. glucocorticoids for asthma) or over-the-counter food supplements [97]. Androgen abuse in the community appears to begin in high school years [98, 99] and mostly subsides by the third decade of life [99]. Recent US surveys suggest that adolescent androgen abuse may have peaked around the turn of the twenty-first century with decreasing prevalence over the past 15 years [99, 100]. The turnaround from around 2000 appears to be related to highly adverse publicity in the US Congress and more widely about the admitted androgen abuse among elite baseball and football players.

There is also evidence that androgen abuse may be more prevalent in various minorities. For example, among gay and bisexual young men, the strong focus on physical strength and muscular appearance may explain the high prevalence of androgen abuse. In a cross sectional survey of gay men frequenting London gyms where one in 7 gay men had used androgens in the past 12 months [101]. A pooled analysis of 14 US jurisdictions showed that sexual minority adolescents were at an increased odds (5.8, 95% CI 4.1–8.2) to report a prevalence of ever-use of androgens compared with heterosexual counterparts [102]. There is also evidence that ethnic minorities are more prone to androgens abuse [94–96]. These findings further illustrate the permeation of androgen abuse into wider society but with uneven foci according to predisposing socio-economic factors.

Types of Androgen Doping

Androgen doping is classified into direct or indirect. Direct androgen doping involves administration of marketed and non-marketed synthetic androgens or exogenous natural androgens such as testosterone, DHT as well as pro-androgens

like DHEA and androstenedione. Initially, marketed synthetic androgens were the commonly abused androgens but these became readily detectable by mass-spectrometry-based urine detection tests. As a result, a variety of other approaches to androgen doping have developed. Although synthetic androgens of known chemical structure are readily detected by exquisitely sensitive, gas or liquid chromatography coupled to mass spectrometry, they require prior knowledge of chemical structure and metabolite profiles of the administered drug. Natural androgen precursors such as DHEA and androstenedione have also been used in doping but their efficacy depends on conversion to potent bioactive androgens and their ultimate ergogenic efficacy remains unclear although their administration is detectable.

Alternative approaches to direct androgen doping comprised use of never-marketed (designer or nutraceutical) synthetic androgens, use of natural androgens (e.g. testosterone, DHT) or pro-androgens (DHEA, androstenedione) as well as indirect androgen doping utilising non-androgenic drugs (e.g. hCG, LH, oestrogen blockers, aromatase inhibitors) to increase serum LH and endogenous testosterone production. Indirect androgen doping may be used either to gain ergogenic advantage or to mask or reverse the deleterious effects of reproductive axis suppression resulting from direct androgen doping [103].

A well-established form of indirect androgen doping is the administration of hCG, a naturally occurring long-acting analog of LH. hCG is a heterodimeric glycoprotein produced by human placenta comprising an α and β subunit. hCG's α subunit is identical with that of LH (and the other pituitary glycoprotein hormones FSH and TSH), while the β subunit of hCG is highly homologous with LH but with a C terminal read-through extension of 29 amino acids. This extra portion contains four O-linked sialic acid capped, glycosylation side-chains which markedly prolong the circulating half-life and potency of hCG compared with LH which lacks this C terminal extension. LH and hCG both act on the common CG/LH receptor expressed on testicular Leydig cells to stimulate testosterone secretion. As a natural long-acting analog of LH, hCG is conveniently able to be administered at longer than daily intervals, whereas LH would require administration many times daily. hCG is available for clinical use either as a product extracted from human pregnancy urine or a recombinant product produced by genetically engineered mammalian cells in culture. The sole therapeutic indication for hCG in men is to restore normal serum testosterone concentration and androgen status in gonadotropin-deficient men undergoing induction of spermatogenesis and fertility [9, 103]. In men, hCG administration produces a sustained increases in endogenous testosterone production with increased serum and urine testosterone but suppressed LH concentrations [104] and unaffected urine T/E ratio [104, 105]. By contrast, LH has no therapeutic indication in men and was never available clinically especially as pituitary gonadotrophins (mainly FSH) extracted from cadaveric pituitaries were reported in 1985 to cause deaths from Creutzfeldt-Jakob disease [106]. More recently, a recombinant LH has been marketed but appears to be an ineffective doping agent as administration failed to increase serum or urine testosterone even at very high (32 times recommended) dose [104]. This lack of efficacy coupled with the need for multiple daily injections and high cost render it an implausible as a genuine doping threat; nevertheless,

vigilance is still required to detect and deter even hard to understand doping practices. Both hCG and LH are prohibited at all times in elite sports although only for male athletes. In females hCG or LH provides no performance enhancement and hCG testing can detect early pregnancies which unreasonably breach privacy.

Another alternative form of indirect androgen doping is the repetitive use of superactive GnRH analogs (not pure GnRH antagonists) in brief periods sufficient to sustain increased endogenous LH and testosterone secretion—known as the flare phase—before the desensitisation and downregulation ensues as expected as a result of sustained, unphysiological GnRH stimulation [107]. The prevalence of such GnRH analog use is unknown.

Oestrogen blockade in men causes a reflex increase in serum LH and testosterone as a result of interference with hypothalamic negative steroidal feedback of testosterone on pituitary LH secretion [108]. Such blockade can be created by use of anti-oestrogens (e.g. clomiphene, tamoxifen, raloxifene, newer SERMs) [109] or by the use of aromatase inhibitors, which inhibit estradiol synthesis. Oestrogen blockers are commonly used as part an adjunct to androgen abuse to prevent or treat gynecomastia and/or to reverse the suppressive effects of androgen abuse on the hypothalamo-pituitary testicular axis. In reality such treatment merely prolongs any underlying gonadal axis suppression while defers its recovery [110]. By contrast, oestrogen blockade by either class of drugs has negligible effect on blood testosterone concentrations in women [103]. This leads to the requirement that female athletes with breast cancer treated with adjuvant anti-oestrogen treatment require a therapeutic use exemption to permit their use of banned anti-oestrogens.

Patterns of Androgen Abuse

There is wide variation in androgen abuse pattern. It is influenced by type of sports involved, geography and drug availability. Commonly used patterns are cycling, stacking or pyramiding.

Cycling is a common practice characterised by “on” cycle when users administer steroids for a period of time followed by rest period called “off” cycle. The duration of cycle varies among users and the rest period is used to recover androgen sensitivity, avoid detection and/or minimise the side effects. Cycling is used more by competitive athletes to avoid detection and intermittently restore sensitivity to androgens, whereas non-competitive bodybuilders, lacking concern for detection, more often use androgen continuously [111]. Pyramiding involves increasing the androgen dose to peak level followed by gradual tapering to the base level before the next cycle. It is often used purportedly to reduce side effects. Stacking involves taking two or more agents with different pharmacological profiles in order to gain synergistic benefit; however, often the various androgens differ mainly or even solely in their trade names.

Androgen abusers also combine use of other banned drugs such as growth hormone (GH), GH secretagogues and erythropoietin for their ergogenic effects, which are claimed to synergise with androgen abuse. In addition they may use other

non-banned drugs to combat adverse effects of androgen abuse such as 5α reductase inhibitors (for male pattern balding) and retinoids and/or antibiotics (for androgen-induced acne).

Laboratory Detection of Androgen Abuse

Androgens have been prohibited in elite sports since the 1970s with deterrence based on sensitive detection by sensitive mass spectrometry-based urine tests. Steroid immunoassay, although widely available, was never sufficiently specific to uphold an anti-doping rule violation due to the lack of specificity of antibodies allowing for cross-reactivity with structurally related steroids. This weakness could never eliminate the possibility of misidentifying banned drug use sufficient to prove a doping offence with sufficient reliability to prohibit a professional athlete from pursuing their occupation for cheating. Furthermore, the mono-analyte approach of immunoassays would require increasing numbers of immunoassays for every new steroid, whereas antibodies were often not available or specific enough rendering this approach nonfeasible. Instead detection methods such as gas chromatography and liquid chromatography mass spectrometry, which had reference level specificity, inherent multi-analyte capability together with high sensitivity became the mainstay of doping detection for androgens with a known chemical structure [55–57]. This covered the complete range of marketed synthetic androgens and could be readily added to when structures of any new steroids were identified. Ongoing research identifying longer-term metabolites of synthetic androgens continues to widen the window of detection of direct androgen doping.

Once the ready detection of synthetic androgens was understood, an alternative approach to direct androgen doping was to use exogenous testosterone or other natural androgens or pro-androgens. This creates problems because unlike synthetic androgens, which have distinctive chemical signatures proving their non-biological origin, exogenous natural steroids cannot be distinguished from their endogenous counterparts at least by conventional mass spectrometry. The first approach in detecting exogenous testosterone use has been to measure the urine testosterone/epitestosterone (T/E) ratio. Both testosterone and its biologically inactive 17α epimer, epitestosterone, are co-secreted by Leydig cells and both are excreted in the urine as phase II glucuronidated metabolites from which the T/E ratio is measured. While epitestosterone production rate is <5% of testosterone, its rapid urinary excretion creates a relatively stable within-individual urine T/E ratio with a mean of about 1.0 and 99% upper confidence limit of 4.0 [112] in a Caucasian population. Administration of exogenous testosterone sufficient to reduce Leydig cell production of both testosterone and epitestosterone increases the urine T/E ratio because the testosterone includes both exogenous and endogenous testosterone whereas the E includes only the suppressed endogenous steroid production. This provides a reliable screening test for exogenous testosterone use. An important limitation of the urine T/E ratio is the relatively common genetic deletion polymorphism of the phase II hepatic enzyme, uridine diphosphate glucuronyl transferase 2B17 (UGT2B17).

This enzyme renders testosterone more hydrophilic by glucuronidation to facilitate the urinary excretion of its more polar metabolites [113]. The homozygous UGT2B17 deletion results in a virtual elimination of testosterone glucuronidation by UGT2B17 so that such otherwise healthy individuals have a population mean urine T/E ratio of ~0.1, a false negative phenotype which may mask exogenous testosterone administration [114]. This deletion variant is rare among Caucasian populations but is highly prevalent in South-East Asian population [113]. Even without genotyping, that deletion phenotype is so distinctive that this challenge to the urine T/E ratio has been met by the introduction of the Athlete's Biological Passport (ABP) [115]. This implements a Bayesian model of serial adaptive individual-based reference limits to supplant population-based reference limits [116]. By this means, an athlete acts as their own control over time with their own individual reference range for urine T/E ratio (or other measurements in the ABP) which is narrower than the population ranges for the same variables. An alternative or corroborative marker is the urine T/LH ratio [104, 105, 117]. However, this measure depends on the validity of urine LH immunoassays but these commercial immunoassays were only ever developed for human serum samples although some LH immunoassays have proven valid with human urine samples [118].

An important tool for detecting administration of exogenous natural androgens is the carbon isotope mass spectrometry, which measures C^{13}/C^{12} ratio of urinary excreted testosterone. This depends on the fact that commercial production of steroids uses plant sterols as a starting material. Over 95% of plants, including those used as starting materials for commercial steroid synthesis, employ C3 photosynthesis which features isotopic fractionation by preferring C^{12} over C^{13} leading to a depleted C^{13}/C^{12} ratio in commercial synthesised steroids. Hence administration of commercially sourced exogenous testosterone product results in a lowered C^{13}/C^{12} ratio in urinary testosterone compared with endogenous testosterone produced by mammalian enzymatic processes, which feature no isotopic discrimination. Hence a significantly low C^{13}/C^{12} ratio in urinary testosterone is indicative of exogenous testosterone administration. An analogous approach can also be applied to detect other naturally occurring androgens (DHT), pro-androgens (DHEA, A) or even epitestosterone administration attempting to mask testosterone doping by manipulating (lowering) the urine T/E ratio. A limitation of this methodology is the recent identification of a small minority of seized testosterone product samples with non-depleted carbon isotope ratio [119]. The complementary development of hydrogen ion ratio MS further refines the ability to distinguish between endogenous and exogenous steroids including in such cases [120, 121]. As noted, suppression of urine LH may provide corroborative evidence for the administration of any exogenous including natural or synthetic androgens.

Another versatile paradigm for detecting androgen abuse is the use of in vitro androgen bioassays [85, 87]. These incorporate the human AR gene with a convenient AR-mediated response read-out indicator into yeast or mammalian cell host cells so that exposure of the host cell to any bioactive androgen will produce a quantifiable signal. These androgen bioassays aligning with AR activation as the mechanism of androgen doping to provide a generic detection for any bioactive

androgen regardless of chemical structure. While mammalian cells provide a more sensitive host, they also express steroidogenic enzymes and other steroid receptors which thereby sacrifice specificity for sensitivity. Conversely, they gain the advantage of also identifying pro-androgens, chemicals without or with minimal intrinsic androgen bioactivity but which may be converted in the body into potent androgens [87]. By contrast, yeast host cell bioassays display high fidelity in detecting solely intrinsic androgen bioactivity of any chemical, a feature that was crucial in securing the first conviction for use of a designer androgen (THG) previously unknown as an androgen [85]. The detection of any androgen bioactivity regardless of chemical structure provides an advantage over mass spectrometry, which requires knowledge of the chemical structure of any analyte. Hence, when coupled, they provide powerful additive detection capabilities [122, 123]. Limitations of in vitro androgen bioassays are their susceptibility to non-specific matrix interference effects from biological fluids such as urine as well as the difficulty in establishing suitably rigorous standardised methodology involving viable cells. Nevertheless, in vitro androgen bioassays are uniquely useful in screening substances for unsuspected androgenic or pro-androgenic bioactivity [124] which athletes may ingest creating inadvertent doping [125].

Another option to detect androgen doping is the use of hair, skin or nail samples which can be minimally invasive, convenient to store dry and with potentially very long detection windows; however, the methodologies have so far been explored only for hair [126–128] and the methodology, while widely used in forensic toxicology, has yet to be accepted for routine anti-doping testing.

Indirect androgen doping is generally more easily detected than direct androgen doping with fewer potential agents and most having established detection methods. hCG has been routinely detected in urine by immunoassays which, unlike LH immunoassay, are validated for human urine having been developed for pregnancy testing and monitoring of trophoblastic tumours [129, 130]. Recently, more accurate, sensitive and specific immunoextraction-mass spectrometry proteomic methods are becoming available [131] with some proof-of-principle clinical studies [132]. As hCG provides no ergogenic advantage in women while potentially invading privacy by detection of unsuspected early pregnancy, hCG testing is confined to men [103]. An important consequence of hCG testing of young male athletes is the detection of early stage, hCG-secreting tumours of the testis or extra-gonadal midline germ cell tumours which require prompt, expert medical management [133] but which are rare, possibly due to the protective effect of exercise on testis cancer [134, 135]. Oestrogen blockade by anti-oestrogens or aromatase inhibitors is routinely included in MS-based urine detection methods.

Adverse Effects of Androgen Abuse

Despite the increasing prevalence of androgen abuse, the literature on its adverse effects is limited, largely due to clandestine nature of abuse that leads to underreporting and a systematic limitation of the inability to establish causation from anecdotal and observational reports. The adverse effects of androgen abuse have been

reviewed in more detail elsewhere [136–138] so are reviewed only in outline here. They fall into several categories including cardiovascular, psychological, reproductive and other effects.

Cardiovascular

A wide variety of cardiovascular harms have been associated with androgen abuse. Pathological findings include lethal arrhythmias, cardiomyopathy, left ventricular hypertrophy, myocarditis, myocardial infarction, cardiac tamponade, cardiac failure, thrombotic and haemorrhagic stroke, subdural hematoma, peripheral artery and venous thrombosis and pulmonary embolism [137, 139]. As the causality of these effects remains speculative, prospective risks remain difficult to define. A recent randomised, placebo-controlled study of testosterone administration at conventional doses for older men with limited mobility was terminated prematurely due to excess adverse cardiovascular events [140]; however, case-control studies and meta-analyses of testosterone administration at conventional doses in older men have produced conflicting findings [141–144]. It remains suspicious but unproven whether cardiovascular effects of androgen abuse exceed expectations for the general population [145].

Reproductive Function

Reproductive effects of androgen abuse are profound but at least initially reversible. Suppressing the hypothalamo-pituitary drive to testicular function leads to hypogonadism with consequential testicular atrophy, impaired spermatogenesis, infertility, sexual dysfunction and gynecomastia in men. In women, suppression of hypothalamo-pituitary axis leads to amenorrhea, anovulation, infertility, breast atrophy and hypertrophy of clitoris. Recovery of reproductive function after stopping androgen abuse hinges on duration and intensity of abuse. With prolonged, high dose use, such as professional bodybuilders often using massive doses continuously for years without interruption, it may take more than a year or in some extreme cases no recover at all. Underground and internet doping folklore claims that using hCG or anti-oestrogens may hasten the recovery of hypothalamo-pituitary testicular axis; however, the evidence for this remains weak. In any case even if such adjunctive treatment has short-term benefits from stimulating testicular function, it will prolong and further delay the recovery from underlying suppression of reproductive function.

Liver

Hepatotoxicity is a serious adverse effect arising from oral 17α-alkylated androgens although not other androgens (natural androgens including nandrolone, 1-methyl androgens) [146–149]. The hepatotoxicity includes hepatic tumours (adenoma,

carcinoma, cholangiosarcoma, angiosarcoma) as well as peliosis hepatis and drug hepatotoxicity (usually cholestatic). Most hepatic tumours are slowly progressive and reversible upon cessation of androgen ingestion but fatal cancers are reported. Peliosis hepatis, focal hepatic necrosis causing vascular cysts, causes hepatic and/or splenic enlargement and serious, even fatal, bleeding either spontaneously or following liver biopsy. Although biochemical and/or ultrasound monitoring for liver damage is theoretically feasible, it is neither cost-effective nor justifiable for a predictable complication of drug abuse.

Psychiatric

Arguably the most alarming adverse effects of androgen abuse is the risk of adverse neuropsychological disturbances. These can affect not only the individual but also their family and the community [150]. Neuropsychiatric side effects can vary from mild mood disorders, poor judgement, uncontrolled aggression, hostility, sleep disturbance, mania and depression [150–153]. Observational evidence correlates psychiatric side effects with the severity of abuse [154]. Direct experimental evidence has proven a risk of hypomania induced by short-term, high dose androgen exposure in apparently healthy volunteers as an idiosyncratic risk affecting an unpredictable small minority (~5%) of individuals [155]. A preoccupation verging on obsession with muscularity is prevalent among androgen abusers and androgen abuse is over-represented among violent men and prisoners [156–160]. However, whether androgen abuse causes the aberrant behaviour or the degree to which prior personality traits predispose to androgen abuse remain unclear [158, 161] due to the limitations of distinguishing these mechanisms based on anecdotal and observational evidence. There is increasing evidence that androgen abuse may involve dependence [153] the nature of which and its relationship to obsessive-compulsive and eating/exercising disorder remains unclear.

Conclusion

Although the appropriate use of testosterone replacement therapy for androgen deficiency due to pathological hypogonadism is well-established and effective, there is growing misuse and abuse of testosterone and other androgens. Dramatic increases in testosterone prescriptions for newer transdermal products but without any new valid indications is apparently driven by aggressive marketing with a focus on ill-defined concept of rejuvenation therapy for male ageing. Increasing prescriptions is a misuse fostered by permissive professional society prescribing guidelines that fail to distinguish between pathological hypogonadism and function disorders associated with a low circulating testosterone and greatly amplified by direct-to-consumer marketing and single-issue anti-ageing clinics. Only better understanding and professional education can reverse this misdirection of medicine highlighting appropriate testosterone prescribing and highlighting the illogicality as well as lack of efficacy and safety evidence for testosterone treatment of male ageing.

Androgen abuse in elite sports and in the community remain ongoing concerns. Effective deterrence of banned androgens in elite sports has been achieved by the WADA's Prohibited List and anti-doping testing regimen which has made effective ergogenic use of natural and known synthetic androgens highly likely to be detected by sensitive urine detection tests. Nevertheless due to efficacy of androgen for doping in power sports mean that such these regulations are always under challenge but novel testing has been meeting these challenges which extend beyond the individual athlete to their coaching and support staff and even state-sponsored corruption. In the community the endemic androgen abuse requires well-designed psychological primary prevention programs aiming to deter adolescents from future androgen abuse but so far these have proven effective at increasing knowledge but not reducing androgen abuse intentions or behaviour [162] suggesting an overlooked but dominant role of coaches. In the community, prevention of androgen abuse requires both demand and supply reductions involving not only well-targeted education of appropriate age cohorts and addressing psychosocial predisposing factors but also enforcing laws to curb illegal networks which maintain and supply androgen abuse subcultures.

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Index

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