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Testosterone

From Basic to Clinical Aspects

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To my family: my parents (Rainoldo and Selma), my sisters (Luciana and Simone), my nephew (Nicholas) and nieces (Luana, Bárbara, and Mariana), and my partner (João).

To Brazilian endocrinologists, who even with so many adversities, practice medicine in a meaningful and special manner; as an example of integrity and commitment, Dr. Marisa H. C. Coral and Dr. Ricardo M. R. Meirelles.

To my colleagues who collaborated on this and all books that provide quality scientific knowledge.

To patients who inspire us to seek the path of healing and relief of suffering.

And to all those who have always believed and supported my work.

Preface

We live in difficult times in society today. The speed for receiving new information is instantaneous. The volume of news and messages is huge and present everywhere: paper publications, television, website searches, social networks, and mobile applications.

If on the one hand we have become a society that increasingly gains weight and becomes sick, it is also difficult to know what "healthy" means. Doctors and other health professionals suggest trendy diets, restrict food randomly, create new diseases, and promise the fountain of youth. Patients come to doctors' offices often distraught.

The main complaints are recurring: weight gain, fatigue, discomfort, lack of energy, and poor memory. Until recently, there seemed to be an obvious explanation: the current intense lifestyle with high levels of stress, unbalanced diet, and lack of regular physical activity. Still, endocrinologic evaluations were performed to rule out subclinical diseases that could be occurring. Normal examinations indicated the path to follow: change the lifestyle!

However, today this is not enough. There must be a "magic" solution. There must be an altered hormone level. Even if for this, a "new cutoff point" for disease diagnosis is established by a physician or a group of physicians with an interest in creating "diseases" in healthy patients.

Furthermore, an increasingly frequent process is occurring: the prescription of hormones! Worldwide, the use of testosterone in eugonadal men has increased considerably. This dangerous phenomenon is occurring in many countries. As soon as there are symptoms of loss of libido or fatigue, there is some hormone-prescribing physician writing a prescription (even without adequately assessing additional tests) and saying they practice "modern medicine".

Similarly, the use of testosterone in women, which has an even more restricted medical indication, is skyrocketing. In this case, the main reason seems to be aesthetic: reduce fat, increase muscle growth, and build a "perfect" body. However, almost always it is accompanied by masculinization and other adverse effects.

Something must be made clear: there is no "magic pill". We live in an era of *evidence-based medicine* and it is not conceivable to prescribe any hormone for healthy patients with normal examinations simply to improve symptoms resulting from another cause or aesthetic reason.

The goal of this textbook is to revisit the entire universe of testosterone; from its molecule to its accurate use in well-diagnosed endocrinologic diseases. So that every doctor or medical student can take advantage of this work to diagnose and properly treat every patient who seeks us, avoiding the inappropriate use of hormones and iatrogenesis.

Florianópolis, Brazil Alexandre Hohl, MD, MsC, PhD

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The History of Testosterone and The Testes: From Antiquity to Modern Times

1

Eberhard Nieschlag and Susan Nieschlag

"The Oldest Key to the Endocrine Treasure Trove: The Testicles"

With that phrase Victor C. Medvei [37], 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 have been 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.

This chapter describes how knowledge about androgenic functions of the testes evolved, that detours and blind alleys that were taken toward the actual discovery of testosterone and finally, how testosterone preparations have been developed for clinical use. An earlier historical report can aid as a supplement to this chapter [45].

Effects of Testis Removal

In Greek mythology, Chronos (Saturn) castrated his father Uranos (Zeus) because he did not allow him to procreate 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

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Vecchio, Florence). In hellenistic times (fourth to first century B.C.) the power attributed to the testes is also reflected in the cult devoted to Diana Ephesina. Seeking favors, good luck, health, and fertility, worshipers affixed the 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 modern times as exhibited in the Archeological Museum of Naples (Italy) (Fig. 1.1). It was clarified quite recently that the bull testes were originally mistaken for 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 they obtained 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 considered among the richest people in history. The last imperial eunuch, Sun Yaoting, died in 1996 at the age of 94. Castration had previously been used to produce obedient slaves who are loyal to their masters and rulers. Over the centuries in Islamic societies castrated slaves, who were predominantly imported from Subsahara Africa as well as from Europe and Asia, were the work force and constituted elite troops who were deployed in wars of conquest.

Fig. 1.1 Diana Ephesina (= Artemis of Ephesus) decorated with sacrificed bulls' testes (Roman creation, 2nd century BC—Archeological Museum Naples)

Castration has also been used as lawful punishment. In medieval Scandinavia high treason was not subject to capital punishment but rather to castration combined with blinding. This was adopted by the Normans who introduced this custom wherever they ruled [70]. After the invasion of England in 1066, William the Conqueror largely abolished the Anglo-Saxon death penalty and replaced it with 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".

For other, more benign purposes, castration was carried out prior to puberty in young boys to maintain the high pitch so that soprano and alto voices resulted with the acoustic volume an adult male. They were featured in operas of the seventeenth and eighteenth centuries, such as those composed by Georg Friedrich Händel (1685–1779) or Nicola Porporra (1686–1768). Such voices could be heard until the twentieth century in the Vatican choirs. Some of those castrated 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 Italian cities of Norcia and neighbouring Preci, secluded in the Sibellini Mountains in Umbria, specialized on delicate operations including castration of young boys. Going back to the thirteenth century, 30 family dynasties formed the Scuola Chirurgica di Preci [17] and monopolized the trade there, guaranteing utmost secrecy concerning this operation which had been 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 deplorable hygienic conditions, in all probability cost the lives of hundreds of boys, however—if the procedure was successful—chances for a lucrative career compensated for the risks. Because of their high-pitched voices, but with a significantly larger volume than in women, castrati were in high demand for opera performances.

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 (Fig. 1.2). The Scuola Chirurgica di Preci was also famous for other surgical procedures such as cataract extraction, and the surgeons were in demand throughout Europe. 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 [21].

Those early castrates served in what can be considered a 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 lives both these groups of artists had to endure, but also revealed no difference in their life expectancy [51].

Fig. 1.2 A butcher's shop in the city of Norcia, Umbria, Italy, advertising for "Specialitá Agnello Castrato" (castrated lamb) (Photo taken in 2015)

In contrast, the lives 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 [39]. 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 the testes in the castrati singers and the Korean eunuchs may also account for the difference.

Lessons from Experimental Testis Transplantation

While removal of endocrine glands is one basic concept of experimental endocrinology, replacing the glands is the other. As a 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 belief, his goal was to demonstrate the survival of the transplant resulting from 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. 1.3 Arnold Adolph Berthold (1803–1861), "father of endocrinology", as depicted on the Berthold Medal, the highest decoration for achievements in endocrinology a warded by the German Society of Endocrinology

At the University of Göttingen Arnold Adolph Berthold (1803–1861) (Fig. 1.3) also 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" [10]. 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 [34].

It would take another 50 years until an endocrine function was clearly attributed to the Leydig cells, when Ancel and Bouin [1] 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 [14]). 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 Ernest Starling [65] in London.

Berthold's experiments were initially not accepted by the scientific community—including his own department director Rudolf Wagner (1805–1864)—until Moritz Nussbaum at the University of Bonn in 1908 [54] and A. Pézard in Paris in 1910 [56] repeated and confirmed Berthold's results using frogs and chickens, respectively.

Erroneous Conclusions from Berthold's Experiments

Possibly prompted by these findings, surgeons turned to testes transplantation as a means to treat hypogonadism and bring about rejuvenation and therapy for all types 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 [36]. Also in Chicago, Victor D. Lespinase (1878–1946) published his experience with transplanting human testes from donors to patients for rejuvenation [33] 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. Stanley later turned to rams as sources for his testicular grafts and reported satisfaction on the part of the patients, which included 13 physicians [63, 64].

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 unleashing a series of lawsuits demanding millions of dollars as compensation. Brinkly declared bankruptcy and died of a heart attack soon thereafter.

In Vienna, Eugen Steinach (1861–1944) performed vasoligation for rejuvenation [66]. One of his followers, Serge Voronoff (1866–1951) turned to xenotransplantation and used monkey testes to be transplanted for rejuvenation [73]. He first offered the surgery in Paris, but after several scandals, continued his questionable operations in Algiers, where he was visited by patients from all over the world. Voronoff's followers xenotransplanted animal testes and pieces thereof to patients demanding rejuvenation in many countries of the world. When unrest among the medical profession grew regarding this quackery, the Royal Society of Medicine (London) sent an international committee to Voronoff in Algiers in 1927. The committee concluded their investigations by declaring Voronoff's claims as unsubstantiated.

Those 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 must be discussed.

Testes for Organotherapy

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 over the centuries. In Rome, Gaius Plinius Secundus (23–79) prescribed the consumption of animal testes for the treatment of symptoms of hypogonadism and impotence. For the same purpose in Baghdad, the Arabic physician Mesue the Elder (777–837) recommended testis extracts. Additionally, 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 an alternate method of consumption [37].

Charles-Edouard Brown-Séquard (1847–1894), the well-known scientist and member of several high-ranking learned societies, gave organotherapy a new dimension when, at the age of 72, he published the results of his dubious self-experimentation in the Lancet [11]. 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, because the testes synthesize testosterone, but do not store it (as e.g. the thyroid does with its hormones), and the amount administered was minute [18]. However, "extracts of animal organs by the Brown-Séquard method" were immediately sold worldwide and factories sprang up in Europe as well as in the United States. 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 J. 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".

The 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). The 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 overall image of the relatively new field of endocrinology. Harvey W. Cushing (1869–1939), a famous neurosurgeon, went so far as to discuss "endocri*mi*nology" in the context of this organotherapy. Nevertheless, many companies worldwide continued to manufacture extracts and pills, well into the period of time when genuine testosterone was already long on the market. It was not until 1961 that Ciba (Switzerland) withdrew Androstin® (= "biologically titrated full extract from male gonads" for oral and parenteral use) from the market, after three decades of successful sales for the treatment of "male gonadal insufficiency, impotence, infantilism, premature aging and endocrine obesity" [58].

Isolation and Synthesis of Testosterone

The pharmaceutical industry, finally reacting to the hype generated by testicular transplantation and organotherapy, started cooperating with academic research to rehabilitate endocrinology and replace organotherapy with proper hormone substitution. In 1935, the era of testosterone emerging as a biochemical and marketable entity, Ciba (Switzerland) and Schering (Germany), pharmaceutical companies both active in the field, began 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 syndicate to share knowledge, to stake their market claims worldwide, and to agree on product pricing [58].

Important steps in the isolation of testosterone were the development of biological tests for androgen activity. Loewe and Voss [35] described androgenic activity in urine and developed the Loewe-Voss-Test for measuring androgenic activity. Moore et al. [40] refined and standardized the capon comb test as the unity of androgenicity. That biological test helped to resolve the question of whether *only one* or *several* androgenic steroids existed and, if more than one—which might be the more potent.

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 then processed by Schering to obtain 15 mg of this first androgen [12]. In 1935 Ernst Laqueur (1866–1947) and his group at Organon and the University of Amsterdam extracted and isolated 10 mg of testosterone (17β-hydroxy-4-androstene-3one) from 100 kg of bull testes and found to be more active than androsterone in biological tests [19]. They called the hormone "testosterone". In the same year Butenandt and Hanisch [13] in Göttingen, (Fig. 1.4) as well as Ruzicka and Wettstein [60] in Zurich/Basel

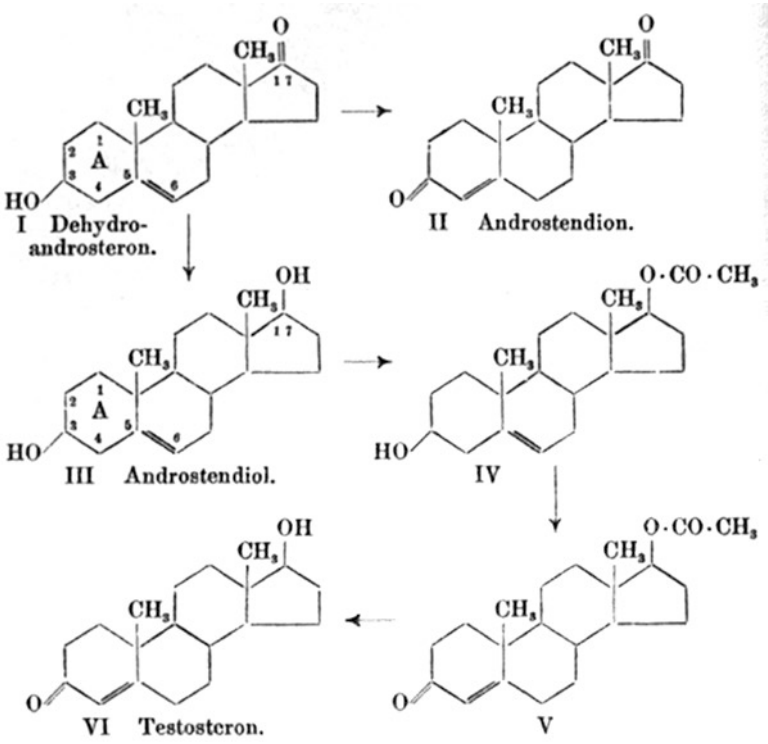

Fig. 1.4 The synthesis of testosterone from dehydroandrosterone as described and shown by Butenandt and Hanisch in their original paper in 1935 [13]

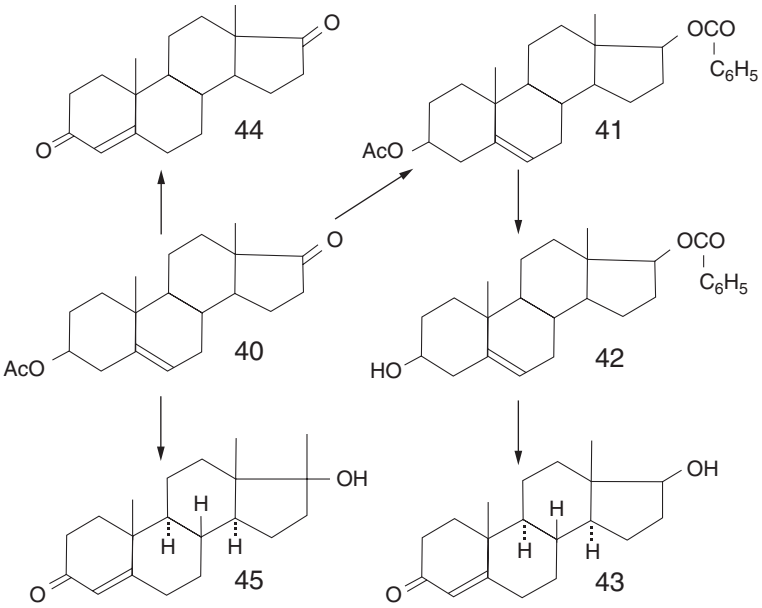

Fig. 1.5 Degradative synthesis of testosterone (43) and 17α-methyltestosterone (45) as described by Ruzicka and Wettstein in 1935. Interestingly, in his autobiography Ruzicka [59] considered the synthesis of 17α-methyltestosterone as "a greater intellectual achievement" than that of testosterone

(Fig. 1.5), released the chemical synthesis of testosterone, marking the beginning of modern clinical pharmacology of testosterone and male reproductive physiology.

The close cooperation of the researchers reinforced by the two pharmaceutical companies may explain why these discoveries were released at approximately the same time. Marius Tausk, one of the former heads of research at Organon who knew the competing protagonists personally, very vividly describes the race to testosterone isolation and synthesis and how the key respective papers were submitted for publication in short sequence in 1935 [68]. In 1939, Butenandt and Laqueur jointly received the Nobel Prize for chemistry, 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 the end of World War II.

The research work was financially rewarding beyond scientific recognition, 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." [59]. During 1939 alone, Ciba transferred 56,744 Swiss Francs to Ruzicka as royalties [58]. Part of this money was invested in a collection of seventeenth century Flemish and Dutch paintings that Ruzicka donated to the Kunsthalle Zürich in 1947 [59].

However, while this pioneering work was well recognized in science and by pharmaceutical companies, clinicians were skeptical about 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 at the time: "The number of testis hormone preparations is still very low. Comprehensive 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" [31]. 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 that were simply not available or were too expensive. We currently know that the lack of oral effectiveness is a result of the inactivation of testosterone by the first-pass effects in the liver (Fig. 1.6) [48, 50]. Three approaches were used to overcome this problem:

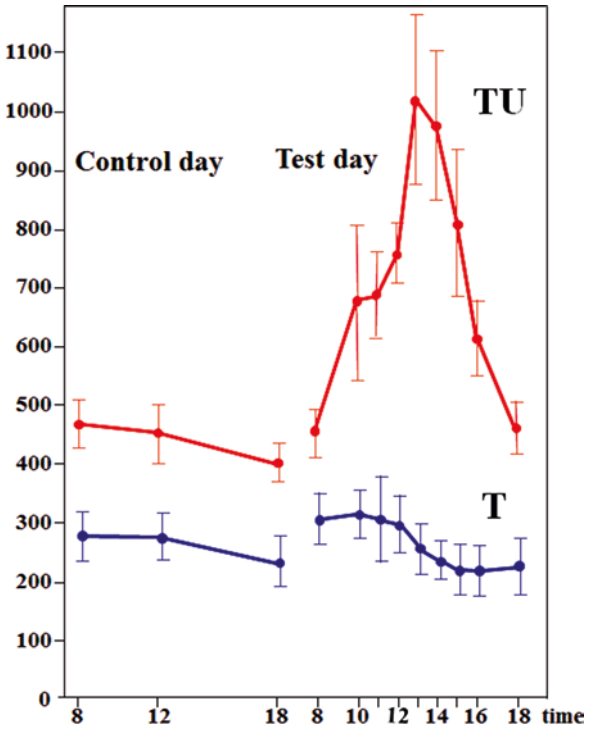

Fig. 1.6 Serum testosterone levels in normal volunteers after ingestion of either 65 mg crystalline testosterone (*lower lines*) or 100 mg testosterone undecanoate capsules (equivalent testosterone doses) (adapted from [48])

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 [43] and Behre and Nieschlag [5] and to respective chapters in this text.

As early as 1935, the year when testosterone was first isolated and synthesized, Ruzicka et al. [61] also synthesised **17α-methyl-testosterone** (Fig. 1.5). Its oral effectiveness was demonstrated and it was soon licensed for clinical use and was well accepted because of the ease of oral application [22]. However, liver toxicity because of the 17α-structure of this molecule soon became evident, particularly with long-term use and at higher doses [79]. It further became clear that the toxic effect would be shared by all 17α-substituted androgens [46], thus giving testosterone in general a bad name among physicians. In the 1980s, however, 17α-methyl-testosterone became obsolete for clinical use—at least in Europe, when another orally effective preparation free of toxic side effects became available (see below).

As testosterone proved to be orally ineffective, parenteral routes were explored. Subdermal **testosterone pellet** implants were the first to be investigated [20] and pellets are still in use today [5]. 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 surgical procedure and harbors the risk of infection and extrusion. However, if enough pellets are implanted they may provide substitution for upto six months. The pellets are predominantly currently used in Australia.

Other parenteral routes were tested in the course of the steroid's existence during which **testosterone suppositories** were marketed by Ferring [23], but yielded rather unpredictable serum levels [49] and are no longer commercially available. The most recent development in this area is bioadhesive **buccal testosterone tablets**, placed on the gingiva and resulting in effective serum levels if applied twice daily [76]. However, because of 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 suitable for substitution. Therefore, the third possibility for making testosterone clinically effective is esterification at the 17β-hydroxy-group of the molecule, making it suitable for 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 and effective serum levels are reached for only 1–2 days. When **mesterolone** (1α-methyl-5α-androstan-17βol-one) became available, Schering used the then well-established name Proviron® for this new oral preparation [24, 41] and continued to market testosterone proprionate as Testoviron®. Because 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 utilized for male infertility treatment—without evidence-based proof of its effectiveness for that indication [28].

Following the propionate ester, **testosterone enanthate** was synthesised by Junkmann [26, 27] at Schering and marketed as intramuscular Testoviron® Depot injection in 250 mg doses, providing substitution for 2–3 weeks [49]. 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 the up and down mood swings and libido between injections, this remained the major testosterone preparation for substitution of hypogonadism for half a century.

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 synthesised, 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. They did, however, retain a shadow existence for doping in sports and bodybuilding, potentially causing considerable undesired effects [46, 47]. 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, not desirable for substitution purposes so that the World Health Organization (WHO) made an appeal for more physiologic modalities of substitution [80].

Unfortunately, the pharmaceutical industry also turned a blind eye on another potentially huge application of testosterone: hormonal male contraception. In the 1970s the WHO Human Reproduction Program and 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, a 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 [42]). To overcome this deficiency both organizations started programs in search of long-acting testosterone preparations. Under the auspices of the WHO, **testosterone buciclate** was synthesised [16] and identified as a long-acting preparation, well suited for male contraception—and by the same token, also for substitution [7]. However, no pharmaceutical company could be inspired to further develop this promising preparation [74], so in its ensuing clinical trials for male contraception, the WHO switched to intramuscular testosterone undecanoate as described below [8].

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 (dihydrotestosterone) and thereby have little effect on the prostate. Because MENT has a 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 in addition to substitution ([53, 67, 72]). 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 continuing the research.

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. 1.6) [15, 25], had been added to the spectrum of testosterone preparations available for replacement therapy. Peter Kicovic, in charge of clinical development at Organon at the time, approached the senior author for first clinical trials with the 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 [44]. Because the assay and the testosterone-antiserum produced for the assay [69] 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 food, the testosterone peaks were short-lived so that 3–4 capsules had to be taken during the day [48, 62]. Oral testosterone undecanoate was introduced to the market worldwide (except in the USA) in the late 1970s under the brand name Andriol®. More recently, an oral self-emulsifying delivery system for testosterone undecanoate has shown better pharmacokinetics than testosterone undecanote in arachis oil [81] and as such, may become acceptable to the FDA.

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, California and first tested in clinical trials in Münster [3, 4, 57], they showed excellent pharmacokinetic and clinical results and, for the first time, physiological testosterone serum levels could be achieved (Fig. 1.7). Patients were satisfied with this physiological pharmacokinetic profile, as long-term substitution revealed [2, 9]. However, physicians were

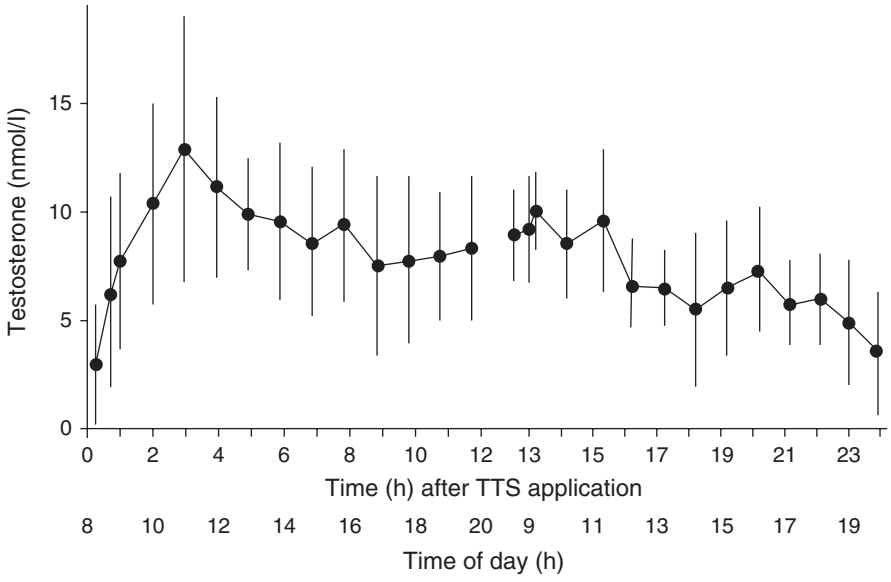

Fig. 1.7 First clinical trial of transdermal testosterone: serum testosterone values in seven hypogonadal men following the scrotal application of a transdermal testosterone film (adapted from [3])

Fig. 1.8 Chinese advertisement for "injectio testosteroni undecanoatis" as exhibited at an andrology workshop in Beijing in 1993. In the Chinese preparation testosterone undecanoate was dissolved in tea seed oil, but showed even more favorable pharmacokinetic properties when dissolved in castor oil

reluctant to prescribe a medication to be applied to the scrotum, preferring a subsequently developed **non-scrotal testosterone system**, Androderm® [38]. That, however, caused unpleasant skin reactions because it required an enhancer to drive testosterone through the skin. For that reason the advent of the first transdermal testosterone gel in 2000 was welcomed for substitution [75]. Of the various gels available currently, 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 [32].

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, as mentioned previously, had been turned into an injectable preparation by Chinese investigators using tea seed oil as a vehicle [77]. When the authors came across it at a meeting in Bejing in 1993 (Fig. 1.8), samples were brought to Germany, injected into monkeys and showed a surprisingly long half-life [55]. 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 as

discussed above, further demonstrating the superior properties of intramuscular testosterone undecanoate [78]. 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 [6]. Several pharmaceutical companies were contacted for further development of the preparation, however, although all were convinced of the excellent pharmacokinetic properties, none were 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 Jenapharm became interested in this fascinating preparation, tea seed oil was replaced by castor oil as a vehicle and the injection intervals could be extended to 12 weeks of physiological serum testosterone levels [6, 9, 52, 71].

Meanwhile, the new testosterone preparation had also been tested in trials for hormonal male contraception and had proved to be effective in combination with norethisterone enanthate [29, 30]. Our 10-year experience with intramuscular testosterone undecanoate has been summarized [82] and has 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 ampules of 750 mg testosterone undecanoate under the brand name Aveed® for the treatment of hypogonadism in the USA.

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Androgen Receptor in Health and Disease

2

Marco Marcelli

Androgenic Hormones

Biosynthesis of Testosterone and 5α-Dihydrotestosterone

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 rise until it peaks in the early second trimester [2]. During this phase of male development, from GW 8–12, 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 rise to the prostate and prostatic urethra, and in the urogenital tubercle, swelling and folds to give rise to the glans, scrotum and shaft of the penis, respectively [3].

At least two generations of Leydig cells have been described in eutherian mammals [4]. 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 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

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fetal population. T production from fetal Leydig cells is Human Chorionic Gonadotropin (hCG)-dependent and Luteinizing Hormone (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 small 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 Adrenocorticotropic hormone (ACTH) 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 into pregnenolone by the first steroidogenic enzyme, CYP11A1. 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 fact that the two pathways run in parallel and entail the same number of enzymatic reactions, most testosterone biosynthesis in the human testis takes place through the conversion of pregnenolone to dehydroepiandrosterone via the Δ^5 pathway, because of 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 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/

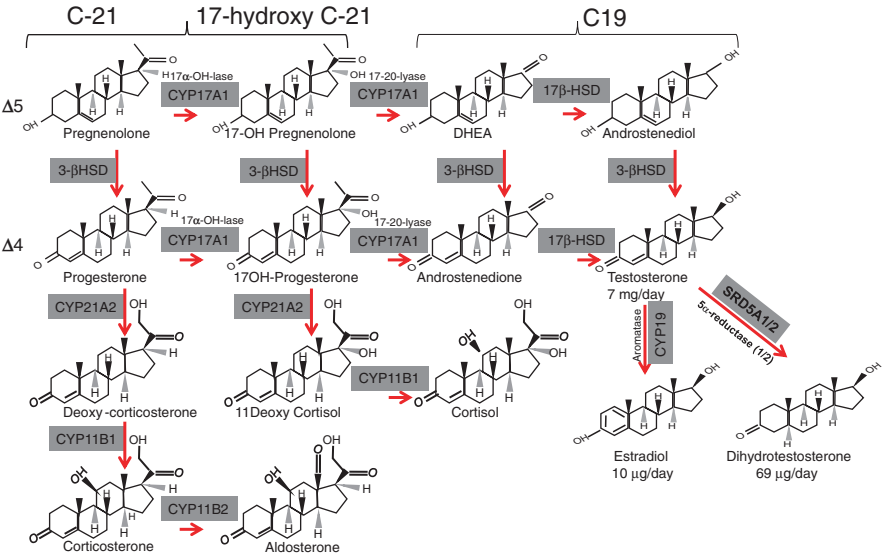

Fig. 2.1 Main pathways of adrenal, ovarian, and testicular steroidogenesis, showing structures of the most important intermediate metabolites and end products, and enzymes involved. Δ(delta)5: Metabolites of the Δ5 pathway characterized by the presence of a double bound between carbons 5 and 6. Δ4: metabolites of the Δ4 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

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 human adrenals produce 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 freely. 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 because of 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 United States), 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, however, this test is not widely available unless for research purposes.

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. The 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 7 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 serum T level is possibly 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 a unique cytoplasmic AR protein with high affinity. 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 as a result of 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 in conjunction with other classic steroid receptors such as glucocorticoid, progesterone, mineralocorticoid, and estrogen receptors α and β (GR, PR, MR, ERα 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 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 with DHT (discussed later), T is a weaker androgen compared with 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, 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 who are 18–50 years old 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, prostate-specific antigen (PSA) levels and sebum production were interchangeably subserved by T and DHT [32]. Based on this information, 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 T and DHT are interchangeable in adult individuals 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 evident for some time that men produce minute amounts of estradiol in their bodies, and that several male organs express ERα and ERβ. However, the physiological meaning of the estrogen → ER axis in males had remained unknown until a unique case of ERα inactivation and several cases of CYP19 deficiency were described [34, 35]. These abnormalities led to conditions of target organ insensitivity or of inability to synthesize estrogens, respectively. 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. The study demonstrated that estrogens also regulate body fat deposition and sexual function in the adult male [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 eight exons (Fig. 2.2a, b). A functioning AR is essential for normal virilization during embryologic development and puberty, as well as 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 about 150 million years to a common ancestor [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

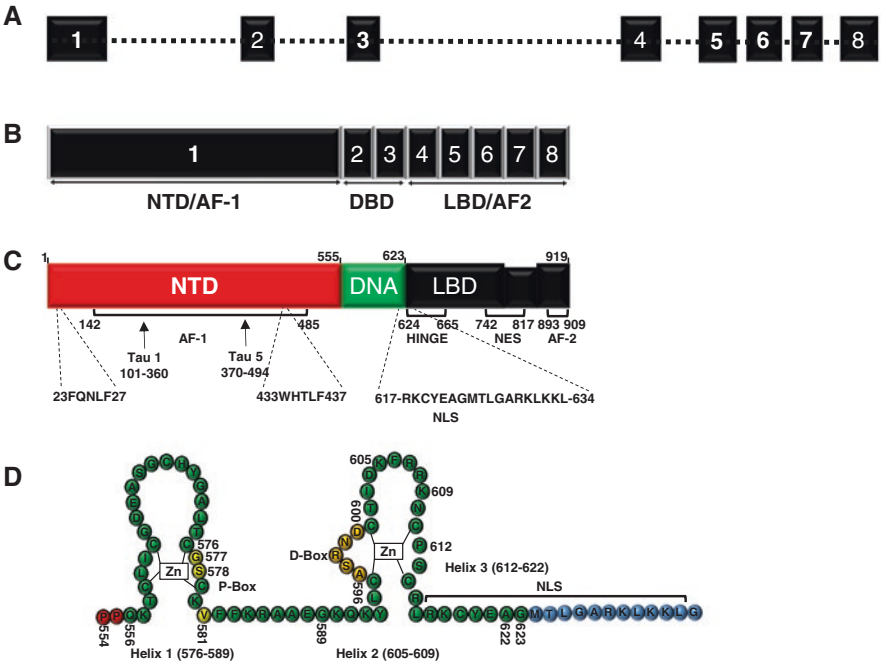

Fig. 2.2 (**a**) AR gene. (**b**) AR mRNA. (**c**) AR functional domains. Color code: NTD *red*. DNABD *green*. LBD *black*. (**d**) Cartoon representation of the AR DBD. Color code: P-Box *yellow*. D-Box *orange*. Exon 1: *red*. Exon 4: *blue*

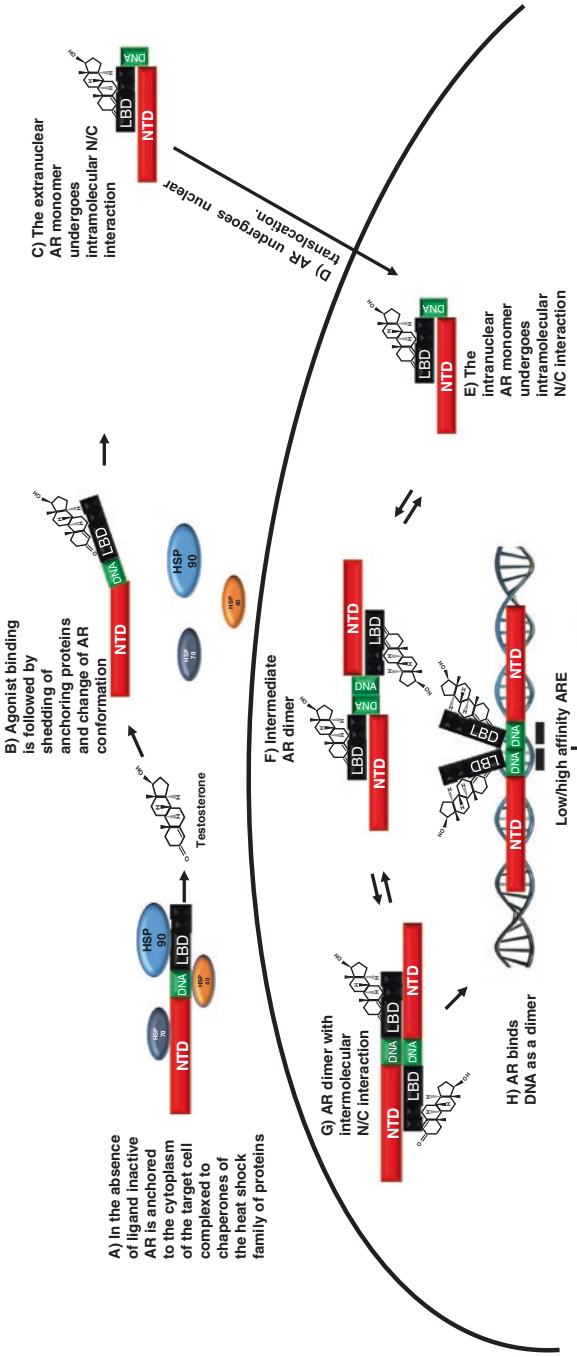

Fig. 2.3 Signaling pathway of the AR schematized in nine steps. Each step is described in the cartoon

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 consists in phosphorylation, acetylation, SUMOylation, methylation, and ubiquitination. It is believed that these modifications 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 manner, and providing a platform for cross communication between growth factors and AR signaling mechanisms. Excellent reviews focusing on the effects of AR post-translational modifications have been published, and the reader is referred to them for additional information on this topic [42–44].

Functional Domains of AR

Similarly to the other NR, AR contains four modular domains (Fig. 2.2c), that include: (1) An 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. In addition to 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 Sect. 2.7. 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 also a significant degree of homology 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 that 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 (see below) [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 bps (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 question, and mechanisms contributing to maintain target specificity of class I nuclear receptors include: (1) Different levels 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 a result of 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, the AR-AR homodimer also 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 have made it possible to pinpoint the entire set of cis-acting targets (named cistrome) of transacting factors on a genome-wide scale. The 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, PSA, occurs through the remotely located PSA enhancer, as opposed to the promoter region [66, 67]. Communication between a distant enhancer with the site where transcription of the gene started, 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 sequencing 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 sequencing 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 has also led to the observation that cis-acting elements for collaborating (or pioneer) factors co-localize 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 small amount 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 a 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 a high affinity binding between AR and T or DHT. The LBD is also 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 four 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 of hydrophobic residues located in β1, H3, H4, H5 and H11, that interact in a non-polar manner with the steroid scaffold of T or DHT [79]. Additionally, 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 the 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 a faster dissociation rate from AR compared with 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 co-activators containing a short leucine rich LxxLL motif (also known as nuclear receptor box) [81]. Presence of the LxxLL motif is a feature of many co-activators, including steroid receptor coactivator-1 (SRC1), TIF2/GRIP1/SRC2 and

SRC3. Co-activator 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 binds 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 Sect. 2.7.

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 receptors, including GR [83], ER [84], and PPAR-α [85]. In the NR, binding of an antagonist causes H12 to rotate clockwise toward H3, block the co-activator binding site, and induce recruitment of co-repressors such as NCoR and SMRT. It is likely that the mechanism of antagonism reported for the NR applies to AR, although the tridimensional structure of antagonist-bound AR has 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 co-repressors [87].

Patients with prostate cancer can experience disease relapse after an initial response to treatment with nonsteroidal anti-androgens such as first generation flutamide and bicalutamide, or second generation enzalutamide. Interestingly, when the antiandrogen drug is discontinued, some patients experience an improvement of their condition. This phenomenon, known as the anti-androgen 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 co-activator 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 10 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, because of 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 (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 (see below), 224–258 and 500–541. AF-1 contains the two transcription units TAU-1 (amino acids 101–360) and TAU-5 (amino acids 370–494) in its sequence [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 (see below). 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 because of deletion experiments performed in the 1990s [100]. Those 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 the effect was AF-1-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 co-activators of the p160 SRC family [107] or CREB-BP [108], co-repressors 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 co-activators (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 because it influences nuclear localization, cofactor binding, DNA binding, and transactivation potential [121]. Types of AR homodimerization that have been discussed in Sects. 2.3 and 2.7 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. It 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 through homology modeling in the amino acids forming the dimer interface between GR monomers and spatially equivalent residues in AR [124].

Coregulatory Proteins

The regulation of AR transcription is structured around completion of two general tasks: (1) Change in chromatin conformation to provide or deny AR and the 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 and are recruited directly or indirectly to DNA by transcription factors. As of January 31, 2016, 320 NR coregulators have been identified (https://www.nursa.org/nursa/molecules/index.jsf). Coregulators can stimulate or repress transcription and they are designated accordingly as co-activators or co-repressors. 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 proteins 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 ATPases and histone modifiers. Nucleosome remodeling ATPases use energy derived from ATP hydrolysis to modify nucleosome organization in a non-covalent manner [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 instances, 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 forms of 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 chapter. We refer the reader to previously published articles for additional information on 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 most well-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]. The 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 domains (AD1 and AD2). AD1 and AD2 act as a scaffold platform recruiting additional coactivators 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 these proteins pharmacologically 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 that information, 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 (testosterone replacement therapy) in hypogonadal patients [136], male pattern baldness [137], and symptomatic BPH [138]. Shorter Q repeats are associated with increased risk to detect higher grade and more advanced stage to detect prostate cancer at diagnosis [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, 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 repeat expansion must be responsible for a toxic gain of function that results in neurologic damage, most likely with a mechanism shared with 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 age at onset and symptoms [141].

Autopsy studies of 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 the 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 past 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 instance, 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 the 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 as 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 it is associated with significant side effects [146]. Hence, the option to target AR in the muscle opens an attractive alternative. Recently, other avenues have attempted to decrease the toxic effect of polyQ AR in SBMA to include the use of microRNA298 (miR-298) [147], anti-androgens [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 androgen insensitivity syndromes (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 the lack of any discernible clinical response to 50 mg/day of methyl-testosterone in a cohort of such patients treated by Dr. Wilkins in the 1940s [152]. The first review of the 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 of AIS 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 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 can be seen in individuals from different families affected by the same missense mutation [155], possibly as a result of 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 co-regulator [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].

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. Patients with CAIS with grade 6/7 external genitalia have a small, blind ending vagina.

Compared with normal males, the endocrine milieu of patients with AIS stands up for two reasons; first, gonadotropins are elevated (LH>FSH), and second, because of 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 because of a lack of an androgen effect in the target tissue. As a consequence, patients with CAIS develop normal feminine shape, breast enlargement, and acne-free complexion at puberty. Many patients with CAIS 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 initiated with the

goal of maintaining secondary sexual characteristics and protecting the skeleton. Bone mineral density is low regardless of the timing of gonadectomy [161], and thus, in addition to ERT (Estrogen replacement therapy), patients with CAIS should be given CA and Vitamin 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. Because these two conditions are at the end of the spectrum of presenting phenotypes, patients with CAIS are typically raised as females and patients with MAIS as males. The decision of gender assignment is much more complex in patients with PAIS, 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 patients with PAIS 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 patients with PAIS 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 one-tenth 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 10,570–23,448 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 having 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 alternative mechanisms within itself 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 beginning in 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 bind with high affinity ligands other than T or DHT. The role of AR mutations in PCa relapse consists of inducing inappropriate agonist responses to non-androgenic 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 particular mutation in the CPRC tissue, and found that this is the case in 17 % of all instances [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 patients 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, which 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 laboratory-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, however, this number is destined to change as new members of this family of molecules are continuously identified. In this article we 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), 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 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 the presence of AR-V7 and AR^{v567es} in 100 % and 23 % of the cases, respectively [186]. Additionally, the study demonstrated that increased AR-Vs expression was associated with shorter time to death [186]. Another study correlated the 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. Furthermore, the 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} develop prostate cancer [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 re-activation, 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 looking at the mechanisms used by AR to remain active after transition to CRPC, it can be predicted that a major mechanism of resistance to currently available drugs consists of 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 were receiving Enzalutamide. AR-V7 was present in 39 % and 19 % of the patients receiving Enzalutamide and AA, respectively. The 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 months vs. 6.1 months and 2.3 months 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], therefore, taxane-based chemotherapy has been studied in detail. A clinical trial described 37 taxane-treated patients with CRPC [228]. Of those patients, 46 % 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 that study, outcome differences between AR-V7(+) or (−) patients treated with taxanes or 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. Xenograft- or cell line-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 sensitivity to taxanes in LNCaP cells [230].

The common AR target of first- and second-generation ADT drugs is the carboxyl terminal 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 the 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 includes 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(−). TNBC are unresponsive to conventional treatments targeting ERα signaling, E_2 synthesis, and HER2/NEU activity, thus their prognosis is poor. The role of AR 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 a mediator of an antiproliferative signaling pathway by binding estrogen responsive elements (EREs) and preventing ER-mediated transcription [237]. In contrast to what was 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]. Additionally, certain histologically defined "molecular apocrine" AR(+) and ER(−) breast cancers display signature microarray profiles 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 anti-androgens 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. For that 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 those models, AR-V7 expression is up-regulated 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 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 patients with CAIS and PAIS [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 women with CAIS compared with controls, however, good compliance with ERT—given to these patients after gonadectomy—was associated with better BMD Z scores compared with non-compliant 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 not 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. In addition, the model overexpressing AR in proliferating osteoblasts had larger bones compared with controls because of 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 anabolic effects on cortical bone and the periosteum. ARKO models where AR inactivation is targeted to the

osteoclast have been reported only in abstract form. These animals have increased numbers 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 laboratory-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 at specific target cells [256]. Cre-LoxP technology involves 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 in shedding further light on the importance of AR in male physiology. Global ARKO mice share the same phenotype as 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 manner 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 entailed reduced testicular size by 25–60 %, followed by developmental sperm arrest at the pachytene spermatocyte stage with no sperm present in the epididymis. In contrast to what was observed in global ARKO mice, Sertoli cells were not decreased in number but were 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 in 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 final confirmation because in this model 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 sperm 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 PTMARKO mice, respectively. Reliability of the phenotypes generated with these promoters is an issue because Welsh et al. published that transgelin does not affect AR expression in peritubular cells [271]. Additionally, 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 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 sperm in the epididymis. The PTMARKO 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 PTMARKO 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 a paracrine modulator of adult Leydig cell function.

The described differences in the phenotypes of PMTARKO and PMCARKO mice could result 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

Even if 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 levels, 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 from the upper and pelvic portions of the urogenital sinus. The epididymis is a storing organ where sperm are collected and undergo final maturation. Prostate and seminal vesicles are located along the vas deferens and contribute the large majority of seminal fluid, along 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]. 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 histologic changes consisting of 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 its phenotype was associated with decreased epithelial in-folding and epithelial cell proliferation. The study also demonstrated that defective development of the prostate in SM-ARKO may be because of 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 AR in both stromal fibroblasts and smooth muscle cells showed reduced size of the anterior prostate (AP) lobes as compared with 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 because of distinctive tissue specificity in Cre expression and 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 those differences, the 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]. As a result of these abnormalities, only 5 of 15 PEARKO males (33 %) were fertile, with only 1 of 15 siring a second litter within a 90-day mating trial.

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

In conjunction, the 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, independent 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 a thinner layer of smooth muscle, reduced epithelial cell height, decreased epithelial cell proliferation, and production of seminal proteins.

AR ablation was also observed in SV smooth muscle cells of PEARKO mice, although it is not clear why in that 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 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 humans, the process of epididymis development consists of the formation of a 6 m duct, coiled and packed into a three-dimensional organ of approximately 10 cm in length [296] and comprised of a differentiated epididymal epithelium consisting in principal, clear, narrow, basal, and dendritic cells throughout the duct. AR is expressed in the periductal mesenchyme in mouse embryos starting at E12.5. Subsequently, AR is expressed in larger amounts in both the epithelium and mesenchyme during WD development between E16.5 to 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 of 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 on, and their phenotype consists of hypoplastic epididymis with dysfunctional epithelial cell differentiation leading to impaired spermatogenesis [293, 299, 300]. In two of those models, Cre recombinase was placed under the control of promoters derived from ribonuclease 10 (Rnase10-Cre) [299] and forkhead 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 rise to the future inguinal canal. The second phase, called the inguinoscrotal phase, occurs between 25 and 35 GW, when the gubernaculum bulges out of the external inguinal ring, reaches the scrotum where it gives rise 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 and 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, gubernaculum-specific ARKO (GU-ARKO) mice were 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 gubernaculums, 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. nl T 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, the animals became infertile and showed abnormal seminiferous tubules, arrested spermatogenesis, and vacuolization of Sertoli cells. The mutant gubernaculum failed to give rise 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].

In conjunction, 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 a matter of controversy. As stated above, AR expression in GU-ARKO mice 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 a model is unavailable because it would require a hemizygous father carrying an

inactive AR allele, and the condition is associated with infertility. Animal models have therefore been instrumental in understanding 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 bred 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 a 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 a 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 down-regulated 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 some times, 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, the 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 up-regulation 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 with 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 ASVs are associated with higher serum and follicular androgen levels, and with a longer menstrual cycle and greater number of antral follicles compared with PCOS women not expressing ASVs, or normal controls. Functionally, the two AR-ASVs demonstrate altered nuclear translocation and transcriptional activity, and, compared with wt AR, a change in the network of transcripts regulated in response to DHT. For example, AR ASVs induce up-regulation 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 the 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, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) stimulate testicular hormone secretion that induces pubertal development and gametogenesis [1]. Normal testicular physiology results from the integrated function of the tubular and interstitial compartments.

Fetus testes are differentiated by the end of the fifth embryonic week, before the gonadotrophs are functionally active. The hypothalamic-pituitary-testicular axis is activated in the third trimester of intrauterine life and during 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 cell function is regulated by the placental chorionic gonadotropin (hCG). Conversely, during the second half of gestation, fetal LH and FSH become major regulators of testicular physiology [2]. FSH stimulates Sertoli cell 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) through 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, then increase and remain high for 3–6 months. Thereafter, gonadotropin levels decrease, resulting in a fall of testicular testosterone secretion to low or undetectable levels during infancy and childhood. Sertoli cells continue to be 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 component in controlling the male hypothalamic-pituitary-testicular axis, a number of other important components in the GnRH neuronal network have been identified. Stimulatory (kisspeptin) and inhibitory (MKRN3) pathways have been described in controlling the GnRH secretion [4, 5].

The discovery of the crucial role of kisspeptin in human puberty completely changed the 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 onset of puberty, sex hormone-mediated secretion of gonadotropins, and fertility [8]. Kisspeptin is a potent stimulator of the hypothalamic-pituitary-gonadal axis and acts directly with 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 neuronal network co-secretes Kisspeptin, neuroKinin B, and dynorphin, a opioid inhibitor. Neurokinin B, via the 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 known 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. Additionally, 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 males caused an increase in LH pulse amplitude and pulse frequency. The authors also found 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, thereby decreasing the responsiveness to GnRH [13].

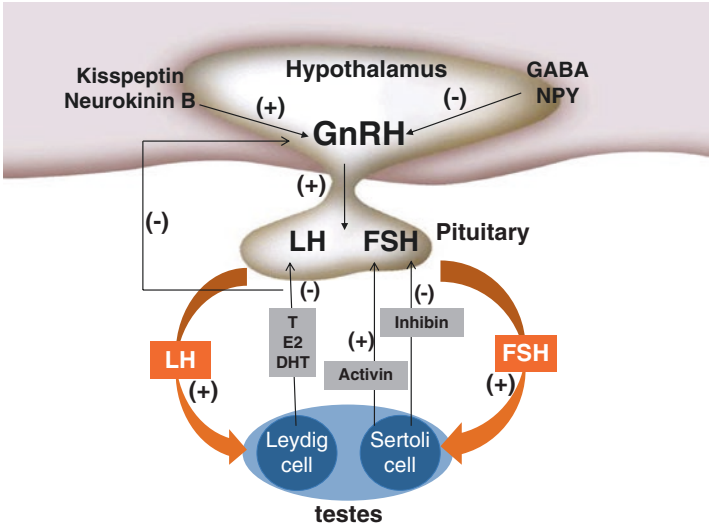

Fig. 3.1 Hormonal control of Male Gonadotropic axis. T = Testosterone; E2 = Estradiol; DHT = Dihydrotestosterone

It has been shown more recently that Kisspeptin also stimulates the secretion of both LH and FSH in humans. While kisspeptin stimulates LH release 2- to 3-fold in most circumstances, the stimulatory effect on FSH levels is much lower 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 as a result of neurokinin B signaling defects [17].

FSH Secretion

It is well known 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 [19]. Two forms of inhibin have been isolated, inhibin A and inhibin B [20]. Both inhibins have the capacity to specifically inhibit FSH secretion through pituitary cells in culture. In contrast, the dimers of the β subunit, termed activins through pituitary cells in culture [21, 22] (Fig. 3.1). Activin, inhibin, and AMH belong to the transforming growth factor (TGF)-β protein superfamily.

Finally, a protein called follistatin has the capability to suppress FSH secretion specifically via pituitary cells in culture [23]. This action is a result of the capability 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 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 particularly because the treated men experienced a concomitant significant increase in testosterone [13].

There is evidence to suggest 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 the male gonadotrophic axis is still emerging.

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 its administration [35]. Additionally, 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. The FSH induces a new Sertoli cell proliferation and the LH re-stimulates the maturation of Leydig cells. The increase of testosterone concentration into the testis incites the maturation of Sertoli cell [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 men with untreated hypogonadotropic hypogonadism, were significantly higher than in normal men and were similar to those previously reported in prepubertal boys. The hCG treatment in these patients induced an increase of plasma T associated with a dramatic decrease of AMH serum. The similar increase in plasma T levels was obtained in those patients treated with exogenous T, but a lesser decrease of AMH serum. These data suggest that intratesticular testosterone concentration can be estimated by measuring AMH serum [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 LH serum 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 anatomic 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 Müllerian structures. Complete absence of virilization results in normal female external genitalia and these patients generally seek medical attention at the pubertal age, because of 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 resulting from factors secreted by the testis (sex differentiation) [43, 44].

At the start of gestation, embryos of the two sexes differ only by 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 induces the anatomic and possibly psychological differences, leading to various behaviors that are ultimately influenced by the social environment. This pool of factors will determine the individual sex.

At 6–7 weeks of gestation, 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 with the androgen receptor in the Wolffian ducts to induce the formation of epididymis, deferent ducts, and seminal vesicles. Testosterone is further reduced to dihydrotestosterone (DHT), which acts with the androgen receptor of the prostate and external genitalia to cause its masculinization. If the testes do not develop normally, and 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 the 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 mediated by DHT [46]. Testosterone and DHT act via a single androgen receptor, and DHT binds more tightly to the hormone-binding domain of the receptor as a result of 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, DHT 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. DHT, in turn, causes 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 patients with 46,XY DSD can be absent, partially or completely dysgenetic, or almost normal [45].

Taking into 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 a 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 which results in patients with ambiguous external genitalia with or without Müllerian 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 non syndromic 46,XY gonadal dysgenesis [48]. Considering that molecular diagnosis is established in just 20 % of patients with DSD, 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 or may not be associated 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.

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 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. Patients affected with the 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 form of Leydig cell hypoplasia, the partial formof Leydig cell hypoplasia has a broad spectrum [50–53]. Most patients have predominantly male external genitalia with a 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].

Table 3.1 Causative genes of abnormalities in testis development

Gene	Simbol name	Locus	Protein	Protein function	Human phenotype
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, hypospadia
MAP3K1	Mitogen-activated protein kinase 1	5q11.2	MAP3K1	Kinase	46,XY gonadal dysgenesis
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' Tumour 1	11p13	WT1	Transcription regulation	46,XY gonadal dysgenesis—Frasier syndrome Denys-Drash syndrome and WAGR syndrome

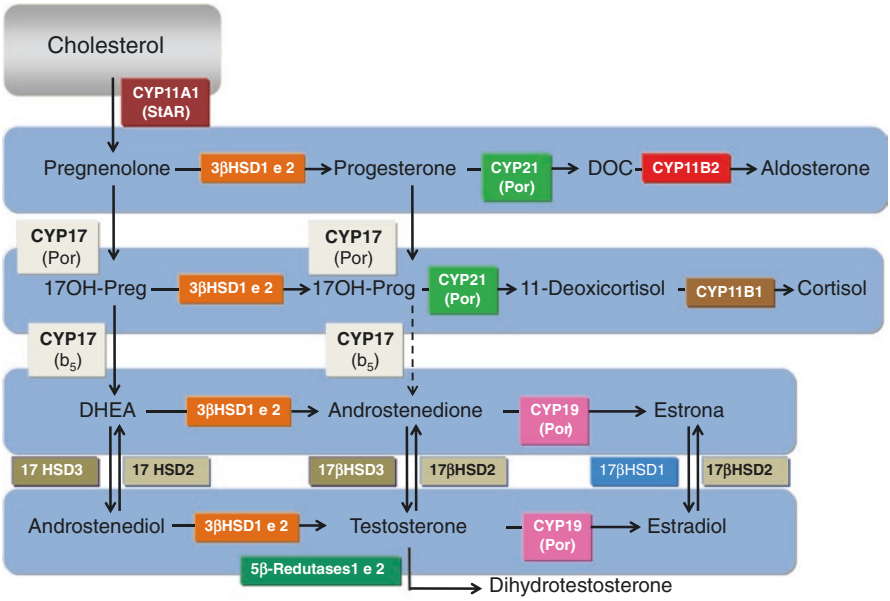

Fig. 3.2 Main biosynthesis pathways of adrenal and gonadal steroids

46,XY DSD Due to Cholesterol Synthesis Defects

Smith-Lemli-Opitz is a 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 thos 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.

Congenital adrenal hyperplasia (CAH) is associated with hypoadrenocorticism or a mixture of hypo-and hyper-corticoadrenal steroid secretion. Synthesis of cortisol only 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 patients with 46,XY [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, hyponatraemia, hyperkalaemia, hypovolaemia, 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. Gynaecomastia is common during the pubertal stage. Male subjects with 46,XY DSD as a result of 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 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 hypokalaemia. Differently from other forms of CAH, these patients do not present signs of glucocorticoid insufficiency resulting from elevated levels of corticosterone, which has a 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 which converts 17OH-pregnenolone into DHEA and 17OH-progesterone into androstenediona. This is a very rare form of 46,XY DSD and patients with isolated 17,20 lyase deficiency present with atypical genitalia with microphallus, perineal hypospadias, and cryptorchidism [56].

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

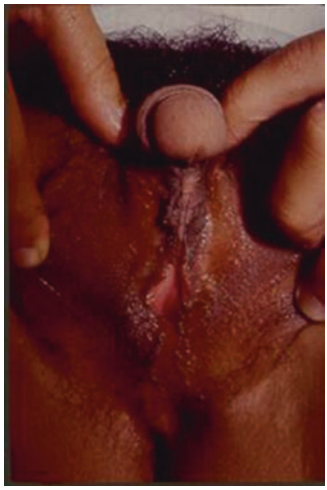

Fig. 3.3 Adult patient with 46,XY DSD due to 17β-hydroxysteroid dehydrogenase type III deficiency

17β-Hydroxysteroid Dehydrogenase Type III Deficiency

17β-hydroxysteroid dehydrogenase type III deficiency is the most common disorder of androgen synthesis. There are five steroid 17β-HSD enzymes that 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 with 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 the extragonadal tissue and of the secretion of testosterone by the testes when levels of LH are elevated in patientss 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 patients with 46,XY are raised as girls during childhood and change to male gender-role behavior during 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 patients and testosterone levels may stay in 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.

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
Wolfian 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 males 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 the male fetus. It has been demonstrated that the 5α-reductase 1 activity is normal in these patients [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].

Affected male 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 because of female-like external genitalia at birth (Fig. 3.4a), but many patients who have not been submitted to orchiectomy during childhood undergo a male social sex change at puberty. In our cohort, all patients were registered in the female social sex except for two cases—one who has an affected uncle and the other who was 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

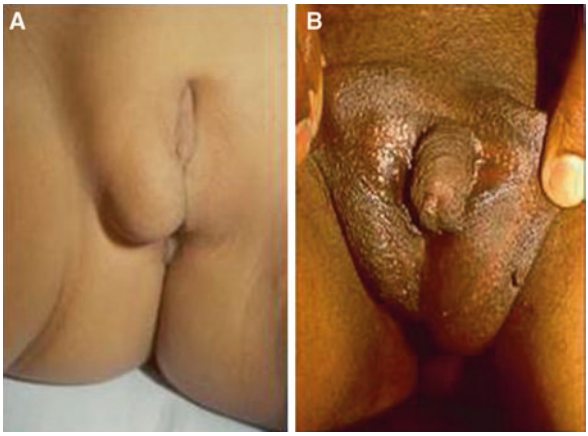

Fig. 3.4 (**a**) Female-like external genitalia at prepubertal age. (**b**) atypical external genitalia and micropenis at pubertal age

and in two cases in vitro fertilization using the patient's sperm cells resulted in twin siblings in one family and in a single pregnancy in the other. None of the 10 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, 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 with 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. An elevated 5β/5α urinary metabolite ratio is also an accurate method to diagnose 5α-reductase 2 deficiency even at a prepubertal age and in orchiectomized adult patients [61].

To our knowledge, there are more than 50 families with this described disorder worldwide. In a few cases of 46,XY DSD as a result of 5-a RD2 deficiency diagnosed by clinical and hormonal findings, no mutations were identified in the SRD5A2 gene.

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

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

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

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
Wolfian 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 pospubertal 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

resulting from complete or partial resistance to the biological actions of androgens in an 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 the prepubertal age, an inguinal hernia in girls can indicate the presence of testes. At puberty, complete breast development and primary amenorrhoea associated with reduced or absent pubic and axillary hair suggest CAIS. Adult women who have intact gonads have the endocrine profile of a 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. Estradiol serum levels arising from testosterone aromatization are higher than those observed in men. Gonadotropin serum 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 patients with CAIS 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 prepubertal gonadectomy as soon as a diagnosis is established and then induction of puberty with estrogens at the appropriate age. This approach diminishes the presence of an inguinal mass that is often painful. In addition, a young child can psychologically better handle 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 patients with CAIS is still controversial [43].

Whereas the clinical picture of CAIS is homogeneous, the phenotype of PAIS is quite variable and depends on the 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 patients with PAIS can cause misdiagnosis with several 46,XY DSDs as a result of defects in androgen production [62]. PAIS diagnosis is unequivocally established by the identification of a molecular defect on the AR gene.

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
Wolfian duct derivatives	Broad spectrum from absent or male
Testes	Eutopic, inguinal or intraabdominal, 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

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

In patients with AIS, final height is intermediate between mean normal male and female, and decreased bone mineral density in the lumbar spine has been demonstrated [66].

Mild AIS 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 [65]. The product of LH serum and testosterone concentrations as an index of possible mild AIS in infertile men could be a useful screening test for the presence of a mutation in the androgen receptor gene [66].

AR mutations were found in the majority of patients with CAIS 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 postpubertal patients with CAIS and PAIS, respectively [68].

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

Patients with CAIS were raised as females and maintained a female gender. Most patients with PAIS who were raised as females maintained a female social sex after postpubertal 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 were raised as females and underwent a change to male social sex at puberty [61, 69].

Table 3.4 summarizes the characteristics of patients with PAIS.

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 reinstated. The use of topical 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 prepubertal age and consequently attaining a higher degree of virilization.

Management of Patients with 46,XY DSD

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

Psychological evaluation is of extreme importance in the treatment of patients with DSD. Every couple who have 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 the 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 the event that the parents and health care providers disagree over the sex of rearing and psychological support was not able to change the 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 Patients With DSD with Male Social Sex

Testosterone replacement begins between 10 and 11 years 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 intramuscularly. The maintenance dose in adult patients 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 then normal dosage is reinstated [61].

The use of topical DHT gel is also useful to increase penis size with the advantage of not causing gynecomastia and promoting faster increase of penis growth 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 Patients With DSD with Male Social Sex

The goals of surgical treatment are to provide an adequate external genitalia and removal of 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 are performed in two or three steps in 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 with satisfactory sexual performance as long as they present with 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 can not be determined through conventional techniques available clinically. The molecular tools have added new possibilities in the investigation of these patients [45]. The majority of patients with DSD present with 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 challenging 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 muscle and bone. Organic hypogonadism resulting from structural hypothalamic-pituitary testicular (HPT) axis dysfunction 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 such as otherwise unexplained weakness, anemia or osteoporosis. Hypogonadism is primarily a clinical diagnosis. Men who present with features suggestive of androgen deficiency should undergo a thorough history and physical examination to determine the degree of clinically significant androgen deficiency. Verification of the clinical impression by confirming low testosterone levels 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 levels [1]. While the diagnosis is relatively straightforward in young otherwise healthy men, it considered 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 examination can be nonspecific. In the European Male Ageing Study, where 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 non-specificity of clinical features in the absence of a biological gold standard of male hypogonadism akin to cessation of menses in females, accurate biochemical confirmation is important.

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This can be fraught with difficulties and pitfalls, and will be discussed in this chapter. This chapter will focus on testosterone measurements in adult men. Measurements of other reproductive steroids 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, such as 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 currently no proven glycemic or symptomatic benefit of testosterone treatment in men with diabetes or the metabolic syndrome [3] and further evidence to support 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 first, 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. Second, 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 (i.e., 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 [4]. Third, a single point in time measurement may not be representative because of biological variability in testosterone levels, and because of technical assay limitations, both further discussed below.

Biological Variability of Circulating Testosterone Levels

As a result of the pulsatility of hypothalamic gonadotropin releasing hormone secretion, testosterone is secreted in a pulsatile manner, but in part because of buffering effects of its carrier proteins SHBG and albumin, the pulse frequency is rapid and the amplitude relatively low, with moment to moment fluctuations of less than

10–15 % [5]. There is circadian rhythmicity, and in men 30–40 years old, testosterone levels are 20–25 % lower at 1600 h than at 0800 h [6]. In fact, up to 15 % of healthy men can have abnormally low levels of testosterone within a 24 h period [7]. While some data suggest blunting of circadian rhythmicity with age [6], a significant proportion of men older than 65 years with low levels of testosterone in the afternoon will have normal levels in the morning [7]. Testosterone levels should therefore be measured before 10 am irrespective of age. This also allows drawing a sample during the fasting state. While current guidelines do not specify fasting [1], recent evidence suggests that food intake can abruptly reduce testosterone levels. In one study of 66 healthy men, glucose ingestion was associated with a 25 % decrease in mean T levels (delta = −4.2 ± 0.3 nmol/L, $p < 0.0001$), reducing testosterone levels to below the reference range in 15 % of study subjects [8]. Consistent with these findings, an observational study of repeated testosterone measurements found that overnight fasting increased testosterone levels 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 [9]. There is also significant day-to-day variability in testosterone levels. 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 [10]. In men with [11] or without [12] diabetes, more than 30 % with low testosterone will have normal levels 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 various individuals. For example, testosterone levels below which increases is fat mass have been reported to range from 6.1 to 13.9 nmol/L in varying studies [4, 13]. However, in a cohort of hypogonadal men receiving testosterone replacement implants, despite a wide range in thresholds for androgen deficiency symptoms among individuals, testosterone threshold levels at the time of return of androgen deficiency symptoms were highly reproducible among individuals [14].

Effect of Illness on Testosterone Levels

Any acute illness or chronic illness, medications (e.g. glucocorticoids or opioids), obesity or malnutrition, and excessive exercise can decrease testosterone levels. Obesity decreases testosterone by 30 %, and in the presence of 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 [15]. Conditions such as type 2 diabetes mellitus, depression, obstructive sleep apnoea, chronic kidney disease, or anorexia nervosa are associated with decreases in testosterone levels of between 2 and 10 nmol/L, depending on the severity of the condition [16]. Any illness can cause non-specific symptoms resembling those seen with true androgen deficiency. There is evidence to show that the age-related accumulation of chronic disease and obesity in particular can accelerate

the age-related decline in testosterone levels [17]. Healthy aging by itself may not inevitably be associated with marked decreases in testosterone levels [9]. In general, however, a repeatedly low testosterone level 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 non-specific symptoms.

Serum Testosterone: What to Measure?

Because of the variability in testosterone levels, the Endocrine Society guidelines recommend making a biochemical diagnosis of androgen deficiency based on repeatedly low testosterone levels [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 can be associated with a decrease testosterone levels. Testosterone levels should be drawn in the morning (before 10 am), in the fasting state, in a medically stable patient without current or recent acute illness. Low testosterone should be confirmed at least once, while a clearly normal level (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 [18]. In practice, a normal fasting early morning total testosterone level (somewhat arbitrarily defined as $\geq$12 nmol/L) is generally consistent with eugonadism, and usually does not need to be repeated. If the total testosterone is $\geq$12 nmol/L, non-specific symptoms will generally not be from androgen deficiency unless SHBG is markedly elevated or if there is androgen resistance, a rare condition.

A low total testosterone, however, needs confirmation because a falsely low level due to, for example, unrecognized intercurrent illness or assay imprecision at the lower range particularly if measured with immunoassay (see below) is more likely than a falsely normal level. Therefore, a diagnosis of androgen deficiency should never be based on a single low testosterone level. Up to 35 % of men with a low testosterone level will have a normal testosterone level on repeat testing [11, 12]. Even among endocrinologists however this is unfortunately not universal practice; in the aforementioned international survey, 25 % of respondents were not confirmed with a low testosterone level but were offered testosterone treatment based on a single low level [18], contrary to guideline recommendations [1].

Free Testosterone

Circulating testosterone is mainly 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 [19], 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 [20]. In this context, normal free testosterone can be reassuring in 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 level should be evaluated with caution to confirm hypogonadism in older men because the risk of overdiagnosis is substantial given that assay reference ranges are usually based on findings in young men.

An extreme case of a "falsely low" total testosterone was recently described in a man who presented with an extremely low total testosterone level but essentially normal masculinization. SHBG levels were undetectable because of a missense mutation in the SHBG gene, and dialyzable free testosterone was in the reference range [21].

In certain instances, men can be androgen deficient despite a normal total testosterone level, and usually occurs if SHBG is markedly elevated most commonly in the setting of anti-epileptic treatment or chronic liver disease, but these men typically have elevated gonadotropin levels and a clearly low free testosterone level.

Testosterone Measurement: Which Method?

The Council of the Endocrine Society 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 [22]. 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 [23]. 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 [24]. Despite improving technologies for testosterone measurements, measurement variability within and across

laboratories is still an issue [25]. The Centers for 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 with improving analytical assay performance (http://www.cdc.gov/labstandards/hs.html) [25]. Traceability of assays to a "gold standard" allows comparability of results across different methods and laboratories and should facilitate establishing 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 utilize an extraction step, they can be prone to cross-reactivity and analytical interference. For example, dehydroepiandrosterone (DHEA), present in male serum in micromolar concentrations can interfere with immunoassay testosterone measurements at concentrations of <10 nmol/L [26]. Reference ranges are generally not well validated, and assays are not standardized, so results and reference ranges are method dependent and cannot be compared across various platforms. Validation of performance and standardization of commercial assays is manufacturer-dependent, given that reagents are proprietary, but is improving, perhaps driven by progress and increasing availability of mass spectrometry-based assays [27]. Immunoassay sensitivity is limited, with deteriorating accuracy and positive bias at total testosterone levels < 10.4 nmol/L [23]. In a study comparing immunoassays with a mass spectrometry-based method, at testosterone levels < 8 nmol/L, methods disagreed by up to five-fold, and immunoassays generally overestimated testosterone concentrations [28].

There is no question that current immunoassays, due to lack of precision and accuracy leading to bias particularly at the lower reference range are not suitable to accurately quantify low testosterone levels in women, children, or men with prostate cancer receiving androgen deprivation therapy. In a study measuring testosterone using various immunoassays, over 60 % of the samples (with testosterone levels 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 [29]. 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 [30]. 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 [31]. However, the

correlation at testosterone levels of < 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 < 11 nmol/L was 75 % and 96 %, respectively [31].

Mass Spectrometry Assays

As a result of logistic advantages, LC-MS/MS assays have largely superseded gas chromatography–mass spectrometry assays and are increasingly available, particularly in research laboratories. Compared with immunoassays, provided they are carefully validated using stringent performance criteria, they offer 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) [24]. They offer improved analytical sensitivity and are the method of choice for accurately quantifying testosterone circulating at low concentrations. While sample through-put, 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 must be validated to yield accurate and reproducible data, including calibration and regular quality control [25]. While an earlier publication reported inter-assay coefficients of variations for different mass spectrometry assays of up to 14 % at total testosterone levels <10.4 nmol/L even in reference laboratories [32], implementation of the CDC HoSt program has, from 2007 to 2011, led to a 50 % decline in mean bias between various mass spectrometry assays [25].

Free Testosterone Methods

Laboratory equilibrium dialysis (ED) is the gold standard for free testosterone measurements, but not widely available because of assay complexity and cost [23]. Importantly, the free androgen index is inaccurate in men and should not be used, and free analog displacement using direct free testosterone (analog) assay is analytically invalid and should not be used [23]. In practice, free testosterone is usually calculated using empiric formulas. The original equations suggested good agreement with ED, but were evaluated in single laboratories using a relatively low number of human samples [33, 34]. A relatively large scale evaluation in more than 2000 serum samples found that the accuracies of five different formulas (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 [35]. There is currently no universally accepted formula that accurately reflects the interaction between plasma protein bound and free testosterone. In addition, these formulas 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 with that of free testosterone and is measured by ammonium sulfate precipitation. While technically relatively simple, the technique can be inaccurate, is not easily automated, and is not widely available [23].

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 [36]. 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 non-specific with high interindividual variation [37].

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" [18]. In 2014, respondents from Northern America reported the highest access to mass spectrometry (19.1 %) and equilibrium dialysis (12.6 %) [18].

Testosterone Level: What Is Low?

In contrast to, for example bone density, where age-dependent reference ranges are defined quite well, there is no general agreement on the acceptable normal range of testosterone, particularly 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 level below which to consider testosterone treatment, with opinions ranging from 6.9 to 10.4 nmol/L [1]. Joint recommendations from the International Society of Andrology and associated societies consider total testosterone levels between 8.0 and 12.1 nmol/L to represent a gray zone, whereas levels >12.1 nmol/L are considered normal and <8.0 nmol/L, low [38].

Using a CDC-certified LC-MS/MS assay, Bhasin et al. reported a 2.5th percentile for total testosterone of 12.1 nmol/L for healthy young men [39]. Population-based studies in healthy Australian men reported lower limits for total testosterone of 9.8 nmol/L for healthy young men [30], and of 6.4 nmol/L for older men reporting excellent health [40], measured by LC-MS/MS assay.

The US Federal Drug administration uses a testosterone threshold of 10.4 nmol/L, the value used to define hypogonadism for clinical trial purposes, without reference to age. This is the threshold level most commonly (in 43 %) chosen by endocrinologists to offer treatment to older man presenting with symptoms compatible with androgen deficiency [18]. This stresses the importance of providing robust reference ranges for older men so as to avoid overtreatment.

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 the analytical shortcomings of testosterone assays. Assay validity and optimization of 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 practice 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.

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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 males 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 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 increase in adrenal androgen production. Adrenarche is a biochemical phenomenon, and the term is sometimes incorrectly interchanged with pubarche. Adrenarche occurs separately from hypothalamic-pituitary-gonadal 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 in pubarche in boys. The most commonly used measure for pubarche is pubic hair Tanner staging. Although developed decades ago [2], the 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

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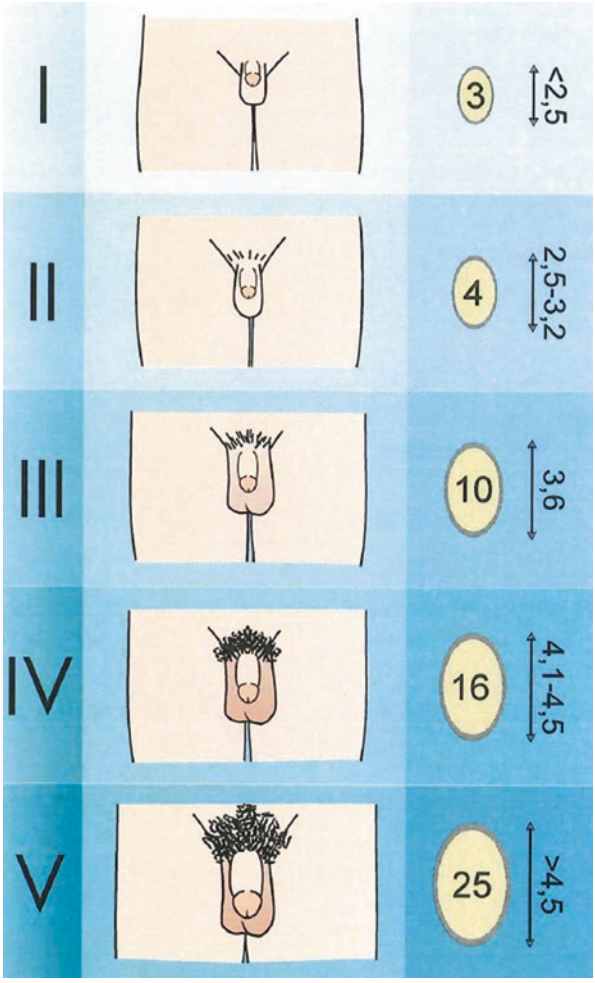

Fig. 5.1 Tanner staging of pubic hair and genitalia in boys with representative testicular volumes at each stage. Figure courtesy of Michal Komorniczak

further progression throughout 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.

A basic understanding of endocrine function during puberty can help physicians better understand and explain the physical changes of puberty to their patients. The hypothalamus releases gonadotropin releasing hormone (GnRH) in a pulsatile manner. This pulsatile release begins about 1–2 years before any physical signs of puberty. The developmental physiology of the process is not well understood. Early in puberty, kisspeptin secretion from the hypothalamus increases, resulting in activation of GPR54, the kisspeptin receptor [3]. 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 onset 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 response increases [4]. 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 males. Additionally, the increase in testosterone concentrations results in lower sex hormone binding globulin concentrations, leading to more bioavailable free testosterone in the circulation [5]. The increased free testosterone permits more testosterone to be converted to dihydrotestosterone, which is more potent because of its slower dissociation from the androgen receptor [6]. This is important for continued development of secondary sexual characteristics, particularly sexual hair growth.

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

Precocious Puberty

Definition

The onset of puberty is defined clinically as a testicular volume ≥4 mL. Traditionally, this stage of testicular enlargement has been believed to occur on average between the ages of 11 and 12 years. Precocious puberty in boys is classically defined as testicular volume ≥4 occurring before the age of 9 years. This timing is based on studies performed between 1950 and 1970 in racially and ethnically uniform populations in the United States and in a group of boys from lower socioeconomic strata living in a children's home in England. The studies found a mean age of 11.64 years at the start of genital enlargement 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 are beginning to 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 [7]. A longitudinal study sponsored by the US National Institutes of Health (NIH) conducted in various centers in the US from 2000 to 2006 assessed 427 boys between the ages of 9.5 and 15.5 years. The mean age at Tanner stage 2 genital development was 10.4 ± 0.6 years for caucasian boys [8]. A study from the cross-sectional US 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 caucasian 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 among 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 [9, 10]. In boys, however, the role of increased body weight is more controversial, with reports associating obesity with both earlier and later onset of puberty [10–13].

There is also significant ethnic variability in the timing of normal puberty. In the NIH study [8], 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 [14, 15]. In the United States, 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, the racial and ethnic makeup of the population under study must be considered 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 [16–18].

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 [19] and may have a higher incidence of polycystic ovary syndrome, at least in some populations [20]. 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 of age and boys less than 9 years of age, the prevalence has been estimated to be 8.6 % and 1.8 %, respectively [21].

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 also may be increased. Testosterone is typically 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 [22]. 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 younger than average but after 9 years. In females, 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 males 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 [23–25]. These results have been attributed to a mismatch between the emotional and cognitive status of the young pubertal child and to an 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 [26].

A number of studies have associated early normal puberty in girls with adverse cardiometabolic outcomes, including adult obesity, hypertension, and dyslipidemia. Data for males 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 [27]. In males, the difference between definitions of pubertal onset was sufficient to render meta-analysis impossible. The investigators noted, however, that most of the analyzed studies reported an inverse association between age of puberty and body mass index in adulthood, although three of the eight included studies did not identify such a relationship. Some studies identified an increased prevalence of

hypertension in males with earlier puberty, although this also was not a consistent finding. Data were insufficient to exclude confounding by childhood obesity. Thus it remains uncertain whether early normal puberty in males 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. It may be difficult to determine the onset of these features, particularly in boys, because they develop gradually and parents 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 with 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 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

Table 5.1 Causes of precocious puberty in males

Gonadotropin-dependent	Gonadotropin-independent
Genetic disorders	Adrenal conditions
Activating mutation of kisspeptin and its receptor	Premature adrenarche
Activating mutation of *MKRN3*	Congenital adrenal hyperplasia
Central nervous system disorders	Virilizing adrenal tumor
Tumors	Glucocorticoid resistance
Hypothalamic astrocytoma	Testicular source of androgen
Central nervous system germinoma	McCune-Albright syndrome
Optic pathway tumors in Neurofibromatosis type I	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	–

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 [28]. Once CPP is diagnosed, a search for anatomic abnormalities should include magnetic resonance imaging of the central nervous system.

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

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 [29]. 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 [3]. 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 [30]. 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 [31–34].

In a search for additional genes that play roles in pubertal regulation, whole exome sequencing was used to analyze 32 individuals with precocious puberty from 15 families [35]. 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 [36]. A number of mutations in the gene have subsequently been reported in children with CPP, and it appears that *MKRN3* mutations are relatively common causes of familial cases of CPP [37]. In those cases, CPP is inherited in an autosomal dominant manner but is transmitted only from the father, consistent with the gene's maternal imprinting [36].

Central Nervous System 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 resulting from one of these disorders may present with accompanying symptoms and signs, such as headache, hemianopsia, papilledema, or characteristic dermatologic findings. It is believed that the more generalized disorders interfere with normal inhibitory inputs that prevent progression of puberty during the quiescent period between infancy and the age of normal puberty. An exception is hypothalamic hamartomas, which are benign congenital ectopic foci of GnRH-secreting neurons that function independently of the normal inhibitory inputs. Patients with CPP resulting from 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 relocated to wealthier countries [38, 39]. Although the majority of reported cases occur in girls, boys may also be affected. The pathophysiology underlying CPP is not completely 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 in these children is significantly earlier than that of non-adopted children in both the native and the adoptive countries [40].

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

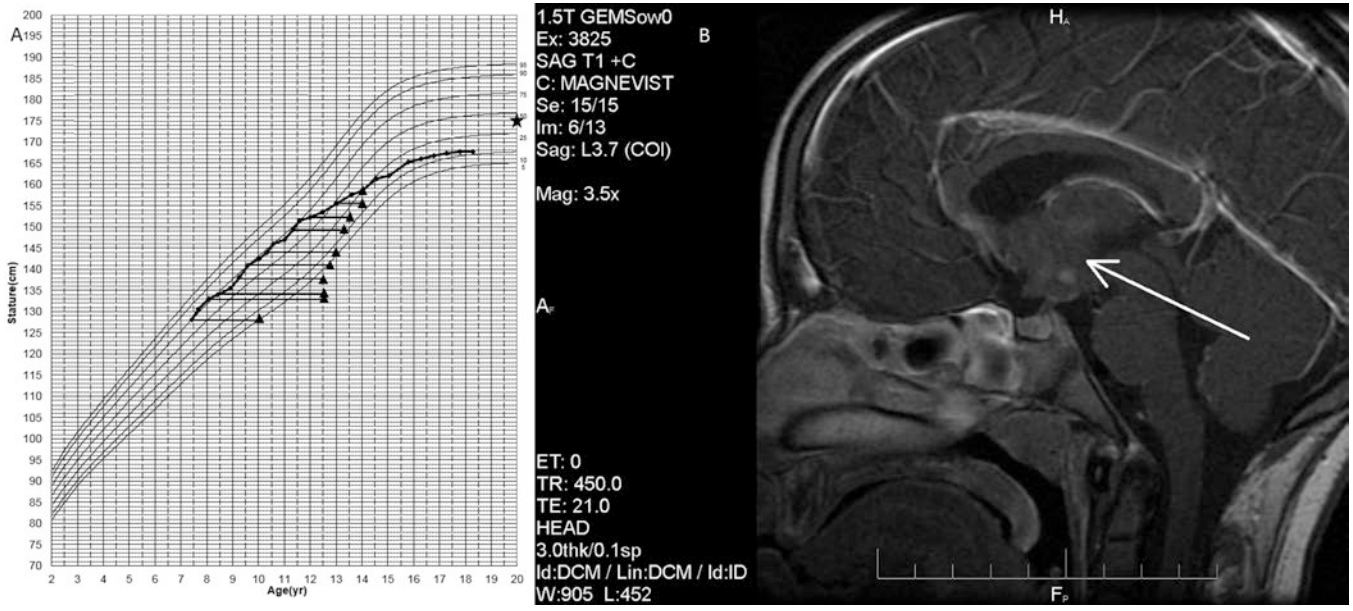

Fig. 5.2 This patient presented at age 7.3 years with a 3-month history of pubic hair. He had had no growth acceleration, axillary hair, apocrine body odor, or acne. He had no history of headache or vision disturbances. He had pervasive developmental delay with autistic features. His height was on the 75th percentile, and his midparental height was at the 45th percentile. (Panel **a**) His physical examination 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 males 0.20–0.3). An ACTH stimulation test was normal, excluding congenital adrenal hyperplasia. A magnetic resonance imaging scan 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**) 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)

is not well understood, but is believed to be the accelerated maturation of the hypothalamus induced by androgens or estrogens.

In the majority of girls, the cause of CPP is never discovered, and remains idiopathic in 95 % of cases. By contrast, idiopathic CPP is relatively uncommon in boys, making up about 50 % of cases. Hence, all boys with CPP should undergo careful physical, laboratory, and radiologic investigation, including magnetic resonance imaging of the central nervous system, as serious undetected pathology is often found.

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 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 with symptoms and signs of precocious puberty until mid-childhood. 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 rare.

Adrenocortical tumors are rare in children. They may present with virilization, features of Cushing syndrome, or both. 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 [41]. 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 adrenocorticotropic hormone (ACTH) and cortisol.

Because of 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 [42].

Testicular Disorders Causing PPP

Familial male-limited precocious puberty (FMPP) was identified in 1993 as being caused by activating mutations in the LH receptor [43] (See Fig. 5.3). The condition is inherited in an autosomal dominant but sex-limited manner. Thus the condition is inherited in successive generations but is only manifested in males. 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 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 and testicular microlithiasis [44].

Tumors secreting human chorionic gonadotropin (hCG) may result in gonadotropin-independent precocious puberty through stimulation of the LH receptor. These tumors include central nervous system 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. Testicular Leydig cell tumors may secrete testosterone in an unregulated manner and usually present with testicular asymmetry and suppressed gonadotropin levels.

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 [45].

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 thyroid stimulating hormone at the FSH

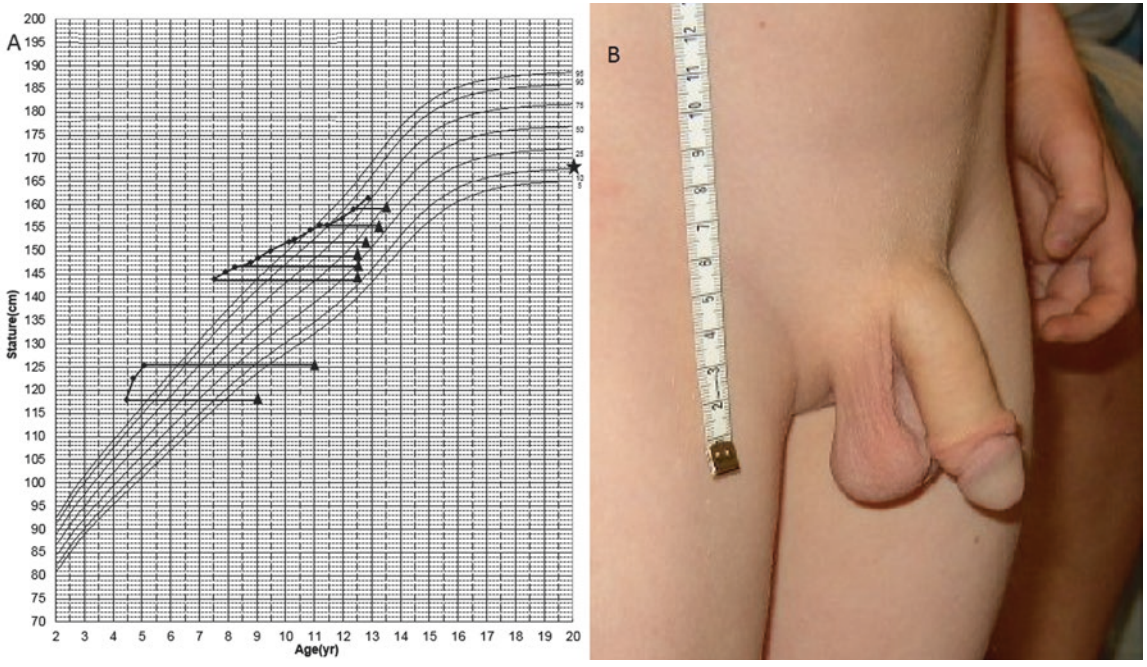

Fig. 5.3 This 4.5-year-old boy presented for evaluation of pubic hair that began at 3.5 years of age as well as mild acne and apocrine body odor. He had aggressive behavior and was occasionally noted to masturbate. His father began puberty at age 6, and the paternal uncle also began puberty abnormally 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 reestablishing 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)

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 [46].

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 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. Commonly used extended release preparations include injections of leuprolide acetate every three months and a subdermal implant containing histrelin. Both preparations effectively suppress central puberty [47, 48]. Although approved by the United States Food and Drug Administration for 1 year, the histrelin implant has shown continued effectiveness for as long as 2 years [49].

There are few data on long-term outcome for boys with CPP, as the majority of clinical trial subjects are girls. Adult height is often reduced in untreated boys with CPP occurring before age 6–8 years, and treatment may increase adult height in this group. Older boys with CPP may have a reduction in adult height regardless of treatment, although this may depend on the rate of pubertal progression [50]. Minimal long-term data exist to assess psychological outcomes of CPP in boys.

Treatment of PPP

Because of the varied causes of PPP, treatment differs among 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 [51, 52] (Fig. 5.3). The treatment of boys with precocious puberty resulting from McCune-Albright syndrome follows a similar approach [44]. 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 by 14 years 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 can 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). These conditions are discussed extensively in Chap. 6 of this text and are only briefly reviewed here. In this chapter, we focus on aspects unique to the adolescent male.

Primary Hypogonadism

One of the most common causes of primary hypogonadism is Klinefelter syndrome, occurring in 1:1000 males and is 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 can be limited, the pace of

Table 5.2 Causes of delayed puberty in males

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	Central nervous system tumor
Infection	Craniopharyngioma
–	Prolactinoma
–	Germinoma
–	Astrocytoma
–	Primary hypothyroidism
–	Prader-Willi syndrome
–	Lawrence-Moon/Bardet-Biedl syndrome
–	Langerhans cell histiocytosis
–	Sarcoidosis

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.

Many of the conditions directly limiting the ability of the testes to synthesize testosterone lead, in their severe forms, to abnormal embryologic 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 are many 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). 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) and Laurence-Moon/Bardet-Beidl syndrome. Both males and females with PWS have central hypogonadism, but data suggest there is also frequently an element of primary hypogonadism [53]. 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.

Table 5.3 Genetic defects associated with hypogonadotropic hypogonadism

Gene	Condition/phenotype	Inheritance
Isolated hormone abnormalities		
KAL1	KS, renal agenesis, synkinesia	X-linked recessive
NELF	KS	AD
FGF8	KS, cleft lip/palate, ear abnormalities, dental agenesis	AD
FGFR1	KS and nIHH, cleft lip and palate, facial dysmorphism	AD, AR
PROK2	KS and nIHH, severe sleep disorder, obesity	AD
PROKR2	KS and nIHH	AD, AR
CHD7	KS and nIHH, CHARGE syndrome	AD
WDR11	KS and nIHH	AD
GPR54	nIHH	AR
KISS-1	nIHH	AR
GNRH1	nIHH	AR
GNRHR	nIHH	AR
TAC3	nIHH	AR
TACR3	nIHH	AR
LEP	nIHH and obesity	AR
LEPR	nIHH and obesity	AR
DAX-1	nIHH and adrenal hypoplasia congenita (AHC)	X-linked
PC-1	nIHH and obesity, ACTH deficiency, hypoglycemia, gastrointestinal symptoms	AR
LHβ	Isolated LH deficiency, delayed puberty	AR
FSHβ	Isolated FSH deficiency, primary amenorrhea, defective spermatogenesis	AR
Combined pituitary hormone deficiency		
PROP1	GH, TSH, LH, FSH, prolactin, and evolving ACTH deficiencies	AR
Specific syndrome		
HESX1	SOD and other pituitary deficits including HH	AR, AD
SOX3	Pituitary hormone deficits including HH, mental retardation	X-linked
SOX2	Anophthalmia/micro-ophthalmia, anterior pituitary hypoplasia, HH, esophageal atresia	X-linked
GLI2	Holoprosencephaly with MPHD including HH, multiple midline defects	AD, AR
LHX3	Variable CPHD including HH, limited neck rotation	AR

ACTH adrenocorticotropic hormone, *AD* autosomal dominant, *AHC* adrenal hypoplasia congenita, *AR* autosomal recessive, *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 [70], McCabe, et al. [71]

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. 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.

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. That is the case for several of the known genetic abnormalities that lead to iHH, including mutations in *FGFR1*, *CHD7*, *GNRHR*, and *PROKR2* [54, 55]. Spontaneous gonadal function has been documented to begin years after it would have been expected and may occur during stable long-term testosterone treatment [54]. It is estimated that 10–20 % of patients may have spontaneous reversal [56]. 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 [57].

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 lead 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 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 boy's 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.

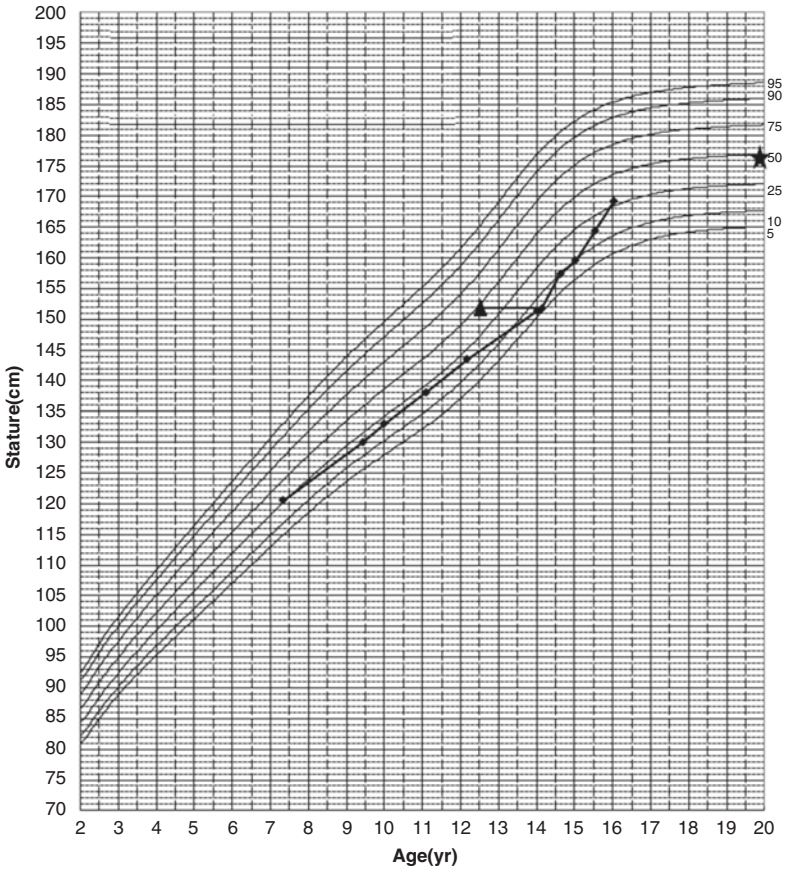

Fig. 5.4 This patient presented at 14.1 years of age for evaluation of delayed puberty. He was otherwise healthy. His sense of smell was normal. His family history was remarkable for delayed menarche in his mother, whose first menstrual period was at age 15. His father recalled that he continued to grow 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 12.5 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)

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, 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 hypogonadotropic hypogonadism (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, and insulin-like factor 3 in various combinations [58–60]. 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 [61], 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, and 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 (SDS) in late adolescence. The adult height of boys with CDGP is typically in the normal range, although it may be less than their midparental height [62, 63].

Bone mineral density is lower than controls during the early and mid-teen years in boys with CDGP, related in part to the delayed exposure to sex steroids and the resulting discrepancy in bone growth and mineralization [64]. Although the rate of increase in bone mineral density during the stages of puberty in boys with CDGP is comparable with norms, the bone mineral density in adulthood may still be somewhat less than normal, even after testosterone treatment [65, 66].

Treatment of Delayed Puberty

The decision to treat boys with delayed puberty must take into account the wishes of the individual as well as the likelihood of the delay in puberty actually being due to permanent hypogonadism. For boys believed 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 CDGP

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 once a month, and 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 years should call into question the diagnosis of CDGP, as the likelihood of permanent hypogonadism increases from this point.

Oral testosterone undecanoate has more recently been shown to be effective in the treatment of boys with CDGP [67]. 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.

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, with a starting dose of 50–100 mg monthly of testosterone enanthate or cypionate. However, the dose of testosterone is usually 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.

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 can result in minor tenderness or bruising at the injection site. Priapism is a rare complication. Transdermal patches are often associated with local skin reactions. A major concern for testosterone gels is the inadvertent passing 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 [68, 69].

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 is often straightforward, allowing administration of any needed treatment and normalization of the maturational trajectory.

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Hypogonadotropic and Hypergonadotropic Hypogonadism

6

Vijaya Surampudi and Ronald S. Swerdloff

Normal Male Pubertal Development

Puberty refers to the maturation of the reproductive axis and the development of secondary sex characteristics. It involves the coordination of multiple hormonal systems including the adrenal gland and the growth hormone axis. The development of secondary sexual characteristics begins when androgen secretions increase during puberty. The sexual maturation process occurs during andrenarche when adrenal androgens are secreted and puberty follows when luteinizing hormone (LH), follicle-stimulating hormone (FSH) testicular and testosterone rise as regulated by the hypothalamic—pituitary axis.

The increase in testicular size is usually the first sign of puberty, reflecting an increase in seminiferous tubule volume. Increasing levels of testosterone deepen the voice and increase the muscle mass. Conversion of testosterone to dihydrotestosterone (DHT) leads to growth of the external genitalia and pubic hair. DHT also stimulates prostate and facial hair growth and recession of the temporal hairline. Growth hormone (GH) increases early in puberty and as a result, GH increases the level of insulin-like growth factor (IGF-1) that enhances linear bone growth.

Regulation of the Hypothalamic-Pituitary-Axis

The interrelationship between the hypothalamus, pituitary, and testis is a vital part of the successful initiation and maintenance of the reproductive axis. The axis is dependent on the pulsatile hypothalamic secretion of gonadotropin-releasing hormone (GnRH) that will stimulate FSH and LH which in turn initiates both

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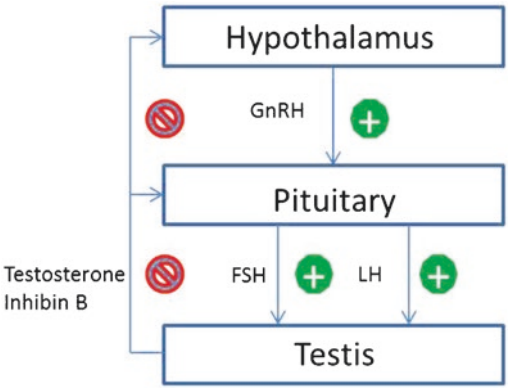


Fig. 6.1 The Hypothalamic-Pituitary-Testis Axis

intra-gonadal testosterone production and spermatogenesis. The testis can exert negative feedback on LH and FSH secretion through secretion of testosterone and inhibin B from the Leydig and Sertoli cells. The hypothalamic-pituitary-testis axis is summarized in Fig. 6.1.

Androgen Synthesis

LH binds to a G protein coupled receptor to activate the cyclic adenosine monophosphate (AMP) pathway in the Leydig cells. Stimulation of the LH receptor induces steroid acute regulatory protein (StAR) and several other proteins involved in androgen synthesis. The major steps involved in testosterone synthesis are summarized in Fig. 6.2. Testosterone can be converted to either DHT or estradiol as shown in Fig. 6.3.

Testosterone Metabolism

In males, the majority of circulating testosterone is derived from testicular secretion and only a small amount of testosterone is derived from the adrenal. Circulating testosterone is bound to two plasma proteins, sex hormone-binding globulin (SHBG) and albumin. Less than 3 % of testosterone is unbound and is referred to as free testosterone as shown in Fig. 6.4. SHBG concentrations are decreased by obesity, insulin, and nephrotic syndrome and SHBG concentrations are elevated by estrogen administration, hyperthyroidism, and chronic inflammatory illnesses, weight loss from excessive exercise, and aging.

Testosterone is metabolized mainly in the liver and via a series of enzymatic steps, it is converted to inactive metabolites which eventually undergo glucuronidation or sulfation before being excreted by the kidneys [2].

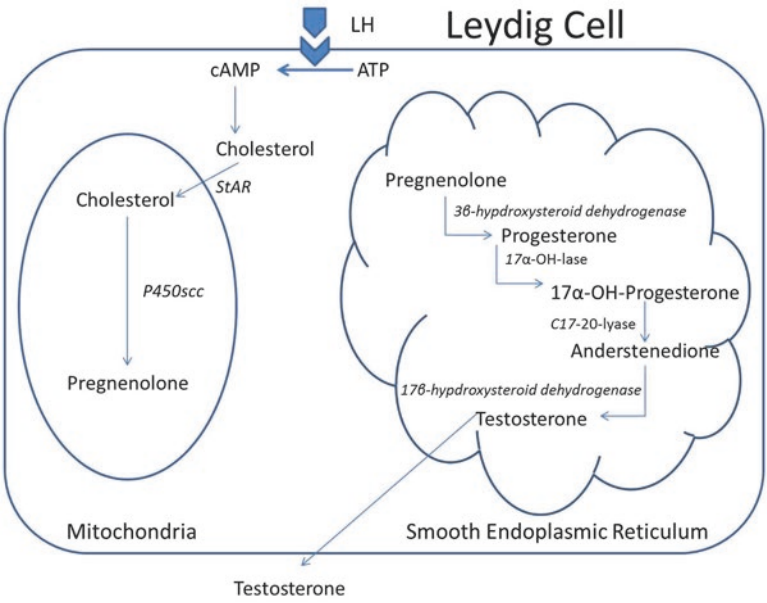


Fig. 6.2 Major Steps in Testosterone Synthesis

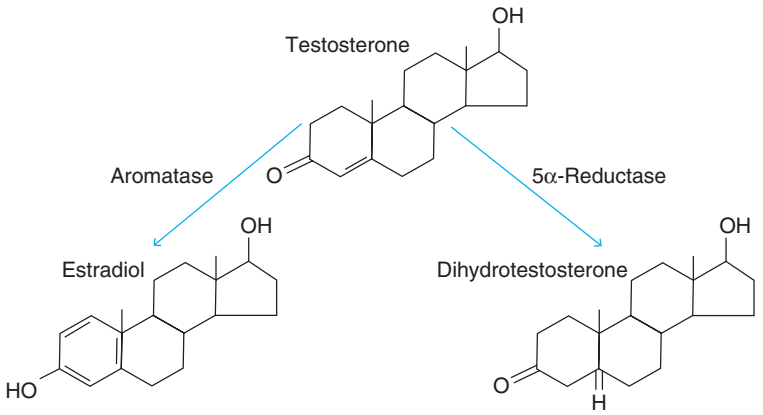
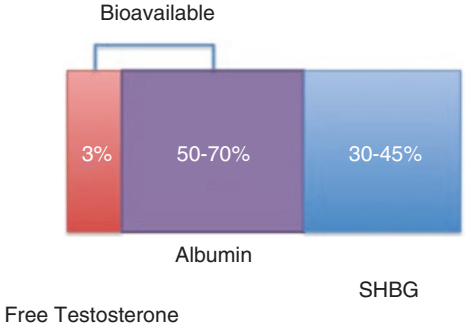


Fig. 6.3 Testosterone conversion to either DHT or estradiol

Fig. 6.4 Circualting Testosterone



Clinical Manifestation of Hypogonadism

History

The medical history for a man being evaluated for hypogonadism depends on age of onset. Onset as an adult leads to a clinical syndrome different than in the fetal or pre-pubertal period. One should begin with a full history from childhood onwards. The history should include information on testicular descent, onset of puberty and pubertal development, shaving frequency, any changes in quality or amount of body hair, and previous systemic illnesses. Men whose hypogonadism is of prepubertal onset and who were not adequately treated will exhibit eunuchoid proportions, delayed development of secondary sex characteristics, and a high-pitched voice.

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 masturbation, sexual activity, and fertility. Information regarding previous orchitis, sinus or pulmonary problems, sexually transmitted diseases, and HIV status should be obtained.

Surgical history is another important aspect in evaluating for hypogonadism that might affect the genital tract along with a thorough drug history that includes recreational drugs, opioids, anabolic steroids, 5-alpha reductase inhibitors, cytotoxic, and psychiatric medications.

Physical Examination

The general physical examination should include height and weight assessments. Characterization of facial, pubic, axillary, and body hair distribution should be included. The presence of acne should be assessed as well a breast examination for evaluation of gynecomastia. Assessment of muscle mass and adiposity should be evaluated. If secondary hypogonadism is suspected, a gross visual field examination should be performed on the patient. A genitourinary examination is needed that includes penile length and location of meatal opening. A scrotal testicular examination should be performed and should include size of testes, evaluation for hydroceles, hernias, and varicoceles. A prostate (digital rectal) examination should be performed in men older than 40–50 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 testosterone level on repeat analysis [6]. Ideally, testosterone should be measured by liquid or gas chromatography and mass spectrometry. Studies have suggested the diagnosis of hypogonadism is more certain at lower levels of testosterone. Most of the circulating testosterone is bound to 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 [7, 8].

Measurement of FSH and LH can help differentiate between hypergonadotropic vs hypogonadotropic hypogonadism. Prolactin should be measured in all men who have a low serum LH level, history of a pituitary mass, or galactorrhea. DHT is measured in cases of abnormal genital differentiation or 5 alpha reductase deficiency. Karyotype is useful in excluding Klinefelter's syndrome particularly in those with testicular volume less than 5 ml, although men with mosaic Klinefelter's syndrome may have larger testicular volumes [9].

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 laboratories but should be between 300 and 1000 ng/dL.

Men suspected of hypogonadism should have their testosterone measured in the morning and if the level is below 250 ng/dL on at least two occasions and symptoms consistent with hypogonadism are visible, 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 one of the measurements of bioactive testosterone is indicated. Serum levels of testosterone above 350 ng/dL indicates hypogonadism is unlikely and further investigation of the symptoms is warranted.

In a man younger than 40 years, as an example, a confirmed testosterone value of <250 ng/dL would warrant magnetic resonance imaging (MRI) but in a man older than 60 years, a value of <150 ng/dL would be necessary to warrant MRI. MRI is warranted for men with hypogonadotropic hypogonadism (low serum testosterone and low or low normal LH and FSH); if they have signs or symptoms suggesting a pituitary mass or hormone excess syndrome, or a serum testosterone <150 ng/dL suggeests the usefulness of MRI even in patients fitting the hormonal pattern without pituitary findings [1].

Classification of Hypogonadism

When there is disruption of the hypothalamic-pituitary-testis feedback system, hypogonadism can occur and can present as hypergonadotropic hypogonadism, hypogonadotropic hypogonadism, or as a combined etiology [10].

Hypergonadotropic hypogonadism is a result of low testosterone secretion caused by 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 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 of testosterone, reveal low levels of testosterone and low or normal levels of LH and/of FSH.

Combined hypogonadotropic and hypergonadotropic hypogonadism can occur in aging and numerous systemic diseases including, but not limited to, type 2 diabetes mellitus, hemochromatosis, metabolic syndrome, obesity, and HIV [11].

Hypergonadotropic Hypogonadism

Hypergonadotropic hypogonadism is when the condition of androgen deficiency is secondary to a pathologic process related to the testis. Such patients differ from hypogonadotropic hypogonadism in that hypergonadotropic hypogonadism is more likely associated with a greater decrease in sperm production than in testosterone production. Many testicular diseases damage both the seminiferous tubules and the Leydig cells, but many damage the seminiferous tubules to a greater degree resulting in low sperm count, and the FSH will be normal or high yet the testosterone level could remain normal. In contrast to hypogonadotropic hypogonadism there is a proportionate reduction in testosterone and sperm production.

Hypergonadotropic hypogonadism is more likely to be associated with gynecomastia, presumably as a result of the stimulatory effect of the supranormal serum and LH concentration on testicular aromatase activity, which results in increased conversion of testosterone to estradiol and enhanced testicular secretion of estradiol relative to testosterone.

Congenital Defects

Hypergonadotropic hypogonadism can be secondary to congenital defects. The most common congenital defects is a chromosomal abnormality such as Klinefelter's Syndrome (KS), but mutations in the FSH and LH receptor genes, cryptorchidism, disorders of androgen biosynthesis, myotonic dystrophy, congenital anorchia, and varicoceles are also possibilites. Primary causes of hypergonadotropic hypogonadism are listed in Table 6.1.

KS is the most common congenital abnormality causing primary hypogonadism, occurring in 1 in 500–1000 live male births [12]. The syndrome is the clinical manifestation of a male with an extra X chromosome and 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, which results in a small testes, very low sperm counts, elevated FSH and LH concentrations, subnormal testosterone concentrations, and decreased virilization [13].

Table 6.1 Causes of hypergonadotropic hypogonadism

Disorders	Examples
Congenital Disorders	Chromosome disorders, Klinefelter's and related syndromes, testosterone biosynthetic enzyme defects, myotonic dystrophy
Developmental Disorders	Prenatal diethylstilbestrol syndrome, cryptorchidism
Acquired Defects	Orchitis, mumps and other viruses, granulomatous disease, HIV, torsion of testis, traumatic injuries
Toxins	Alcohol, fungicides, insecticides, heavy metals,
Drugs	Cytotoxic agents, opioids, alcohol, ketoconazole, spironolactone, flatamide, cimetidine
Autoimmune Testicular Failure	Isolated, autoimmune syndromes
Androgen Resistance Syndromes	
5α-reductase Deficiency	
Systemic Diseases	Chronic renal failure, cirrhosis, HIV, sickle cell disease, amyloidosis

Cryptorchidism refers to testes that are not descended into the scrotum, and 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 be severely subnormal and the serum testosterone may also be reduced.

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, the enzymes 3 beta-hydroxysteroid dehydrogenase and 17 alpha-hydroxylase are present both in the adrenal gland and the testes. Each of these mutations can result in decreased testosterone secretion which can result incomplete virilization.

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 and can result in a decrease in the size of the testes. They can return to normal size and function or they can atrophy. Spermatogenic defects occur more often and can result in normal LH with increased FSH levels. Over time, low testosterone levels and lower LH levels can develop [14]. 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 a degree sufficient to cause azoospermia and markedly elevated serum FSH concentrations. After chemotherapy,

particularly with alkylating agents such as cyclophosphamide, testosterone secretion is also impaired, leading to a decrease in the serum concentration of testosterone and increase in serum LH [15].

Trauma

The testes are located in the scrotum which can make them susceptible to injury. Surgical injury and type of scrotal surgery including hernia repair, varicocele, and vasectomy can result in permanent testicular damage resulting in hypogonadism and infertility.

Medical or self-treatment with androgens and synthetic anabolic steroids can lower serum LH/FSH and subsequently intratesticular testosterone and sperm count [16].

Autoimmune testicular failure is a result of antibodies against the microsomal portion of the Leydig cells that occur either as an isolated disorder or as part of a multiglandular disorder [17].

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 Tables 6.2 and 6.3.

Congenital Hypogonadotropic Hypogonadism

Congenital abnormalities from decreased secretion of gonadotropins are rare but fairly easy to recognize due to the resulting abnormalities of sexual development. It can be the result of isolated hypogonadotropic hypogonadism, congenital GnRH deficiency such as Kallman's syndrome, leptin, or leptin receptor mutations, syndromes associated w/ mental retardation like Prader Willi Syndrome, gonadotropin subunit mutations or hypogonadotropic hypogonadism associated with other hypothalamic pituitary hormonal deficiencies such as the PROP-1 mutation which results in hyposecretion of anterior pituitary hormones [16, 17].

Table 6.2 Causes of idiopathic/congenital hypogonadotropic hypogonadism

Disease	Examples
Isolated Deficiency of Gonadotropin-releasing hormone	Kallman's syndrome, Prader-Willi syndrome, basal encephalocele
Partial deficiency of gonadotropin-releasing hormone	Fertile eunuch syndrome
Multiple hypothalamic and/or pituitary deficiency	PROP-1 mutation
Pituitary hypoplasia	

Table 6.3 Causes of acquired hypogonadotropic hypogonadism

Disease	Examples
Traumatic brain injury	
Neoplastic	
Pituitary adenoma	Functional or nonfunctional tumors
Craniopharyngioma	
Infiltrative and infectious diseases of hypothalamus/pituitary	Sarcoidosis, tuberculosis, coccidiodomycosis, hemochromatosis, syphilis, histocytosis X
Autoimmune hypophysitis	
Obesity	
Malnutrition	
Exogenous hormones and drugs	Antiandrogens, estrogens, antiestrogens, progestogens, glucocorticoids, cimetidine, spironolactone, digoxin, drug-induced hyperprolactinemia

Acquired Hypogonadotropic Disorders

Anorexia nervosa and weight loss causes functional defects resulting in low serum testosterone levels. Men can present with hypogonadotropic hypogonadism due to decreased gonadotropic secretion.

Stress and illness also lower gonadotropin and testosterone levels. There are organic etiologies including neoplastic, granulomatous, infiltrative, and post-traumatic lesions in the region of the hypothalamus and pituitary. Treatment of the underlying disease process may improve gonadotropin secretion.

Prolactinomas manifest differently in men where the tumors are usually macroadenomas by the time they are detected. Male patients with prolactinomas often present with hypogonadism, erectile dysfunction, and visual manifestations from supracellar extension. In small tumors, hypogonadotropic hypogonadism may be caused by suppressive effects of elevated prolactin levels on GnRH secretion which subsequently can suppress testicular function.

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.

Drugs including androgens, glucocorticoid treatment, and opiate administration can disrupt the axis. All three have been known to suppress gonadotropin and testosterone secretion.

It can also be idiopathic when no cause can be identified in some men with acquired secondary hypogonadism. This is more common in older men and the severity can range from mild to severe [18].

Hypogonadism Associated with Systemic Diseases

HIV infection and hemochromatosis are associated with both hypogonadotropic and hypergonadotropic hypogonadism. Previously, when successful treatment was not available, many patients with AIDS had low serum testosterone levels. The pathophysiology may be complex with both hyper- and hypogonadotropic patterns evident. Additionally, there may be alterations in the SHBG levels resulting in low testosterone levels. Fortunately, HIV infections are being successfully treated in many instances and the frequency of low testosterone states has greatly reduced. It has been observed in hemochromatosis that iron deposition can occur in the testis and the anterior pituitary resulting in either hyper- or hypogonadotropic hypogonadism [21, 22].

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

The etiology of testicular dysfunction in liver disease is complex and may be either independent of, or associated with, the direct toxic effects of excessive use of alcohol. Gynecomastia, testicular atrophy, and impotence are concomitant signs of cirrhosis. Estradiol levels are usually elevated which results in an increased ratio of estradiol to testosterone which can lead to gynecomastia [23, 24]. In obesity there is a decrease in SHBG resulting in lower testosterone levels and in diabetes mellitus type 2 there are data suggesting that low testosterone levels are due to hypothalamic-pituitary dysfunction [25, 26].

During hypogonadism with aging, testosterone falls from maximal concentrations to the end decade of life; free testosterone falls faster than total testosterone. The incidence of men at any age with serum levels below 275 ng/dL on two occasions appears to be less than 5 % this before is considerably lower than predefined from earlier studies. Recent data indicate that testosterone replacement can enhance sexual drive and function in older men [26].

Treatment

The main medical indication for androgen replacement therapy is hypogonadism. Absolute contraindications to androgen replacement therapy include prostate cancer and breast cancer. Androgen therapy should be used with caution in older men with an enlarged prostate and severe urinary symptoms, elevated hematocrit, and obstructive sleep apnea.

There are several preparations of testosterone replacement that are available and are summarized in Table 6.4. They include testosterone esters, which can be given as an intramuscular injection every 2 weeks. Testosterone undecenoate injections administered every 10–12 weeks are available in many parts of the world. A slightly different regimen of depot testicular undecenoate is available in the United States but treatment under careful observation is required because of Food and Drug Administration concerns about oil embolization to the lungs and possible anaphylactic reactions.

Table 6.4 Testosterone formulations

Formulation	Regimen	Advantages	Disadvantages
Testosterone enanthate or cypionate	100–200 mg every 2 weeks	Inexpensive Flexibility of dosing Corrects symptoms	IM injection, can result in peaks and troughs in testosterone levels
Scrotal testosterone patch	One patch delivers 6 mg/24 h applied daily	Corrects symptoms	Scrotal skin needs to shave scrotal skin
Nongenital transdermal	One or two patches delivers 5–10 mg testosterone/24 h applied daily	Ease of application, lesser increase of hemoglobin, corrects symptoms	Skin irritation, some men may only achieve low normal range and need two patches
Testosterone gel	Testosterone gel containing 100–200 mg testosterone should be applied daily	Corrects symptoms, flexible dosing, ease of application	Potential of transfer to a female partner or child
17α-alkylated androgen	Orally active compound with concern of liver toxicity		Potential for liver toxicity
Buccal Adhesive	10 mg tablet applied to buccal mucosa twice daily	Ease of application	Limited experience

Oral Androgens

Modified 17α-alkylated androgens are available in oral preparations but are not recommended as androgen replacement. Oral testosterone undecanoate capsules have been available for over 20 years in many parts of the world. The main limitation of oral testosterone undecanoate is the requirement for 2–3 times daily administration. New oral testosterone formulations are under investigation.

Transdermal Androgens

Transdermal testosterone delivery through skin patches and gels have been available in the United States for over 15 years. The patches are less frequently used because of a high rate of skin irritation; skin irritation is less frequent with gels. Transfer from the user to others is possible for gels during routine use [19]. Special care should be exercised when young children come into contact with the user's skin. The label inserts strongly recommend that gel users with a female partner should wear a shirt over the application areas at night, and apply to skin in the morning before dressing and beginning their daily activities.

Androgen replacement leads to the development and maintenance of secondary sexual characteristics in hypogonadal men. Testosterone has important anabolic effects on muscle and bone; it improves libido and sexual dysfunction. It has no major short-term effects to the prostate tissue except when prostate cancer is present.

Erythrocytosis is a common side effect of testosterone treatment. When present, dose reactions or polycythemias may be required. There is ongoing controversy regarding testosterone replacement therapy and the risk of cardiovascular events.

Concern about increased incidents of prostate cancer has not been substantiated and caution is an appropriate useful prospective, large scale double blinded safety studies are preferable. It is generally agreed that long-term safety studies will help clarify the issue.

Summary

Hypergonadotropic hypogonadism results from disease of the testes while hypogonadotropic hypogonadism results from the pituitary or the hypothalamus. The distinction between these two disorders is made by the measurement of LH and FSH. There are disease processes that can result in hypogonadism via both etiologies. It is imperative that a thorough history and physical and laboratory assessment be performed because hypogonadism may be reversed with appropriate treatment of the underlying disease process. The diagnosis of hypogonadism should be only made after confirmation repeat laboratory assessments in conjunction with signs and symptoms. There are many testosterone preparations available around the world to treat clinically significant hypogonadism.

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

7

Ricardo Martins da Rocha Meirelles

Introduction

Male hypogonadism can be primary, when caused by diseases or lesions of the testes, or secondary, when a result of pituitary or hypothalamic disorders. In both cases, hypogonadism can result from genetic, neoplastic, infectious, granulomatous, vascular, actinic, traumatic, or drug-induced injury. High levels of 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.

Functional hypogonadism is prevalent in metabolic disturbances, remarkably in type 2 diabetes mellitus, obesity, and metabolic syndrome [3]. In this chapter we will discuss the prevalence, pathophysiology, clinical aspects, and treatment of low testosterone levels and these diseases.

Epidemiology

Diabetes

Many authors have reported association between hypoandrogenemia and diabetes mellitus [4–9], and this disease is one of the conditions for which the Endocrine Society suggests measurement of serum testosterone levels [10]. Questionnaires

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have been developed to screen for male hypogonadism: The St. Louis University Androgen Deficiency in Aging Male (ADAM) [11], the Aging Male Survey [12], and the Massachusetts Male Aging Study [13]. Comparison of the three questionnaires showed that ADAM has more sensitivity (97 %) although less specificity (30 %) than the others [14]. We found that the ADAM questionnaire shows less sensitivity (85 %) and almost no specificity (2 %) in men with diabetes [15], not being useful for screening and reinforcing the suggestion of Endocrine Society for measuring testosterone levels in patients with type 2 diabetes.

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, and tobacco and alcohol consumption [16]. Cross-sectional research is not suitable to ascertain a cause-consequence relationship, but the higher risk of type 2 diabetes in Klinefelter syndrome hypogonadism [17] suggests that the age-associated lowering of testosterone is the cause of increasing insulin resistance.

Dhindsa et al. [18] 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, normal total testosterone (TT) concentrations are found, and only 6 % have low calculated free testosterone (cFT) [19]. However, Grossman [20] found 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 patients with type 2 diabetes have lower levels of total free testosterone (FT) when compared with patients with type 1 diabetes matched for age [21]. Notwithstanding, a trial with 181 male patients with type 1 diabetes showed a prevalence of 8.3 % of hypogonadotropic hypogonadism in this sample, and an association with age, waist circumference, and insulin requirements was found [22]. Previously known and newly diagnosed patients with type 2 diabetes showed the same prevalence and risk factors of testosterone deficiency in a cross-sectional study comprising 186 men [23]. In the group of previously known patients with type 2 diabetes, those with better control (glycated hemoglobin [HbA1c] < 7 %) had higher levels of TT and lower risk of low levels of this hormone [23].

Glucose metabolism is affected by the levels of serum testosterone even when 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 (TT ≤ 12.6 nmol/L and cFT ≤ 234.1 pmol/L) [24]. It seems as if there is a continuum in the prevalence of normal glucose metabolism to diabetes mellitus, according to the progressive reduction of serum testosterone levels in men. In 775 men ≥55 years of age, those with impaired glucose tolerance diagnosed by 75 g oral glucose tolerance test showed significantly lower TT levels in an age and body mass index (BMI) adjusted analysis [8].

Some studies showed that low testosterone levels are predictive of the development of type 2 diabetes in men [25–30].

Obesity

The association of low levels of testosterone and hypogonadotropic hypogonadism has been known for approximately four decades [31–34]. Although some authors postulated that, despite TT levels being low, the cFT concentrations were normal in obese men [33], Zumof et al. [35] showed that free and bioavailable testosterone also presented identical percent decrease as BMI increases.

In the Tromsø Study, comprising 1548 men 25–84 years of age, after adjustment for age and BMI, men with waist circumference (WC) ≥ 102 cm had significantly lower levels of TT than those with WC < 94 cm [36]. Considering BMI instead of WC, in the European Male Aging Study (EMAS), 5.2 % of 3219 men, 40–79 years, with a BMI ≥ 30 kg/m^2 had LOH and only 0.4 % of men with a BMI < 25 kg/m^2 had TT <320 ng/dL and/or FT <6.4 ng/dL [37].

The association of low testosterone levels and obesity is true not only among middle-aged male adults but also in younger men. Young obese pubertal and post-pubertal males, 14–20 years of age, with Tanner stage ≥4, present testosterone and cFT concentrations 40–50 % lower than non-obese men matched for age and pubertal status [38]. Once both testosterone and FT are reduced in these obese young men, it is not reasonable to attribute to lower sex hormone binding globulin (SHBG) on the difference found when compared with lean counterparts.

Metabolic Syndrome

The components of metabolic syndrome (MetS) are abdominal obesity (determined by the waist circumference), dyslipidemia, high blood pressure, and high fasting glucose. The National Cholesterol Education Program—Adult Treatment Panel III (NCEP-ATP III) [39] defines MetS as the presence of at least any three of these risk factors, and the International Diabetes Federation [40] requires central obesity associated with two or more other risk factors. Table 7.1 shows both criteria.

Table 7.1 Metabolic syndrome in men: comparison of the NCEP-ATP III and international diabetes federation (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 then 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] and [4]

Many studies have demonstrated the association between low levels of testosterone in men and MetS. In a small sample of 130 of nonsmoking men from the Quebec Family Study, the frequency of men presenting three or more features of MetS increased with decreasing testosterone levels from 8.9 % in the third tertile, to 44.2 % in the first tertile ($P < 0.0005$) [41]. In a case control study of 832 hypogonadal patients with Klinefelter Syndrome (KS) compared with 4033 randomly selected age-matched men, KS subjects were at significantly increased risk of type 2 diabetes and obesity [42].

In the Third National Health and Nutrition Examination Survey comprised of 1226 men ≥20 years of age, those in the lowest quartile of TT were more likely to have MetS than men in the highest quartile (prevalence ratio 2.16, 95 % confidence interval [CI] 1.53–3.06) [43]. However, EMAS, performed 2 years later on 2966 community-dwelling men 40–79 years of age, found a significant association between low testosterone levels and MetS (odds ratio [OR] 9.94; CI 2.73–36.22) only in men with severe LOH [44].

A meta-analysis of 52 observational studies comprised of 22,043 men concluded that TT and FT are lower in men with MetS (TT mean difference = −2.64 nmol/L, 95 % CI −2.95 to −2.32; FT standardized mean difference = −0.26 pmol/L, 95 % CI −0.39 to −0.13), and men in the highest tertile of blood TT levels had lower MetS risk (relative risk RR estimate = 0.38, 95 % CI 0.28–0.50) [45]. The presence of ED, obesity, peripheral vascular disease, and alcohol intake significantly increase the probability of MetS in men with testosterone deficiency [46]. Sexual symptoms, even when not associated with hypogonadism, can be accompanied by a higher prevalence of MetS. Almost half of 280 men with ED had MetS, and the ED was more severe among those with MetS [47].

Not only is MetS associated with low testosterone levels, but also the intima-media thickness (IMT). A trial in 935 men, median age 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 [48].

A few long-term prospective trials can be found in the medical literature. Laaksonen et al. [26] followed 702 middle-aged Finnish men participating in a population-based cohort study for 11 years without diabetes or MetS at the time they entered the study. More men in the lowest quartile of TT developed MetS (OR 2.3, 95 % CI 1.5–3.4) and diabetes (OR 2.3, 95 % CI 1.3–4.1). The authors hypothesized that hypoandrogenism is an early marker for disturbances in insulin and glucose metabolism that may progress to MetS or diabetes [26].

EMAS, with 3369 community-dwelling men, 40–79 years of age, established a link between greater risk of MetS in men with low levels of testosterone, independent of SHBG, BMI, or insulin resistance [49]. A lower estradiol/testosterone ratio was associated with protection against the risk of developing MetS [49]. A Korean study of 2172 men, 21–79 years of age showed a negative association of TT and the prevalence of MetS independent of age and BMI [50]. However, one study of 203 men with type 2 diabetes showed no difference in the proportion of patients with MetS in the group with and without hypogonadism, using the International Diabetes

Federation criteria [51]. Some authors believe that MetS-associated hypogonadism needs clarification, once it is not defined if the low levels of testosterone are cause or consequence of MetS [52].

Pathophysiology

The diagnosis of hypogonadism comprises the presence of low testosterone levels associated with clinically compatible signs and symptoms [53]. Although the most common symptoms are sexual in nature (loss of libido, ED, 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, fat mass increase and muscle mass decrease, their consequences may be the basis for the disturbance leading to type 2 diabetes mellitus, obesity, and MetS. Insulin resistance is present in all these conditions, however, the inverse relationship between low levels of testosterone and high insulin is found independent of age or obesity [16].

It has been known for centuries that animal castration causes weight and fat gain. There are many pathways through which testosterone can interfere with adipose tissue. The differentiation of stem cells into myocytes or adipocytes depends on the level of circulating testosterone. Testosterone induces differentiation of mesenchymal stem cells into muscle cells and inhibit adipocyte formation (Fig. 7.1) [54, 55]. Because this is a dose-dependent phenomenon, some athletes take supraphysiological doses of androgens as anabolic agents, with well documented adverse effects [56].

Higher relative muscle mass is inversely associated with insulin resistance and lower levels of HbA1c, and 10% elevation of Skeletal Muscle Index corresponds to 11% reduction of homeostatic model assessment of insulin resistance (HOMA-IR) [57]. The authors also found that the prevalence of glucose intolerance reduced by 12% [57].

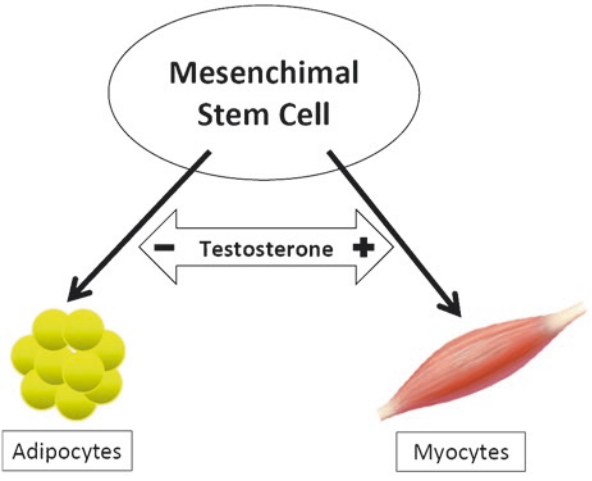

Fig. 7.1 The action of testosterone on mesenchymal stem cell differentiation

A study carried on in male Sprague–Dawley rats indicated that testosterone replacement treatment decreases visceral fat cell size characteristic of the central obesity and MetS [58]. In men, testosterone (but not dihydrotestosterone) inhibits lipoprotein lipase activity and incorporation of triglycerides to adipocytes in abdominal but not in femoral fat [59]. Testosterone also influences the catecholamine-induced lipolysis, through increasing the number of β-adrenoceptors and the activity of adenylate cyclase in adipose precursor cells from male rats [60].

The relationship between obesity and hypogonadism is bidirectional. Obesity is associated with high levels of inflammatory cytokines, produced by adipocytes. Tumor necrosis factor-α and interleukin-6 reduce gonadotrophin releasing hormone (GnRH) and luteinizing hormone (LH) secretion by hypothalamus and pituitary, respectively, both in animals and in vitro [61, 62], and can contribute to low testosterone levels seen in obese men.

Higher content of aromatase in abdominal fat of obese men could contribute to 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 [63]. However, this was not confirmed by Dhindsa et al. [64]. Notwithstanding, the LH pulse amplitude is decreased in obese men pointing to a hypothalamo-pituitary abnormality in these subjects [65].

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 is inhibited by leptin, which can contribute to the low levels of testosterone found in obese men [66–68]. It is worth mentioning that the concentration of serum leptin 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 [69].

Compromised mitochondrial function induces metabolic alterations characteristic of insulin resistance [70–73]. Pitteloud et al. [74] demonstrated an association of impaired mitochondrial function with low testosterone levels promoting insulin resistance in men using a hyperinsulinemic-euglycemic clamp in 60 men.

Bellastella et al. [75] suggested that the anti-pituitary antibodies targeting gonadotropin-secreting cells shown to be present at high titers in patients with 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. 7.2).

Testosterone Replacement Therapy

Although some authors believe that the risk-to-benefit ratio of testosterone therapy in patients with type 2 diabetes requires further elucidation [76–80], most of the published trials points out the benefit to overcoming risks. Three papers that advocated for higher cardiovascular risk with testosterone replacement therapy (TRT) [81–83] were criticized for methodological issues or misinterpretation of data, and many world endocrine societies and individual researchers asked for a retraction of

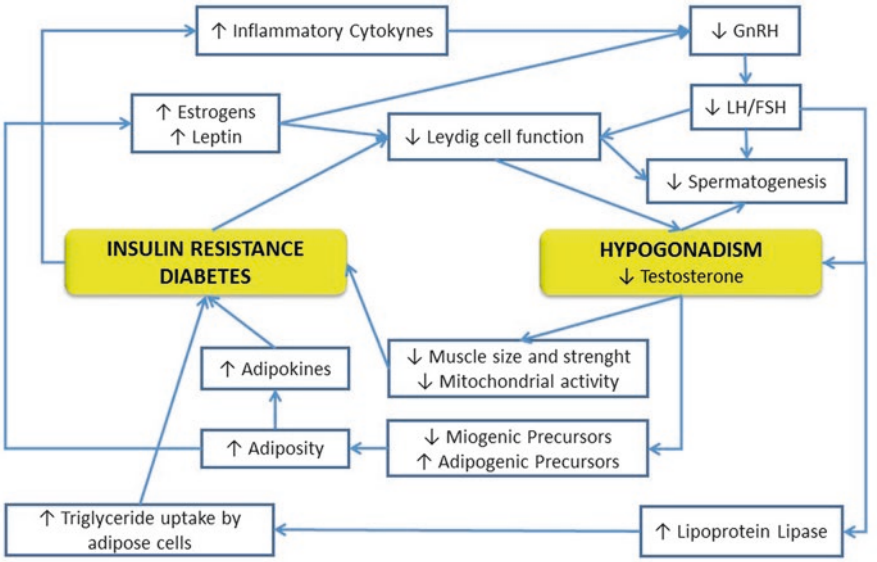

Fig. 7.2 Bidirectional relationship between hypogonadism and insulin resistance (Adapted from references [3] and [74])

one of them [84, 85]. In fact, 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 [86].

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 time span of 12 months for increasing muscle mass and strength, 24 months for fat mass and waist circumference, and up to 36 months for increasing bone mineral density [87]. 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 better levels within 24 months [87].

Reducing excessive fat mass and increasing lean mass is one of the main goals of the treatment of obesity, type 2 diabetes, and MetS, with the objective of ameliorating cardiovascular risk factors and outcomes. Interestingly, in a long-term study of 255 hypogonadal men during a 5-year period, those who were overweight or obese lost weight and those within the normal BMI range gained weight, probably by increasing muscle mass [88]. It is noteworthy that the study was not designed to investigate weight change in hypogonadal men treated with testosterone. Another study of 261 hypogonadal elderly men with ED during a a 5.5-year period concluded that TRT reduces obesity and improves MetS parameters: total cholesterol, high-density lipoprotein-cholesterol (HDL-cholesterol), low-density lipoprotein-cholesterol (LDL-cholesterol), triglycerides, fasting glucose, HbA1c, systolic and diastolic blood pressure [89].

Mortality is higher in men with low testosterone levels. A study of 794 older community-dwelling men, 50–91 years of age, followed up for an average of 11.8 years, showed 40 % higher mortality in men with serum testosterone in the lowest quartile when compared with those in the higher quartile, mainly by cardiovascular and respiratory disease [90]. These data were confirmed by another study of 3637 older community-dwelling men 70–88 years of age, with a mean follow-up of 5.1 years [91]. The Health In Men Study 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 [92].

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 in increased risk of all-cause mortality, in a study of 5077 patients with median age of 69.5 years [93].

It is known that low serum testosterone levels are associated with higher mortality [94] and treatment of hypogonadism improves risk factors in patients with obesity, diabetes, and MetS. However, evidence of TRT could reduce mortality was first shown when Shores et al. [95] 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) [95]. 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 %) [96].

Conclusion

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

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Male Hypogonadism and Aging

8

Pedro Iglesias, Florentino Prado, and Juan J. Díez

Abbreviations

Bio	Bioavailable
BMD	Bone mineral density
BPH	Benign prostatic hyperplasia
CV	Cardiovascular
CVD	Cardiovascular disease
CVRF	Cardiovascular risk factors
DHT	Dihydrotestosterone
E1	Estrone
E2	Estradiol
FSH	Follicle-stimulating hormone
FT	Free testosterone
HPG	Hypothalamic-pituitary-gonadal
hs-CRP	High-sensitivity C-reactive protein
IL-6	Interleukin 6
IL-10	Interleukin 10
LH	Luteinizing hormone

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LOH	Late-onset hypogonadism
MS	Metabolic syndrome
SHBG	Sex hormone-binding globulin
T	Testosterone
TNF-α	Tumor necrosis factor α
T2D	Type 2 diabetes

Introduction

Gonadal function in men decreases with age. Serum testosterone (T) levels begin declining from the fifth decade of life from defects in both testicular and hypothalamic-pituitary function and a high percentage of men older than 60 years have such concentrations below the lower limit of normal for a young adult male [18, 31, 134]. This clinical situation is known as andropause, age-related hypogonadism or late-onset hypogonadism (LOH) [51, 52]. On the other hand, as it occurs in middle-age men, classical hypogonadism, both primary (hypergonadotropic hypogonadism due to testicular failure) and secondary (normo- or hypogonadotropic hypogonadism due to hypothalamic or pituitary insufficiency) can develop in the elderly. There are also several clinical situations of reversible hypogonadism and multiple risk factors for developing hypogonadism in aged men. Clinical benefits of hormone replacement therapy with T in aged men with classical hypogonadism are well established; however, long-term benefits and risks of T therapy in aged males with LOH are under study.

Aging and Gonadal Function

Androgens in men are necessary for the proper functioning of the body at different stages of life, also in the elderly. The decrease of gonadal function with age is more evident from the fifth decade of life [18, 31, 134]. From that point, T secretion gradually decreases throughout life. Multiple causes have been associated with serum T reduction [1, 124]. However, recent studies have shown that, in the absence of serious disease, serum T in older men may be comparable with those found in younger men [36, 102]. The main changes in hypothalamic-pituitary-gonadal (HPG) axis function associated with aging are summarized in Table 8.1.

Definition of Hypogonadism in the Elderly

Male hypogonadism is the failure of the testis to produce normal amounts of T, with the presence of symptoms and signs of androgen deficiency, and a normal number of spermatozoa resulting from a disruption of one or more levels of the HPG axis [64]. Maintaining normal T levels is important in sustaining male secondary sexual

Table 8.1 Changes in hypothalamus-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 homorne, *T* testosterone, *SHBG* sex hormone binding globulin, *FSH* follicle-stimulating homone, *LH* luteinizing hormone
[a]Allan and McLachlan [1], Veldhuis [124]

characteristics, bone mass, muscle mass and strength, erythropoiesis, sexual and cognitive function, and well-being. 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.

Patients with primary hypogonadism often have a decrease in T levels, sperm count, or both, along with an increase in the concentration of pituitary gonadotropins, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Hypogonadotropic hypogonadism is characterized by a reduction of T production, sperm, or both, in the presence of normal or low concentrations of gonadotropins. Combined primary and secondary testicular failure may occur in several conditions, such as hemochromatosis, sickle cell disease, alcoholism, glucocorticoid treatment, and also in older men [14, 15].

Several longitudinal studies have shown that aging is accompanied by a decrease in T levels [31, 87]. 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 % per year for a normal lower limit of 325 ng/dl [46]. The rate of fall in serum T with age varies among individuals and is affected by chronic diseases and medications [46]. 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].

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 [5, 113]. This situation has been described as androgen deficiency in the elderly male, andropause, or LOH [88]. 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 [74, 91, 127]. 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 [127].

Epidemiology and Risk Factors for Hypogonadism in the Elderly

The exact prevalence of hypogonadism in the elderly is unknown. It varies according to the definition used, the population studied, the method of analysis, and the cutoffs used. Given the steady increase in life expectancy it stands to reason that the prevalence of hypogonadism will also increase. Epidemiologic studies have shown that the prevalence of hypogonadism would be around 6–9.5 % of healthy men living in the community between the ages of 40 and 70 years and would increase to 15–30 % in diabetic and obese men [119]. Other studies have shown different prevalence ranging from 5.6 to 39 % depending on the diagnostic criteria for hypogonadism, geographic area, and age of the studied subjects (Table 8.2).

Various factors can promote androgen deficiency and negatively influence the HPG axis in men. Several risk factors for classical hypogonadism in the elderly can be considered including all those processes that can affect the integrity of the HPG axis, such as hemochromatosis and testicular involvement (mumps, injury, tumors, and previous chemotherapy, or radiation therapy). On the other hand, comorbidity is highly prevalent in the elderly and it is known that several diseases, such as those related to metabolic syndrome (MS) (obesity, hyperglycemia, insulin resistance, hypertension, and dyslipidemia), acute diseases, and several drugs including corticosteroids and opiates can negatively impact serum T concentrations in the elderly [55, 103].

Table 8.2 Prevalence of hypogonadism according to various criteria for diagnosis and age of the study subjects

Study (Author, year)	Criteria for diagnosis (serum testosterone) (ng/dl)	Age (years)	Prevalence (%)
Mulligan et al. [90]	<300	≥45	39
Araujo et al. [3]	<200	40–70	6–12
Hall et al. [44]	<300	30–79	5.6
Wu et al. [135]	<320	70–90	2.1–5
Liu et al. [73]	<374	40–79	9.1

Hypogonadism and Morbidity

Hypogonadism occurs in middle-age men, therefore, in the elderly it may negatively affect not only sexual function but also different clinical aspects such as body composition, muscle function, mood, cognition, health-related quality of life, nutritional status, BMD, blood pressure, lipid profile, carbohydrate metabolism, and erythrocyte count [23, 26, 33, 48, 50, 60, 116, 121, 123, 135, 136]. Additionally, an inverse relationship between T deficiency and systemic inflammation and cardiovascular (CV) comorbidity has been also reported in elderly men [23, 40, 43, 63, 75, 117, 118, 139–142] (Table 8.3). However, it is often difficult to discern whether many of these clinical manifestations are symptoms related to hypogonadism per se or the aging process and associated comorbidity.

Sexual Function

Low T levels contribute to sexual dysfunction in the elderly. In fact, the symptoms more significantly related to low serum T concentration in this population seem to be sexual symptoms [26]. In this setting, the European Male Aging Study (EMAS) performed in 3369 men between 40–79 years of age, 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 and a FT level of less than 64 pg/ml [135]. Primary hypogonadism seems to be more likely associated with low sexual thoughts, irrespective of age, than those with secondary or compensated hypogonadism [116].

Nutritional Status and Body Composition

Aging is associated with reduction of appetite and food intake that leads to weight loss, preferably at the expense of muscle loss predisposing to undernutrition [86]. A cross-sectional study performed in patients older than 70 year showed that geriatric men with compensated hypogonadism (normal T and increased LH) had worse nutritional status compared with healthy controls [121]. Furthermore, T is a well-known anabolic hormone that acts by increasing fat-free mass and decreasing fat mass. A relationship between body composition and testicular function in older men has also been reported [38, 125]. 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 [13, 59]. 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 [18].

Table 8.3 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

Bone Mineral Density

Age and hypogonadism, separately, are well-known risk factors for decreased BMD in men [58, 99]. Hypogonadal older men have increased bone resorption, impaired static and dynamic balance, higher risk of falls, and a slightly lower BMD [115]. Furthermore, male aging is associated with variations in reproductive hormones which seem to be related to longitudinal changes in BMD [50]. Cross-sectional and longitudinal studies suggest that serum estradiol (E2) levels are more strongly associated with BMD, bone turnover, and bone loss than T levels in middle-age and older men [71, 123]. A prospective (4.6 years of follow-up) study of 1238 men 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 [19]. These same changes have also been associated with a higher risk of nonvertebral fractures in a prospective cohort study of men older than 65 years [71]. More recently, an observational (6 years of follow-up) study performed in 1705 men 70 years old and older from the Concord Health and Ageing in Men Project study showed that lower SHBG, 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 [50].

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

Low T levels have been related to depressive symptoms in older men [60]. The Rancho Bernardo Study [8], a cross-sectional population-based study performed in 856 community-dwelling older men, 50–89 years old, 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 including 608 men, ≥65 years of age (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 [61]. To date, there is not enough evidence linking low T levels with major depressive disorder in older men [60].

As occurs with endogenous T secretion, neuropsychological function decreases substantially with age in men. On the other hand, as reported in hypogonadal young men [2] and in men receiving androgen deprivation therapy for prostate cancer [80], low serum T levels in older men may negatively influence some aspects of cognitive

function, memory, executive functions, and spatial performance [12, 48]. A prospective, longitudinal (10-year) study performed in 407 men, 50–91 years of age, investigated the relationships between age-associated decreases in endogenous serum T and FT 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 [82].

It is well-known that aging in men is accompanied by worsening of general health. Age-related decline in T and other male reproductive hormones could be associated with impairment in general health status in older men. A cross-sectional and longitudinal study examined the relationships between reproductive hormones and self-rated health, and quality of life in community-dwelling older men at baseline as well as changes over a 2-year follow-up period. Low serum T and E1 were associated with poorer self-rated health, whereas lower serum levels of E1 were predictive of subsequent deterioration in self-rated health over time. No association between T levels and longitudinal changes in general health status was found [49]. A more recent cross-sectional study performed in 788 participants 65 years old or older with evidence of sexual dysfunction, diminished vitality, and/or mobility disability, and an average of two TT < 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 [26].

Erythrocyte Count

Anemia, mainly resulting from iron deficiency, chronic disease/inflammation, and chronic kidney disease, is common (~11 %) in older men and has been adversely associated with morbidity and mortality [41, 47]. T has a stimulatory effect on erythropoiesis [107]. This effect might explain, at least in part, the association of anemia in severe hypogonadism in males. For the first time in 2006, Ferrucci et al. [33] reported that serum T level is a risk factor for anemia in older people. In this setting, 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.

Inflammation, Metabolic Derangements, and Cardiovascular Disease

Observational studies have shown lower risk of CV events in older men with higher T [54, 92, 137]. On the contrary, T deficiency in aging men has been associated with CV risk factors (CVRF) and CV morbidity.

It has long been recognized that T has an immune-modulating action. On the other hand, chronic low-grade inflammation is a risk factor for atherosclerosis [101]. Low T levels have been related to an inflammatory condition that is associated with (1) increased high-sensitivity C-reactive protein (hs-CRP) [133], proinflammatory cytokines [tumor necrosis factor α (TNF-α) and interleukin (IL-)6]

[66], as well as fibrinogen and plasminogen activator inhibitor activity promoting a hypercoagulable state [39], and (2) decreased IL-10, an antiinflammatory cytokine, promoting a pro-atherosclerotic condition [76].

Results from EMAS have shown that severe LOH in community-dwelling men who were 40–79 years old is associated with larger waist circumference, insulin resistance, and MS [63, 117]. Other studies have reported that low serum T concentration is independently associated with insulin resistance in non-diabetic older men [139, 140] and has a predictive value for the development of not only MS but also visceral obesity [23] and type 2 diabetes (T2D) [40]. The relationship between low T and diabetes could be via a bidirectional relationship with visceral fat, muscle, and possibly bone [40]. Hypertension has also been associated with increased prevalence of low T [112]. 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 [28, 43, 63, 89].

Some observational studies [75, 118, 139–142] have reported a higher incidence in CV events in older men with low T levels [138]. In this regard, serum T behaved like an independently significant risk factor for incident stroke and transient ischemic attack [139, 140]. Other studies found a positive association between low serum T and high serum E2 with lower extremity peripheral arterial disease [118] and lower FT and higher LH levels with abdominal aortic aneurysm in this population [142]. In one study performed in 2703 men who were between 70–89 years old, lower levels of T or DHT, but not E2, were associated with the presence of intermittent claudication independent of age, smoking, obesity, and other CVRF [141]. 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 [55]. In that study congestive heart failure and acute cerebrovascular disease were the second and the third causes of hospitalization, respectively. Finally, T deficiency was strongly associated with atrial fibrillation risk in very old men (≥80 years) [75].

On the other hand, a number of studies [54, 97], but not all [45], have reported a relationship between gonadotropins, mainly LH, and an adverse CV disease (CVD) risk profile in older men. In this setting, high LH levels have been positively associated with increased incidence of major adverse CV events in older men with sexual dysfunction [54, 97].

Hypogonadism and Mortality

Some, but not all reports [45], have shown an inverse relationship between LOH and mortality in the elderly male population [4, 70, 72, 96] (Fig. 8.1). A case-controlled prospective study performed in 11,606 men, 40–79 years of age, showed an inverse relationship between serum T levels and mortality from all causes [68]. Furthermore, low serum T levels in older men were predictors of mortality in the long-term (20 years), independent of multiple risk factors and several preexisting health conditions [70, 72]. A recent cross-sectional study showed that low T at admission in aged hospitalized male patients admitted for acute disease was

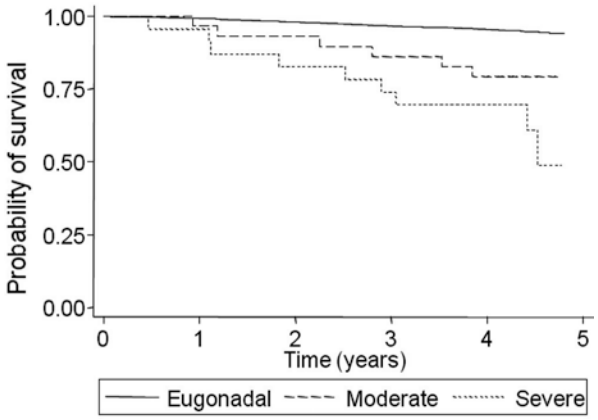

Fig. 8.1 Unadjusted Kaplan-Meier survival curves for late-onset hypogonadism (LOH) status in community-dwelling aging men showing a strong association between LOH and all-cause mortality with a progressive decline in the probability of survival over time. Data from [96]. Reproduced with permission of The Endocrine Society

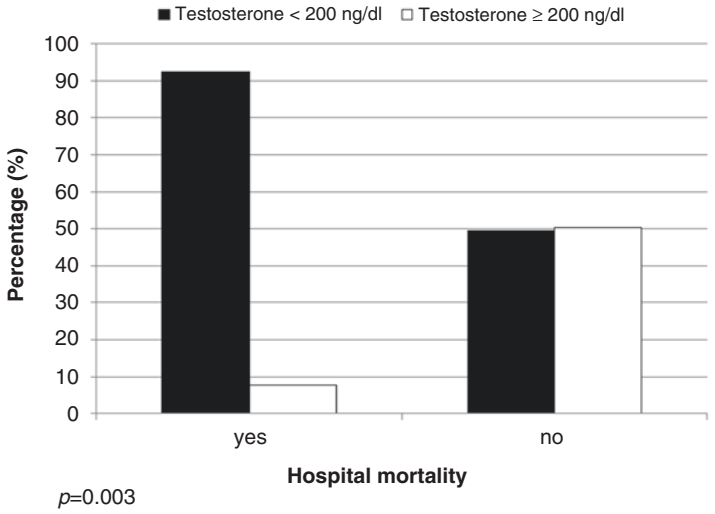

Fig. 8.2 Percent distribution of 150 aged male patients hospitalized for acute disease according to gonadal status at entry and hospital mortality (13 patients [8.7%] died during hospitalization). Data from [55]. Reproduced with permission of Springer Science + Business Media

associated not only with prevalent morbidity (personal history of heart disease, cancer, respiratory pathology, and renal insufficiency) but also with in-hospital mortality [55]. In fact, the likelihood of death during hospitalization in this population increased at lower serum T concentrations (Figs. 8.2 and 8.3). In spite of the fact that the probability of recovery of gonadal function is high after discharge [56], the presence of low levels of reduced serum T concentration at hospital admission also

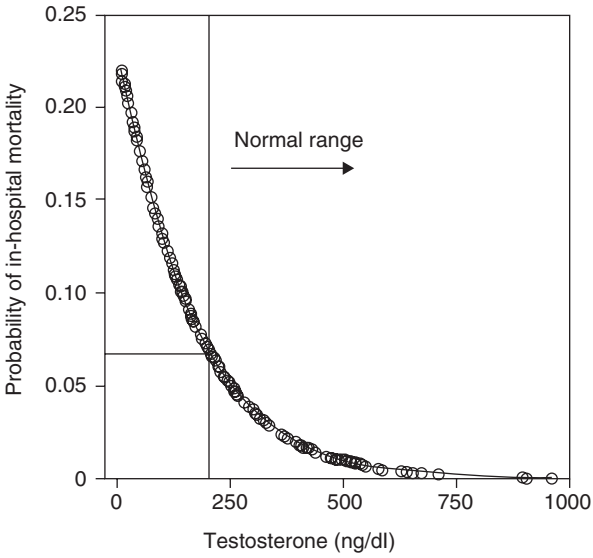

Fig. 8.3 Probability of death during hospitalization in 150 aged hospitalized male patients as a function of serum concentrations of total T at the time of hospital admission, according to a model of logistic regression analysis. Data from [55]. Reproduced with permission of Springer Science + Business Media

behaves as a powerful predictor, even more than age, not only for hospital mortality but for long-term (5 years) all-cause mortality [57] (Fig. 8.4).

Similarly, some studies have shown an association between low serum T concentrations and CV mortality in community-dwelling aged men [53, 68]. 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 were accompanied by a median survival time for CV mortality significantly lower than eugonadal patients after hospital discharge. In those patients, hypogonadal status was also independently associated with 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 [57] (Fig. 8.4).

Replacement Hormonal Therapy

Objectives of Therapy

T treatment aims to restore normal serum levels of male hormone and relieve symptoms caused by androgen deficiency. Therefore, the objectives of the treatment of androgen deficiency are maintaining virility, restoring sexual function and libido, restoring a sense of well-being, optimizing bone density and preventing osteoporosis, and improving CV risk [95]. 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 [14, 15, 91]. Improvement in signs and symptoms of T deficiency should be

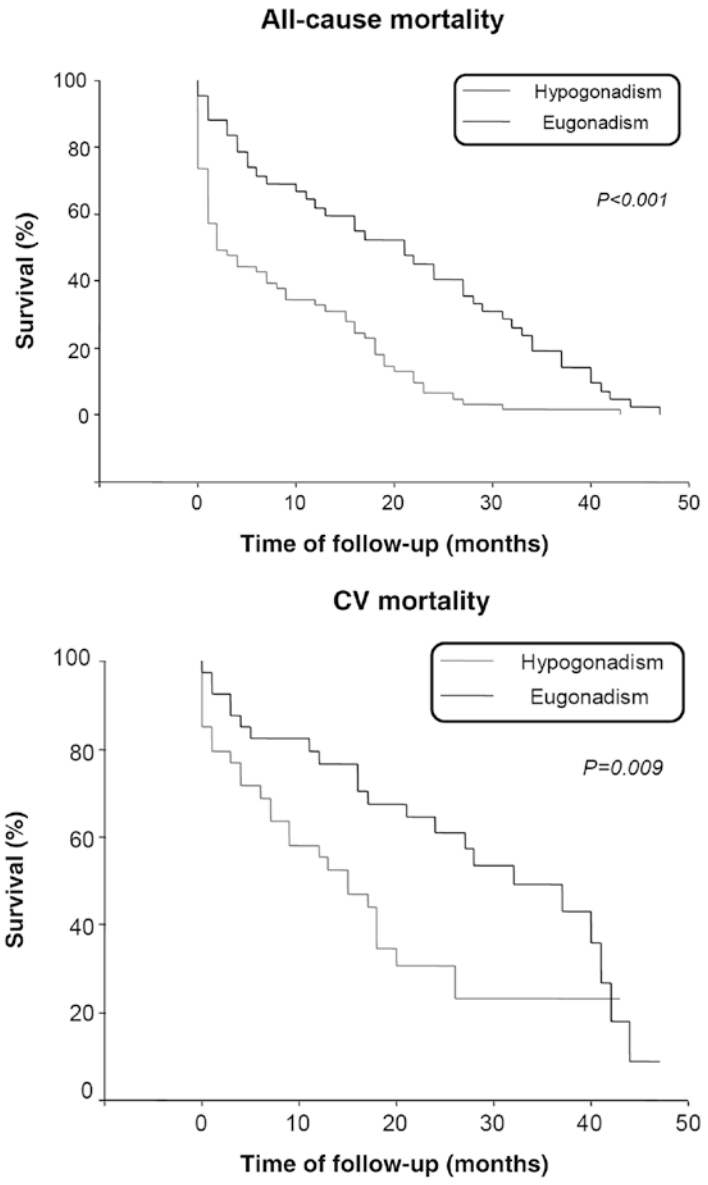

Fig. 8.4 Kaplan-Meier survival analysis for all-cause and CV mortality in 150 elderly male patients admitted for acute illness stratified according to gonadal status at hospital admission. Data from [57]. Reproduced with permission of Thieme

sought and failure to benefit clinical manifestations within a reasonable time interval should result in discontinuation of treatment [91].

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 [15]. 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 [127].

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 [14, 15]. 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 [78]. LOH is often comorbid to obesity and several chronic diseases. For this reason lifestyle modifications should be encouraged in patients with obesity or T2D [24]. The potential clinical benefits, risks and contraindications should also be discussed with the patient. T should not be prescribe 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 [51, 52].

There is no evidence-based consensus on the need for T treatment in aging men, therefore, current recommendations are based on expert opinion [64]. 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, 50, 60, and 70 years, respectively [83].

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 [95]. Morley [85] 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 [85]. The Endocrine Society does not clearly define serum T levels below which T therapy should be offered to older men. Some panelists favored treating symptomatic older men with T level below the lower limit of normal for healthy young men, i.e., 280–300 ng/dl, whereas other panelists favored a level less than 200 ng/dl [15].

Contraindications

Androgen therapy is contraindicated in men with breast cancer or prostate cancer [74, 91]. 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 [15]. In men over 40 years with baseline PSA >0.6 ng/ml, digital examination of the prostate and PSA measurement before initiating treatment, at 3–6 months, and then in accordance with guidelines for prostate cancer screening is recommended [15]. 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 [127].

Androgen treatment is not recommended in men with hematocrit >50 %, blood viscosity, and untreated severe obstructive sleep apnea syndrome [15]. 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 [127].

T Preparations

T is well absorbed from the digestive tract, but is rapidly metabolized by the liver, making the oral route not suitable for treatment. This therapeutic drawback has been bypassed by changes in the T molecule or changing the route of administration. Various androgenic formulations are currently available for hormone replacement (Table 8.4).

Alkylated androgen therapy has been accompanied by hepatic adverse effects such as cholestatic jaundice, peliosis, and hematoma [93, 131]. 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 [6]. Therefore, alkylated androgens such as methylT, fluoxymesterone, and oxandronole should not be used in androgen replacement therapy for hypogonadal males.

Table 8.4 Advantages and disadvantages of the main T formulations for the treatment of male hypogonadism

Formulation	Dose	Advantages	Disadvantages
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
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)
Scrotal patch	A daily 6-mg patch	Avoids IM injection	Elevated DHT and reduced T:DHT ratio
			Shaved scrotal skin
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 haemoglobin than with IM T esters	
T gels	5–10 g of gel per day (50–100 mg of T per day)	Avoids IM injection	Moderate increase in DHT
		Dose flexibility	Potential transmission to another person by skin contact
		Easy application	High cost
		Good skin tolerance	
Bioadhesive bucal tablets	30 mg bid	Absorption at buccal mucosa	Gingival adverse effects
		Unchanged T:DHT ratio	Twice daily application

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

T undecanoate, currently used intramuscularly, is also available in oral preparations. The recommended dose is 40–80 mg, two or three times daily, with meals. However, there is considerable inter- and intra-individual variability in absorption and produces erratic serum T levels. Furthermore, it requires frequent dosing and causes gastrointestinal adverse effects, which limits its usefulness.

T pellets are implanted subcutaneously and provide a prolonged release of T for 3–6 months [65]. 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 [22]. Because pellets release T over several months and are not removable, they are less desirable for treatment in older men [78].

T enanthate and cypionate have been used for many years as oily solutions for intramuscular injection [37, 109]. 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 8.4). 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 [111].

T undecanoate, administered by intramuscular injection at doses of 1000 mg every 3 months, has shown to maintain T levels within the normal range [104].

Transdermal drug preparations provide the closest approach to the normal circadian rhythm of T [122]. 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 8.4). Furthermore, because scrotal skin is rich in 5α-reductase, patients can have high levels of DHT [34].

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 [16, 27], and application at night is recommended with DHT levels remaining within the normal range [81]. 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 [128]. 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 8.4).

Gels are generally well tolerated, although can cause skin irritation that usually does not require treatment discontinuation [114]. The gel dries quickly after application, albeit it is possible to transfer to another person if there is direct skin contact.

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 [69].

The choice of a T preparation depends on the availability, cost, convenience, dose needed, and preference of the patient while taking into account the advantages and drawbacks summarized in Table 8.4. 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 [91, 127].

Monitoring of Treatment

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 [127]. 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 [15]. Metabolic parameters such as blood glucose and lipid profile can also be measured [24]. 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 [78]. 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 [120].

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 [98]. A recent meta-analysis of 15 studies including 739 patients that received T

Table 8.5 Risks associated with therapy using various 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

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 [62]. 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 [27, 67, 77, 94, 105, 108]. However, some men may have an exacerbation of symptoms of BPH (Table 8.5), and in those cases an appropriate urologic evaluation should be performed before proceeding with the androgen treatment.

Prostate Cancer

A summary of prospective studies in hypogonadal men of different etiologies undergoing T replacement therapy showed only five cases of prostate cancer in 461 men followed between 6 and 36 months, which represent a 1.1 % rate, similar to that found in the general population [98].

A meta-analysis [17] 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 [32], 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 compared

with the placebo group. Despite these reassuring data, a screening for prostate cancer is recommended before starting androgen treatment in hypogonadal men. 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 [127]. 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 per year also requires a urologic evaluation [14, 15, 20, 21, 98]. Urologic consultation should also be performed in patients with severe lower urinary tract symptoms (American Urological Association /International Prostate Symptom Score above 19).

CV Effects

Epidemiological studies have shown that low testosterone levels are more predictive of cardiovascular disease than high levels [43]. Other studies suggest that high levels of testosterone may even have a favorable effect on cardiovascular risk [29, 30, 43, 130]. Overall mortality and CV event rates did not differ among T- and placebo-treated men in a recent meta-analysis [32]. 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 [32]. Nevertheless, a recent 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 [9], particularly in men who achieved high serum T levels [10]. Two recent studies have also reported increased CV risks in men who received T therapy [35, 126]. However, 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 [84]. A recent 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 [11]. 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. Therefore, long-term controlled trials with sufficient statistical power are needed to assess the real risk of T treatment on CV events.

Lipid Profile and Blood Glucose

T replacement produces a neutral effect on plasma lipids, sometimes accompanied by a minimal reduction in HDL-cholesterol and a reduction in total cholesterol [7, 27, 42, 105, 106, 110, 132]. 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 LDL-cholesterol decreased or remained unchanged in 14 of the 15 studies analyzed [132]. The decrease in HDL-cholesterol has been shown to be more marked in studies enrolling older patients using intramuscular T preparations [32].

A recent meta-analysis has shown that 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. 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 [24]. Other authors have confirmed an improvement in MS in older men with LOH [136].

Sleep Apnea

T treatment may worsen sleep apnea syndrome [74]. 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 [79].

Erythrocytosis

Erythrocytosis (hematocrit >50 %) is associated with supraphysiological concentrations of Bio T and E2 and occurs more frequently in patients treated with intramuscular esters [27, 32, 74, 110]. Indeed, its incidence has been estimated at 3–18 % of patients treated with transdermal formulations and up to 44 % of patients treated with injectable preparations [98]. In patients treated with transdermal preparations there is a direct relationship between the dose of T and the incidence of erythrocytosis [129]. Two meta-analysis showed that the relative risk of having hematocrit >50 % in men who were treated with T was 3.69 [17] and 3.15 [32], respectively, compared with men treated with placebo or without intervention. If hematocrit is >54 % during T therapy, treatment should be stopped until hematocrit decreases to a safe level, and evaluation for hypoxia and sleep apnea is indicated [15].

Liver Toxicity

Liver toxicity effects, including the development of benign and malignant tumors, have been associated with oral preparations of T, particularly alkylated T [131]. Intramuscular and transdermal preparations have not been associated with hepatic injury [6].

Other Adverse Effects

Gynecomastia and infertility have been described in patients receiving T replacement [6]. Androgen treatment has also been associated with acne, increased skin fat, and increased body hair. T levels above the physiological range may cause irritability, impulsive aggression, and signs of major depression [74]. There is no evidence that androgen replacement causes clinically significant fluid retention. 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 [100].

Conclusion

Hypogonadism is a hormonal deficiency commonly seen in elderly men. It can be permanent or reversible and can manifest as the classic form of hypogonadism or LOH. On many occasions the symptoms of hypogonadism are difficult to distinguish in those who are associated with the aging process. Decline in serum T concentrations has been associated with several CVRF, 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 characterized by typical symptoms and a deficiency in serum T levels. Androgen treatment would only be indicated in older men with signs and symptoms of hypogonadism and a decrease in serum T concentration on more than one occasion. Therapy aims to achieve T levels in the mid-lower part of the normal range of young men and relieve symptoms caused by androgen deficiency. Androgen therapy is contraindicated in men with breast cancer or prostate cancer. Age per se would not be a contraindication to initiate T treatment, although an individual assessment of comorbidities and potential risks is necessary.

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Hypogonadism After Traumatic Brain Injury

9

Alexandre Hohl and Roger Walz

Traumatic Brain Injury: A Silent Epidemic

Traumatic brain injury (TBI) is common and a serious social and public health problem [1, 2]. Among the different types of traumatic injuries, 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, the US, Germany, and Australia, TBI is the leading cause of death in people under the age of 45 years [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 and sports accidents [12], and violence [13].

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A deeper knowledge of TBI may be useful to develop new strategies for diagnosis and treatment, including rehabilitation. The lack of knowledge about 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 cases of trauma—about 80 %. The hospital care ends up being directed for victims of severe and moderate TBI, representing 20 % of cases [14], and most patients with severe TBI die before being hospitalized.

In the 1990s, the mortality rate resulting from TBI in the US was three times higher for men than for women. Half of all hospitalizations for TBI in the US in 1994 were caused by traffic accidents and 25 % were from 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 Centers for Disease Control and Prevention 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, approximately 15–20/100,000. TBI-related deaths in the US account for approximately one third of deaths caused by trauma [3].

On 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 those, 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 all cases. The number of hospital admissions (50–60/100,000) is lower when compared with other countries such as 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 have managed to reduce the number of TBIs in developed and developing countries [23]. How patients with TBI are rescued is also crucial to reduce the mortality rates and the costs of care 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 with 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 loss of consciousness resulting from 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 injury mechanisms, clinical severity, or morphological changes (Chart 9.1) [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 because of 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 anatomic, physiologic, metabolic, immunologic, and genetic attributes, 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 9.2) [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 9.1 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 on Glasgow Coma Scale	
	Moderate	Score 9–13 on Glasgow Coma Scale	
	Severe	Score 3–8 on 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 9.2 Glasgow Coma Scale

Eye opening		Motor response		Verbal response	
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 9.3 Computed tomography (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 neurologic 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 are imaging exams used to evaluate the morphology of lesions [28, 33]. A 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 on CT (Chart 9.3) [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

TBI and neurologic 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 from 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 neurologic 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 because of 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 8th and 14th 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 extremely 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, 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 during 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 as a result of 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 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]. Following 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 which focused on long-term hypopituitarism have reported high rates of hormone deficiencies of the anterior hypophysis, particularly deficiency of gonadotropins [54, 55].

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

Table 9.1 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

Cases of post-TBI hypopituitarism may occur only through 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 for the onset of hypopituitarism [58]. Therefore, 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 because 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 has been reversed spontaneously after a few years [60].

There are some controversies regarding the severity of the trauma and the hypophyseal dysfunction as a consequence. 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 GCS score 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 present as several signs and symptoms in adult men [64]. The patient may experience loss of libido, worsening of erection quality, increased body fat, abdominal obesity, dyslipidemia, insulin resistance, hypertension, increased thrombotic factors, and cardiovascular mortality and decreased bone mass and muscle strength. The patient may also experience a decrease in physical energy, memory, and attention span, and some patients may even experience emotional disorders, social isolation, and decrease in psychological 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 9.1 provides a summary of the attempts to evaluate the gonadotropic function.

There is still no consensus about those patients who have had TBI need to be investigated. Table 9.2 describes a recent investigation proposal.

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 done with the hormone deficiency alone or together

Table 9.2 Inclusion criteria for investigation of hypopituitarism in patients with mild, moderate or severe traumatic brain injury (TBI)

Inclusion criteria
1. Patients requiring hospitalization for at least 24 h or who were in the intensive care unit (ICU) should be investigated in the acute phase and prospectively.
2. Patients with a 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 anatomic change in the initial computed tomography or magnetic resonance imaging scans (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 antihypophyseal antibodies and antihypothalamus antibodies

in case of a panhypopituitarism [9, 65]. Several beneficial effects can be seen with hormone replacement therapy, 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 recovery from TBI [57, 66].

Testosterone replacement in post-TBI hypogonadism is not different from other forms of treatment of male hypogonadism. It can be performed with injectable testosterone formulations, transdermal (gel or adhesive), or subcutaneous implants. Oral testosterone is not often 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].

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

In that 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; and renal, hepatic, or respiratory failure may contribute to improving the prediction capacity of the model. In this context, considering the anatomic location of the hypophysis and the hypothalamus in the central nervous system, 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. The 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].

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 recently been investigated, both being used as markers of oxidative stress. Plasma levels of Thiobarbituric Acid Reactive Substances (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 score and have been proved 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 were 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. Similarly to 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].

Evidence suggests 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 within 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].

Wagner and colleagues have recently 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. The 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, Insulin Growth Factor 1 (IGF-1), and total testosterone was measured 10 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 and 30 h. In conclusion, the hormones plasma levels, excepting the LH, are not highly consistent with the hospital mortality of male patients [79]. Additional studies are needed to understand the behavior of the gonadotropic axis during the acute phase of TBI in male patients.

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 quality of life. 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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Testosterone Therapy: Oral Androgens

10

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List of 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 testosterone is 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 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. 10.1).

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].

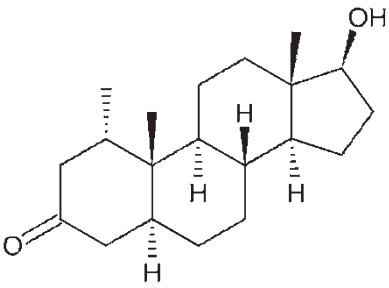

Fig. 10.1 Mesterolone chemical structure

Methyltestosterone (17-α-methyltestosterone, 17α-methylandrosten-4-ol-17β--one-3) was first synthesized in 1935, and has remained 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. 10.2).

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].

A new oral testosterone preparation—*fluoxymesterone* was synthesized in 1956 (Fig. 10.3). 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.

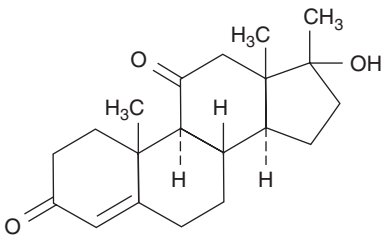

Fig. 10.2 Methyltestosterone chemical structure

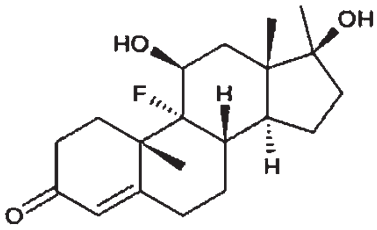

Fig. 10.3 Fluoxymesterone chemical structure

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 twentyfold. 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.

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) (Fig. 10.4).

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].

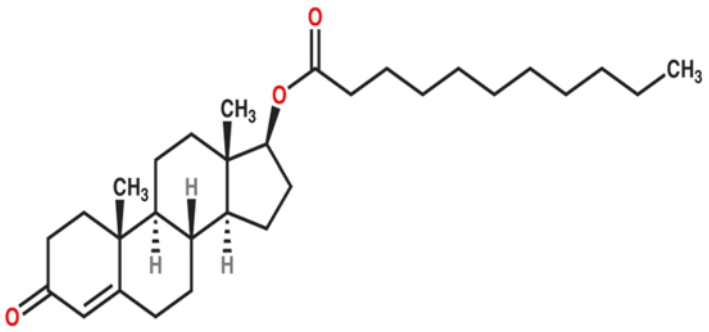

Fig. 10.4 Testosterone undecanoate chemical structure

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 US for clinical use [26] (Table 10.1).

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, 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.

Table 10.1 Comparison of oral (methyltestosterone, mesterolone, testosterone undecanoate), transdermal, and injectable drugs (testosterone propionate and testosterone undecanoate)

Drugs/effects	Methyl-testosterone	Mesterolone	Oral testosterone undecanoate	Testosterone gel	Testosterone 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

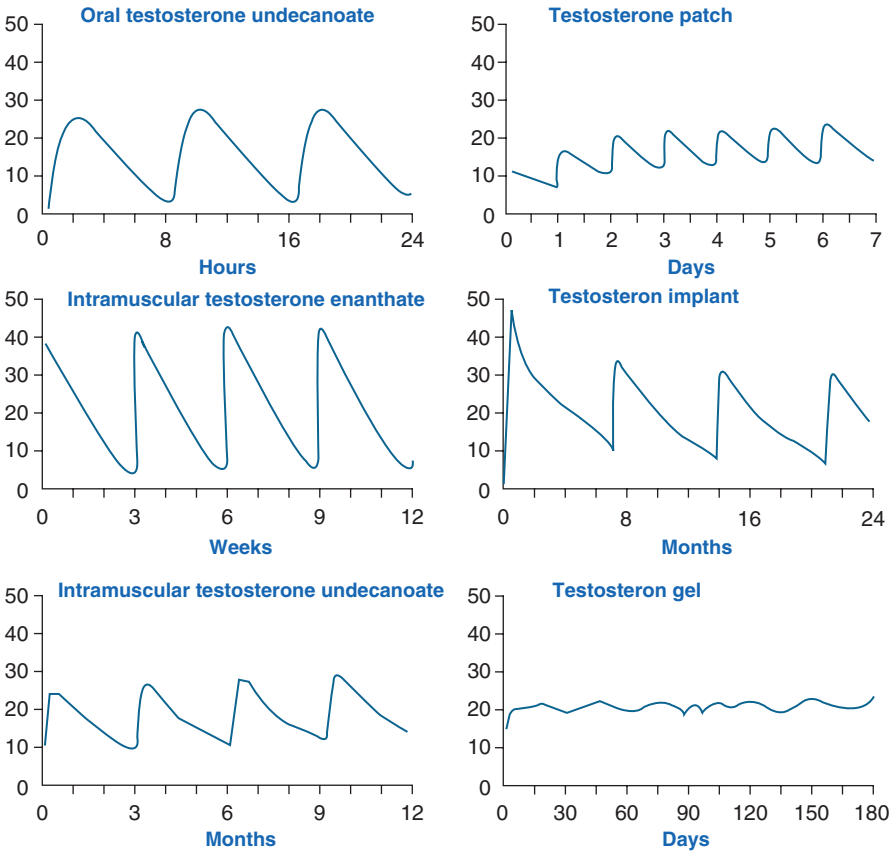

Fig. 10.5 Comparative pharmacokinetics of various formulations of testosterone [28]

Figure 10.5 shows a patient receiving oral testosterone undecanoate has fluctuations in the plasma level of testosterone during the day [29, 30].

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) [31, 32]. 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.

Efficacy of Oral Testosterone Undecanoate in Men

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 [33].

Interesting data were obtained during the use of oral testosterone undecanoate in the surgical treatment of severe forms of hypospadias in boys [34]. The efficacy and safety of testosterone undecanoate has been observed during the treatment of disturbance of puberty and induction of puberty in boys [35–38].

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 [39, 40].

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 [41] 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 [42–44]. 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 [45, 46].

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 [47]. Additionally, this therapy was accompanied by improvement of psychoemotional background and quality of life of men [48, 49].

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 [50, 51].

According to the latest 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 [52].

Dosing Regimens

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 [53]. 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.

S.Y. Kalinchenko et al. (2016) 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) [54].

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 [31, 32]. 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 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: Indications for Use, Dosage, and Monitoring of Efficacy and Safety

The main indication for use of testosterone preparations in women, according to the available guidelines, should be the treatment of inhibited sexual desire (previously defined as hypoactive sexual desire disorder or HSDD) [55–57].

However, the testosterone effects are not limited to only a positive effect on the libido and sexual function in women [58–62].

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 [63]. 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 [64, 65].

The interest in the problems of female androgen deficiency has been growing recently, many modern clinical and experimental studies suggest the important but underappreciated role of testosterone deficiency 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 [66–73].

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 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% [74–76].

There is an age-related decline in serum dehydroepiandrosterone sulfate, dehydroepiandrosterone, and testosterone in women, which parallels the age-related decline in reproductive ability [77]. Some authors consider that poor response represents an androgen-deficient state [78].

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 [79, 80], promoting the initiation of primordial follicle growth, resulting in the improved number of growing preantral and antral follicles [81, 82] (Fig. 10.6).

In humans, granulosa cells in vitro testosterone is able to positively modulate FSH receptor expression at the gene and protein level [85]. In a recently published meta-analysis, testosterone treatment led to statistically significant increase in pregnancy and live birth rate in women with poor ovarian response [86].

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 [87]. The clinical pregnancy rate was 27% per cycle and 43% per embryo transferred after administration of testosterone undecanoate.

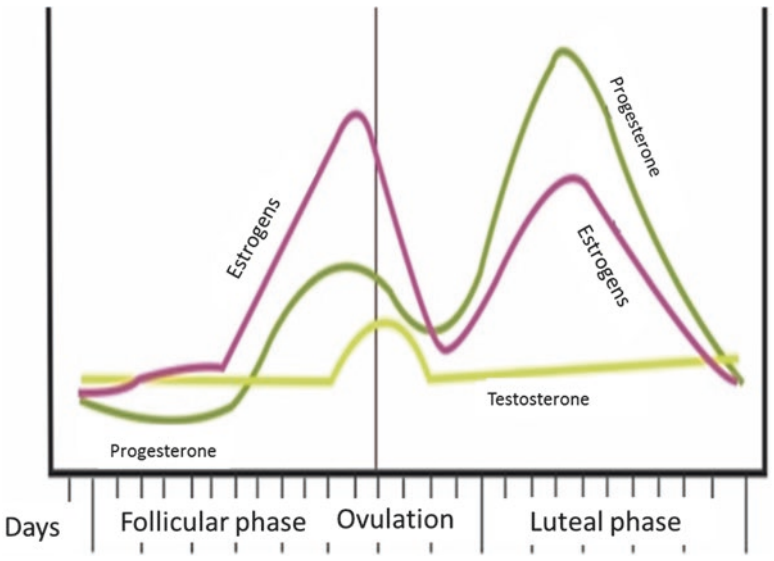

Fig. 10.6 Interaction of sex steroid hormones in the regulation of the menstrual cycle and ovulation in women of reproductive age [83, 84]

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 ($p=0.18$) [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 [89] (Fig. 10.7).

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 [90–92].

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 [93]. 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 [94].

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 [95]. A small study of testosterone therapy in women with congestive heart failure has demonstrated beneficial cardiovascular effects [96].

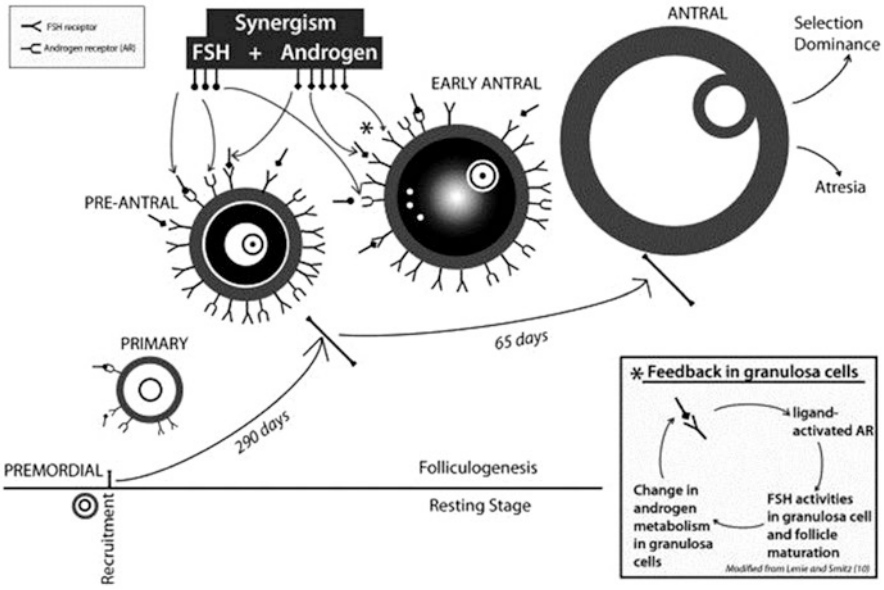

Fig. 10.7 Synergism between androgen and FSH in oocytogenesis [89]

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 [97]. In addition, the relationship between testosterone levels and the severity of chronic fatigue syndrome in postmenopausal women has been established [98] and the balance between estrogen, testosterone, and their metabolites was shown to be essential in maintaining the cognitive function in postmenopausal women [99].

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 [100].

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 [101].

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 [102, 103].

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 [104].

Dosing Regimens

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, 2016), 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] [56]. If the testosterone therapy prescribed for a woman based on clinical indications has a clinical effect, it should

be performed long-term or even for life, particularly in postmenopausal women along with traditional estrogen-progestin menopausal hormone therapy.

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 testosterone deficiency associated with the therapy.

Monitoring of the Safety of Oral Testosterone Undecanoate Therapy in Women

The side effects of testosterone therapy are dose-dependent and may be prevented by using dosage forms and strengths suitable for women. Modern 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 [105–108]. 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 [109, 110].

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 [58, 111].

Summary

Therapy with oral testosterone undecanoate in women can be considered a rather effective and safe 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.

Oral Forms of Testosterone: Optimization of Therapy 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 [112].

Studies have shown that the buccal delivery of testosterone is characterized by a rapid rise and equally rapid decline in plasma testosterone concentration [113]. In this regard, the drugs for hormone replacement therapy must be taken several times a day, similarly to oral testosterone undecanoate [114]. 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 [115].

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, rising the question about the feasibility of their usage in treatment of male hypogonadism [116–118].

Another new direction in optimizing oral forms of testosterone is incorporation of testosterone in special PE (polyethylene) matrices having limited water solubility—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 [119].

Serum concentrations of total testosterone, 5-α-DHT, and estradiol (E2) return to normal in hypogonadal men as a result of such therapy [120]. 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 [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 creation of new unique testosterone preparations with a good efficacy and safety profile. Presently, testosterone undecanoate is the only oral product which remains 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. 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

11

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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, through a permeable membrane, or directly applied to the skin in the form of a gel or lotion. The subcutaneous tissues serve as a depot as small doses are being constantly released into systemic circulation, thus achieving sustained serum levels. There may be a small peak of testosterone within the first few hours after application and then the transdermal testosterone preparation usually maintains serum testosterone within the adult male range for 24 h. Transdermal delivery systems have been available as patches or spray for estrogen replacement in women and as patches, gels, or lotions for androgen replacement in men [1, 2]. Transdermal testosterone gels are the most commonly used formulation to treat hypogonadism in the US and several other countries [3, 4], while long-acting injectables are more widely used in European countries. Some 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

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"patch" delivery systems [5]. Currently available topical products vary by their application methods and dosage adjustment strategies, and are generally expensive. 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 With Other Delivery Systems

Table 11.1 shows the advantages and disadvantages of transdermal testosterone preparations. Transdermal testosterone preparations usually result in less fluctuation of serum testosterone levels compared with oral preparations [6, 7]. However, recent studies suggest that serum testosterone varied with fluctuations within a day in older men after testosterone gel application [8]. 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 [9]. Additionally, transdermal administration helps to avoid first-pass liver metabolism and has less effect on liver secreted proteins such as lipoproteins. Slow-sustained delivery of testosterone may help to avoid adverse effects related to 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 [6, 10]. It has also been suggested that transdermal preparations may have a better cardiovascular safety profile than injectables [11]. Discussion on testosterone replacement therapy and cardiovascular disease risk is found in Chap. 17.

Table 11.1 Advantages and disadvantages of transdermal testosterone for replacement in hypogonadal men

Advantages	Disadvantages
1. Ease of application	1. Possibility of skin to 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 with gels
3. Provides steadier and more sustained release of testosterone into the circulatory system	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 physiologic testosterone secretion with some preparations	5. Expensive

DHT - 5α Dihydrotestosterone

Preliminary data also show lower levels of spermatogenesis suppression in comparison with injectable treatment [12], but these findings need to be validated in larger cohorts.

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 respective sections.

Transdermal Patches

Scrotal patches were the first commercially available transdermal formulation, but have since been phased out in the US 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 the mid normal range. Testosterone levels peak at 2–4 h and remain within the reference range of adult men for 24 h after the application of the scrotal patch. 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 [13, 14]. Scrotal skin has high 5α-reductase activity which results in serum 5α dihydrotestosterone (DHT) levels in the high upper or above the reference range of adult men [15]. Although serum levels of DHT do not correlate with intra-prostatic DHT levels and on long-term follow up there is no known increase in incidence of prostatic cancer [16], there remain concerns in the minds of some clinicians or regulatory agencies with regard to slightly higher DHT levels [17]. Scrotal patches are rarely used by patients mainly because of inconvenience of poor adherence to skin.

Non-scrotal patches (Androderm®) deliver 2 or 4 mg of testosterone. The usual starting dose is one 4 mg patch applied daily before bed time. Testosterone is continuously released for 24 h with maximum concentration ranging within 4–12 h after application of the patch. After removal of the patch, serum testosterone decreases with an apparent half-life of approximately 70 min [9, 18]. 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 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 a 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 skin allergic contact dermatitis [19]. Mild

allergic skin irritation is noted in up to two thirds of patients, while up to 10–15 % of patients have been reported to discontinue the treatment. Topical corticosteroids have been suggested to decrease the discontinuation rate of the skin patches [19, 20]. More serious skin reactions are rare, however, localized skin necrosis has been reported [21]. It is recommended that prior to a magnetic resonance imaging procedure a patch is removed because it may cause skin burns because the patch contains aluminum. Another larger non-scrotal matrix patch without enhancer was developed (Testoderm TTS®). Although 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 the 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 it by rubbing the finger around the edges. In case the patch falls off completely, it is advised to apply a new patch if this occurred before noon, otherwise waiting until regular evening application.

Transdermal Gels

Transdermal gels are becoming increasingly popular and have surpassed injectable preparations as the most common form of testosterone replacement in the US and United Kingdom over the past decade [3].

Testosterone gel is applied directly to the skin avoiding the requirement of a patch or a membrane and resulting in less skin irritation than that observed with transdermal patches. Testosterone gel is available as prepackaged single dose packets or multi-dose pumps. Some manufacturers provide both options (Table 11.2). Most testosterone gel preparations are formulated as hydroalcoholic gel, others use other enhancers in lotions. When applied to the skin, testosterone is absorbed into the stratum corneum over time, which serves as a reservoir. Testosterone is slowly released into the circulatory system over several hours resulting in steady state serum levels of the hormone [22]. 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 circulatory system 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 [23]. Long-term studies with testosterone gel have shown that steady and relatively consistent serum levels of testosterone levels are attained [7], which results in significant improvement of sexual and body composition parameters [24–26].

Several formulations of testosterone gels are available on the market [1, 2, 27]. Currently available gels vary in testosterone concentration and are usually applied once a day. Their pharmacokinetic profiles are also similar: Androgel 1 %®/ Testogel 1 %® [7], Testim® 1 % [28], Axiron®2 % [29] Fortesta Gel® 2 %/Tostran® 2 % [30], and Androgel 1.62 %® [31]. These transdermal preparations have been proven to be efficient in normalizing serum levels, as well as reversal of androgen

Table 11.2 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 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 (eg, genitals, chest, back, abdomen, axillae, or knees)	5 h
Androgel 1.62 % (AbbVie Inc.)	40.5 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 (eg, genitals, chest, abdomen, axillae, or knees)	2 h
Axiron 2 % (Eli Lilly and Co.)	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

deficiency symptoms for long periods of treatment [24] and have been considered an acceptable form of testosterone substitution by users [5]. The maximum concentration of testosterone achieved is variable depending on the preparation but usually within 2–5 h of application and is maintained for 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 have shown that after testosterone gel application there were large fluctuations in serum testosterone concentration both within and between patients [8]. Skin structural differences may be one of the causes of these significant variations in the bioavailability of the drug, which poses challenges in predicting effectiveness of medication and determining an adequate dose, as well as appropriate time for testing serum testosterone levels [8, 32]. 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 [33]. Sampling of blood after testosterone gel applications should be away from the application sites.

Different sites for drug application have been studied with various degrees 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 the axillary region may enhance absorption and may cause less skin transfer, and has been shown to be beneficial to patients who failed other transdermal preparations in a single study [34]. However, because the skin is sensitive in the area, skin irritation, edema, and erythema have been observed as in other transdermal preparations [35]. On the other hand, even though application of 1.62 % testosterone gel on abdominal skin led to 30–40 % lower availability than on the upper arms and shoulders, application on all of these sites resulted in eugonadal testosterone levels [36]. While selection of an application site may not be an issue for most patients, those failing to achieve sufficient systemic levels may benefit from a change of site.

Additionally, some gels include emollients that prevent skin drying and ensure better testosterone absorption. There are data to suggest that this may help achieve better bioavailability and higher serum concentrations [37]. Differences in gel formulations and their pharmacokinetic profiles are a reason 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 11.2. It should be noted that some gels are marketed in various countries under different names but are in fact 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 to skin transfer of the 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, the gel on the application site 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 [38]. However, showering within a short period of time (15–30 min) after application of the gel may result in lower serum testosterone levels [39] and should be avoided. Manufacturer recommendations for minimum time before washing after application vary from 2 to 5 h among different formulations (Table 11.2). It must be noted that washing within that time resulted in approximately 30 % decreased bioavailability of testosterone, however, serum testosterone levels within normal range were sustained. Even with these precautionary recommendations in place, skin to skin transfer continues to pose challenges including reports of virilization of prepubertal children [40–43]. Therefore, physicians prescribing the use of transdermal testosterone gels or lotions must discuss with the participants the risks of transfer and the measures to prevent transfer, as well as other potential adverse events of testosterone discussed in Chaps. 14, 16, 17.

Elevation of DHT has been found to be more pronounced in transdermal gels compared to other formulations possibly due to high 5-α reductase expression in skin (especially when applied on scrotal skin) [7]. In contrast to transdermal patches, a much larger area of skin is exposed to testosterone, thus 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 testosterone ratio is increased after transdermal testosterone application, there are no data showing the association between higher DHT levels and adverse effects on prostatic hyperplasia or cancer of the prostate [17]. Elevation of DHT has been associated with a higher risk of cardiovascular events in observational studies [44] but 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 remains within the reference range limits in healthy adult men and has not been related to adverse effects on primary DHT targets, such as the prostate.

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 from Canada reported large variations of testosterone levels in these preparations [45] and standardization strategies have been suggested [46]. Increasing availability of generic testosterone gels may lead to decreased costs and improve affordability in the near future.

As discussed above, there are distinct differences among the various transdermal preparations. Decision on the most appropriate treatment strategy should be based on an individual patient profile and personal preferences after all available strategies are discussed. It is of utmost importance that the patient is comfortable with the selected treatment as compliance is one of the major challenges with long-term treatment of chronic asymptomatic conditions [47].

Other Topical Testosterone Delivery Systems

Similar strategies to those used in transdermal testosterone delivery systems have been used in developing trans-mucosal preparations. Currently available systems include the trans-buccal system and intranasal gel. The mucous membrane of the nose is more permeable than skin, and therefore because of a 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 for intranasal gel) [48]. The 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 [49]. Although 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 of continuous use of this buccal delivery system [50] 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.

Conclusion

Transdermal testosterone delivery by applying gels or lotions onto the skin is the preferred method of many men. This delivery method 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 the number of actuations of a canister or a number of sachets or tubes. The main issue with transdermal testosterone for replacement therapy is the potential of skin to 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 treatment is optimal for the patient depends on the patient's preference and whether there are contraindications to other therapies. In older men with comorbidities, it may be prudent to commence treatment with lower doses of transdermal testosterone. If adverse effects develop, the application of the gels or lotions can be stopped and the patient's testosterone will return to the prior levels within a 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

12

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Abbreviations

BMI	Body mass index
BPH	Benign prostatic hyperplasia
CC	Clomiphene citrate
CV	Cardiovascular
CVD	Cardiovascular disorder
FDA	Food and Drug Association
FSH	Follicle stimulating hormone
HDL	High density lipoprotein
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
QoL	Quality of life
SARM	Selective androgen receptor modulator
SERM	Selective estrogen receptor modulators

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T Testosterone
TE Testosterone enanthate
TRT Testosterone replacement therapy
TU Testosterone undecanoate

Introduction to Testosterone Preparations for the 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 in 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 T is approximately 110–575 ng/dL in men who are 18–69 years old.

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 T concentrations, hypogonadism may also be the result of a consequence of autoimmune or genetic disorders, accidents, infection, prolonged exposure to heavy metals or alcohol, radiation, tumors and chemotherapy [1], and obesity [2]. Various 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 T in the form of implantable T gel patches, followed in the 1980s by injectable preparations. Other means of T delivery are 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]. However, these transdermal delivery systems may cause moderate to severe skin reactions because of the T delivery systems used, with regard to T patches, while caution is advised with regard to T gels to avoid inadvertent exposure to women and children [9–12]. Furthermore, T absorption via the transdermal route can vary greatly among individuals, and 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 T molecule, which allowed for oral delivery routes such as T 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 has been 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 T cypionate and T enanthate (TE), T propionate and T cyclohexanocarboxylate. 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 their approximately 85 years of availability, 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 of a medium-term solution with improved efficacy, balanced symptoms, and reduced side-effects, long-acting testosterone undecanoate (LA-TU) with intramuscular administration was initially developed in the 1970s 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 reduced to 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 hydrolized 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 T fatty acid esters such as T-propionate (three carbon atoms), T-enanthate (seven carbon atoms), T-cypionate (eight carbon atoms) and T-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 with 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, residence time, terminal half-life, maximum T concentration and time to maximum T concentration, in contrast to 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].

A number of independent research groups have reported findings in humans looking at pharmacokinetics of injectable TU in a variety of concentrations and using a number of delivery vehicles. The first pharmacokinetic investigation by Zhang and colleagues, lasting over 8–9 weeks, 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 T 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. There was one exception in where 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. Those findings 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 two volumes of 4 ml each at two 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 that was supported by another study showing that the bioavalability 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 three 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 that was administered TU 1000 mg every 12 weeks (former LA-TU group) and the group that was 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 two 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 with 26 injections per year for T 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 has a requirement to be taken with fatty meals in order to achieve acceptable plasma T levels [36].

Efficacy of Injectable TU and Comparative Studies

Although a number of TRTs 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 47–74 years old, indicated that while both preparations meet the requirements of present day androgen treatment, higher plasma T levels are achieved with TU compared with T gel [37]. These include improved positive effects on the International Index of Erectile Function, the Aging Males' Symptoms Scale, and International Prostate Symptoms Score 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 MS 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, the 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 one-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, although the majority of these studies are of short duration [42, 43, 44]. A prospective registry study of 261 men treated with T has however provided long-term data on metabolic parameters following T 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 resulting from 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 MS. The group reported improvements in anthropometric parameters, such as Body Weight (BW) and Waist Circumference (WC), in a stepwise yearly manner [44]. Additionally, a continuous decrease in anthropometric parameters in those patients was observed when compared with 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 with the use of short-acting T 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, those 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 comparewith TE treatment, TU treatment improved sexual parameters (spontaneous morning erections, total erections, and ejaculations) and psychological parameters for depression, fatigue, and anxiety. Additionally, an independent study of 33 hypogonadal men treated with TU confirmed some of those findings where patients presented normal serum PSA levels and improved mood, sexual function, and QoL [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 they were, they 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. TE 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

Because T is an endogenous protein, the pharmaceutically active component is T itself, therefore injectable TU is well tolerated. Indeed, only minor complications with TU treatment have been reported, and those were generally limited to include local irritation at the site of injection, not usually lasting more than 3 days [22]. There have been no patient reports of disrupted treatment due to problems or local discomfort. In contrast, conventional injectable TE often leads to mood swings or emotional instability, most likely resulting from 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 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 cardiovascular disorders (CVD) and Prostate Cancer (PCa) and 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 development of comorbidities, T use has been associated with conditions such as prostate cancer, worsening benign prostatic hyperplasia (BPH), male breast cancer, policytemia, an increased risk of obstructive sleep apnea (OSA) [54], and CVD. Indeed, the supposition that patients receiving TRT have an increased risk of prostate cancer is controversial. In this context, although there is no evidence that T therapy increases the risk of prostate cancer, over the decades, physicians have been trained with the notion that T is the fuel for prostate cancer because it is known to be driven through the Androgen Receptors (AR). To address this, the incidence of prostate cancer was evaluated in three independent observational studies in more than 1000 hypogonadal men treated with T 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 [56].

These data suggest that if the European Association of Urology guidelines for prostate screening and monitoring are followed, T therapy should be a safe and effective treatment in hypogonadal men. Further large-scale, randomized, controlled, long-term studies are needed to more completely address the linkage between T levels and prostate cancer.

Increasing evidence suggests that TRT 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 been noted in several studies [57, 58], although PSA levels and prostate size remained within the normal range despite a significant increase being observed. The increase in hypogonadal men is associated with subnormal PSA values and small prostate sizes at baseline [59], and is observed with all T 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, it is an observation not yet made in men undergoing T therapy [66]. Similarly, no documented evidence exists of polycythemia in studies using more traditional T esters despite increases of erythropoiesis parameters to eugonadal values [35].

An examination of the literature reveals evidence clearly suggesting that low T concentrations are associated with CVD risk and known risk factors for CVD such as obesity, diabetes, and the MS [70, 71]. Of 11 longitudinal studies, nine have demonstrated increased mortality rates in men with low T levels and improved survival in those with higher T [72], while two studies showed no effect [73]. In contrast, a recent study by Layton and collaborators investigating the CV safety of T injections, patches, and gels revealed an association between T injections and an increased risk of CV events compared with T gels and patches. However, the study did not assess whether patients met the criteria for use of T and did not assess the safety of T among users compared with non-users [74, 75].

Two studies reporting risks with T gel preparations concluded that there is a significant direct correlation between T therapy and CVD risk [68, 69], although these studies should be interpreted with caution due to their study design limitations [67].

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 [72, 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 with almost bi-monthly visits for TE therapy. Furthermore, because TU requires only four injections per year compared with 26 injections per year with TE, there is a greater compliance rate in patients treated with TU.

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].

Most of these compounds are in the very early phases of pharmaceutical development with combined research and clinical goals to produce reductions in catabolic consequences of hypogonadism and/or aging 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 T 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. Pre-clinical models looking at SARMs have shown a positive levatorani/bulbocavenous muscle complex/prostate ratio, demonstrating an improved anabolic/androgenic ratio with limited side effects [88–90].

Conclusion

All T 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 with 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 Food and Drug Administration (FDA) stated they are investigating the potential link between T therapy and several comorbidities, ‘FDA-approved T 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.

Injectable T can produce improvements in QoL, energy level, libido, muscle mass, cognition and bone density when given to appropriately selected patients with vigilant monitoring. 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 Adverses Effects of Testosterone Therapy

13

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Introduction

Testosterone is a steroid hormone important in many aspects throughout the development and life of men. 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 appear 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 gonadotropin-releasing hormone (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 dihydrotestosterone (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 increased thickness of the vocal cords, growth of long bones and bone epiphysis closure, prostate enlargement, and seminal fluid production by the seminal vesicle [6].

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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 testosterone levels [7, 8]. Other established or controversial indications include constitutional delay of growth and puberty (CDGP), men with sexual dysfunction, female to male transgender persons, hypogonadism secondary to drugs or chronic illness, and 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 detailed. Serum testosterone levels exhibit a circadian variation with peak values in the morning; the circadian rhythm is blunted with aging. Because of the circadian variation in testosterone levels and the fact that normal ranges for serum testosterone are usually established using morning blood samples, testosterone measurement should 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 sex hormone-binding globulin (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 variou 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 improvement of athletic performance, with aesthetic goals, or for attempted anti-aging therapy. Androgen replacement for these purposes has not been proved to be effective and is not exempt from side effects.

There are currently several available preparations of testosterone, with various routes of administration, pharmacokinetics, and half-lives such as transdermal patches, gel, and the classic intramuscular injections (short-acting and long-acting formulations). Characteristics of each of them are detailed in Chaps. 10, 11 and 12.

A summary of the available preparations, their advantages and disadvantages, and specific adverse effects is detailed in Tables 13.1 and 13.2.

Table 13.1 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 supraphysiological 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 supraphysiological levels of testosterone within the first days after injection
Injectable testosterone undecanoate	1000 mg/12 week	4 injections/year	High cost
		Does not provide supraphysiological 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 13.2 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 rarely been reported 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 Various Conditions

Constitutional Delay of Growth and Puberty

CDGP is the single most common cause of delayed puberty in both sexes, being responsible for approximately 65 % of all 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, feelings 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, with 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 fractioning of the dose. The data suggest that short courses of low-dose testosterone (3- to 6-month course of 50–100 mg intramuscular [IM] 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 [14, 16–18]. Even at the initial doses used for pubertal induction, there is a decrease in total fat mass, percent body fat, and whole body proteolysis once IM injections of testosterone are initiated, however, it 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 the course of 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 is begun 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 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 pathophysiologies, although they may coexist 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 occurrences of intercourse and orgasmic and ejaculatory function [7].

Older Men with Low Serum Testosterone Concentration

Testosterone concentrations decline an average of 1–2 % per year with age [25–27]. A significant fraction of 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, a 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 because there is a lack of controlled studies. A recent clinical trial found that in men who were 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 has also shown to consistently increase muscle mass and to decrease fat mass. 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 a slightly better mood and lower severity of depressive symptoms [28]. Elderly hypogonadal men have an increased 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].

Hypogonadism Associated With 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. The recommendation for testosterone replacement in those patients follows the same criteria as for classic 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 using various testosterone formulations [33–35], whereas others did not confirm such 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 IM testosterone treatment in men with type 2 diabetes and hypogonadism 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 hypogonadism is discussed in Chap. 7.

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, prostate-specific antingen (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 in 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 guidelines 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]. Additionally, male chronic opioid users have a higher prevalence of

cardiometabolic abnormalities, therefore, 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 Transgender Persons

Testosterone treatment is essential for the induction and maintenance of virilization of female-to-male (FTM) transsexuals. Testosterone therapy is recommended after the diagnosis is confirmed in patients who are 16 years or older [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 IM short-acting testosterone enanthate in a dose of 200 mg biweekly was effective and safe for FTM transsexual 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 in this hormonal transition are usually associated with an improvement in psychological well-being and, in general, life satisfaction [43]. Patients are typically pleased with the virilization achieved. The IM 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) with dihidrotestosterone 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 however 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 low density lipoprotein (LDL), decreased high density liprotein (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 substantial concern for women who are affected. Evidence supports the short-term efficacy and safety of high physiological doses of

testosterone treatment of postmenopausal women with sexual dysfunction due to hypoactive sexual desire disorder. However, to date 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 the subject is shown in Chap. 18.

Benefits of Testosterone on Specific Organs and Systems

Sexual Function

It it well known that testosterone treatment of hypogonadal men improves general sexual function, increases libido, sexual thoughts, response to erotic stimuli, and erectile function [8]. Testosterone therapy has been more consistently associated with improvement of libido than of erectile function [8]. In men with late-onset hypogonadism who are 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]. The topic is discussed in detail in Chap. 8.

Quality of Life, Mood, and Cognition

The exact role of testosterone therapy on quality of life and cognition remains unclear. Nevertheless, most studies have reported improvement in mood, energy, well being, physical function, and quality of life. The quality of life of older men with hypogonadism who use testosterone compared with those who do not, 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, particularly in older men with low testosterone and cognitive impairment [8, 49]. However, testosterone administration leading to supraphysiologic serum levels negatively affected cognitive function [50].

Bone, Body Composition, Muscle Strength, and Physical Function

Testosterone replacement in hypogonadal men has a positive effect on bone mass and has shown 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 in 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 undergo further urologic evaluation before initiating testosterone administration. Other conditions that can be worsened by testosterone therapy include untreated severe obstructive sleep apnea, severe lower urinary tract symptoms, uncontrolled or poorly controlled congestive heart failure, men at high risk for acute myocardial infarction, cerebrovascular accident, or acute coronary syndrome over 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 a hematocrit level above 50 % should undergo further clinical evaluation before considering testosterone therapy [7].

Table 13.3 summarizes the main contraindications to testosterone therapy.

Table 13.3 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 urologic assessment is negative
Severe lower urinary tract symptoms associated with benign prostatic hypertrophy as indicated by american urological association (AUA)/international prostate symptom score (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 physiologic doses. Common drug-related adverse effects 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 at onset 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 13.3. 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 mood and sex drive. Additionally, IM 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 IM testosterone preparations a compared with 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 greater than 54 % during testosterone therapy, treatment should be discontinued and disorders that cause hypoxia should be investigated. Once hematocrit normalizes, resuming treatment with lower doses or a change from IM to transdermic testosterone administration preparations can be considered.

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 growth of preexisting 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]. The topic is discussed in detail in Chap. 15.

Cardiovascular

Testosterone therapy and cardiovascular risk remains a controversial issue. The studies investigating tong-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 that 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 the topic can be found in Chap. 16.

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 supraphysiologic doses of IM 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

Table 13.4 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

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 begin 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 gonadotropin 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 into consideration before testosterone replacement therapy is begun 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 of both Sertoli and Leydig cells. In normal men, recovery of testicular function typically occurs in 6–18 months. Azoospermia can be persistent in some patients, with significant negative consequences for fertility in the future [61, 62].

Table 13.4 shows potential adverse effects of testosterone replacement.

Conclusions

Since 2000, the number of men started on testosterone therapy has increased considerably in the USA [63]. Middle-aged and elderly men have been increasingly submitted to routine serum testosterone testing in clinical practice over the past several years. Recent studies suggest that many patients who begin testosterone replacement may not have a clear medical indication. The majority of these patients have been submitted to only one testosterone test before beginning 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 in 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 determined 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 on an individual basis before starting 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

14

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Introduction

There is much evidence supporting 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 which included 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, even more recently we confirmed that the simultaneous presence of reduced morning erections and desire is the cluster of symptoms that, along with

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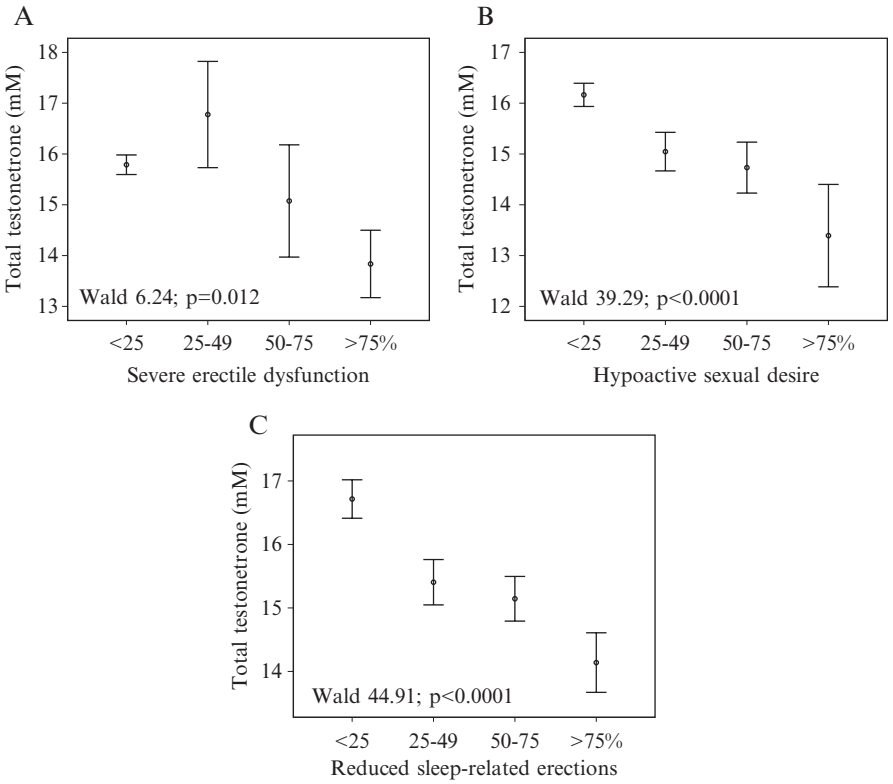

Fig. 14.1 Age-adjusted relationship between total testosterone and severe erectile dysfunction (**a**), hypoactive sexual desire (**b**), and sleep-related erections (**c**). Data were derived from a consecutive series of 4793 subjects (mean age = 51.1 ± 13.3 years) seeking medical care at our unit for sexual dysfunction

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. 14.1 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 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 the 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, bulbocavernosus 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 directly or indirectly controls 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. 14.2). 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 ref. [2]; Fig. 14.2, panel B). Furthermore, T also negatively regulates 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. 14.2; panel A).

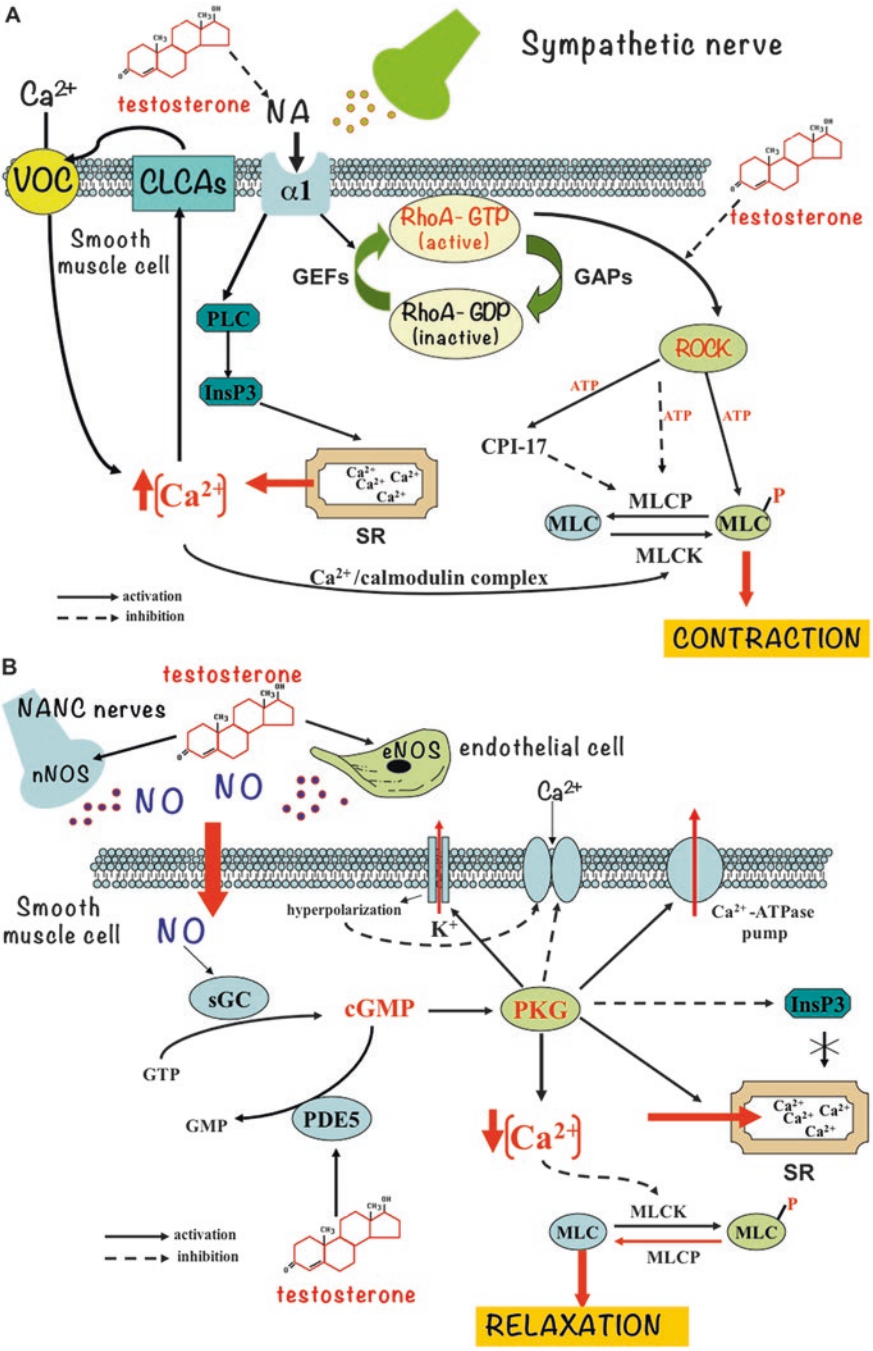

Fig. 14.2 Schematic representation of the biochemical events leading to penile flaccidity (*upper panel*) or erection (*lower panel*) along with the proposed events regulated by **testosterone**. Panel **a**. Noradrenaline (NA) binding to <1 receptors generates inositol 1,4,5-trisphosphate (InsP3), which, 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,

Finally, T positively controls the expression and the activation of phosphodiesterase type V (PDE5; Fig. 14.2, panel B; [22–25]; see for review ref. [2]).

Another recognized mechanism of androgen action is the regulation of α1-adrenergic responsiveness of smooth muscle cells (Fig. 14.2, Panel A; see for review ref. [2]). Consistent findings point toward T as having an effect 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 denervation following prostate surgery in men [2].

Hypogonadism and Male Sexual Dysfunction

Sexual Desire

There is evidence documenting 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 replacement therapy (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 14.1). 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, 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.

Fig. 14.2 (continued) 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 through a series of kinase activation increases the sensitivity of MLC to Ca^{2+}. **Testosterone** is intended 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

Table 14.1 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;0.1.12]**
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]**

[a]Including coital and non-coital erections
[b]Only coital erections considered
*$p<0.001$, **$p<0.0001$
Adapted from ref. [3]

It is important to recognize that although T plays 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].

Erectile Function

Because 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, resulting from impaired NO production, is most probably counterbalanced by reduced PDE5 activity and cGMP hydrolysis. Accordingly, it has been reported 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 those trials had only a few men enrolled, 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-to-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 Total testosterone 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 testosterone supplementation 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 enrollment were analyzed (see also Table 14.1). 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 cases of ED hypogonadal men, the association between TTh and PDE5i is thus mandatory [2, 3]. Additionally, because 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 prescribing 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 to the normal range (about 12.0 nmol/L). Accordingly, it has previously been 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 advised in order to better clarify the role of TTh as an add-on to PDE5i in the treatment of ED.

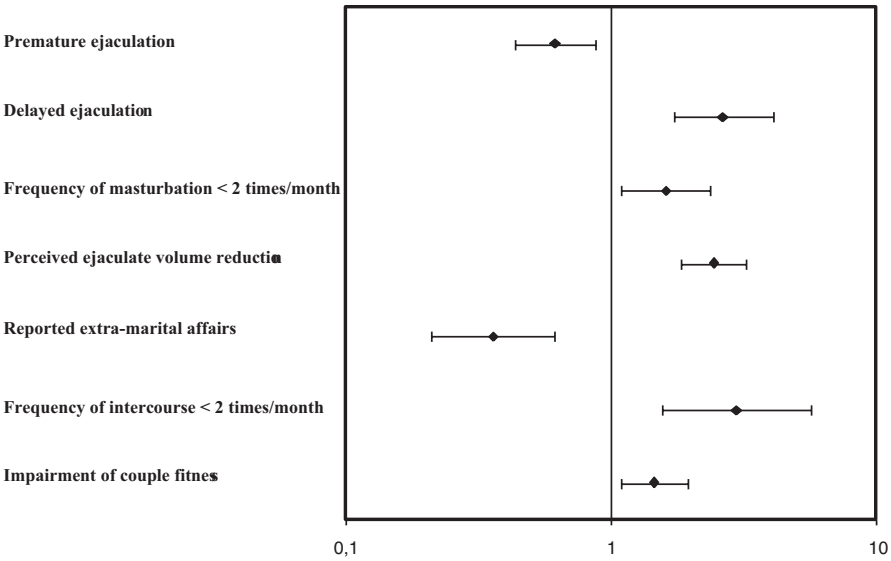

Fig. 14.3 Ageadjusted risk of hypogonadism according to European Male Aging criteria (see ref. [8]) of several sexual parameters. Impairment of couple fitness was evaluated using SIEDY Scale 2 score (see refs. [51, 52]). Data were derived from a consecutive series of 4793 subjects (mean age = 51.1 ± 13.3 years) seeking medical care at our unit for sexual dysfunction

Orgasm

In 2006, we originally reported that hypogonadism represented a risk factor for delayed ejaculation [53]. In a further study we documented that different T levels could be linked to various subsets of ejaculatory disturbances as they are higher in subjects with Premature Ejaculation (PE) and lower in those with Delayed Ejaculation (DE) ([54]; see also Fig. 14.3). Similar results were confirmed by other authors [55]. T might control ejaculatory reflex acting both at the central and peripheral levels [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 amygdale, and the posterior thalamus as well as SNB [19]. Additionally, 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 also documented a positive effect of T in ameliorating orgasmic function ([3]; see also Table 14.1). Furthermore, 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 14.3 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. 14.3 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. 14.3). 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. 14.3). 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

There is evidence to suggest 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, Jannini and colleagues robustly substantiated the hypothesis of an 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 increase in T was independent from the kind of therapies used, 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]. Accordingly, we confirm that reduced frequency of sexual intercourse is associated with a higher risk of hypogonadism (Fig. 14.3). In addition, we found that the impairment of sexual activity due to relational complaints (as assessed by a higher score in Scale 2 of Structured interview on erectile dysfunction structured interviewed) was associated with overt hypogonadism [67]. Similar results were confirmed in a larger sample of patients with sexual dysfunction (Fig. 14.3).

Conclusions

Testosterone plays a major role in regulating male sexual function. TTh is capable of improving all aspects of male sexual function and should be considered the first line treatment in ED patients with overt hypogonadism. However, TTh as a monotherapy might not be adequate in all cases because of the multifactorial nature of the pathophysiology of ED. In those 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

15

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Abbreviations

DHT	Dihydrotestosterone
EBRT	External beam radiation therapy
LH	Luteinizing hormone
LHRH	Luteinizing hormone-releasing hormone
PCa	Prostate cancer
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 sub-normal testosterone (T) 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 becomes more prevalent as men age [1]. It is estimated that TD affects 10 % of men older than 40 years, increasing to 50 % after 70 years (defined by using a total T less than 325 ng/dL). Although there is no consensus as to what threshold should be used to establish normal total T 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 T 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 T 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 T are necessary for the development of the prostate. 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 T therapy may stimulate PCa. 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 luteinizing hormone-releasing hormone (LHRH) agonists. Furthermore, most PCa 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 T administration in supraphysiologic doses to healthy men did not result in significant increase in prostate specific antigen (PSA) nor to prostate volume [6]. PSA also does not seem be influenced by the circadian rhythm of T levels [7].

A variety of studies on PCa, including experiments in animals, cell lines, and humans indicate that prostate tissue (benign or malignant) responds quickly and vigorously to the addition of T when it is in an androgen-deprived state. However, at higher concentrations, prostate tissue becomes unresponsive to this hormone [8].

The Testosterone-Prostate Dependence Myth

The concept that PCa is androgen-dependent was established by the work of Charles Huggins in 1941, which demonstrated dramatic biochemical responses to castration in men with metastatic PCa, 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 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 PCa was androgen-dependent, and that T administration was risky in men with PCa [8, 9]. Indeed, the transient initial increase in T 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 resulting from cancer progression [5, 8].

The idea that T 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 T levels and the risk of PCa, in addition to the rate of growth of existing PCa. The observation that malignant prostate tumors become increasingly prevalent as men age and experience a decline in serum T levels was largely ignored as an inconvenient fact [1–9].

The androgen hypothesis only began to undergo scrutiny in the 1990s, with increasing awareness of the benefits of T therapy, and increased numbers of men receiving treatment. Since then, a large number of publications have shown physical and sexual improvements in hypogonadal men using T therapy. Surprisingly, these studies have failed to show increased PCa rates in subjects treated with T compared with control groups or the general population. Those observations led to a reexamination of the relationship between T and the prostate, particularly PCa. Over the last 20 years we have witnessed a new era in the field of prostate physiology, carcinogenesis, and its relationship with T [5–8].

The Saturation Model

Endogenous serum T appears to have limited effects on the prostate cells. Several studies suggest that T stimulates the growth of PCa only at very low levels, and variations in the endogenous T concentration within the physiologic range or above do not appear to influence prostate physiology. In other words, there appears to be a limited ability for androgens to stimulate prostate growth, or PCa growth, with a maximum achieved at fairly low serum T concentrations. The 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 [8]. The initial PSA elevation seen in hypogonadal men on T therapy is thus a physiologic response.

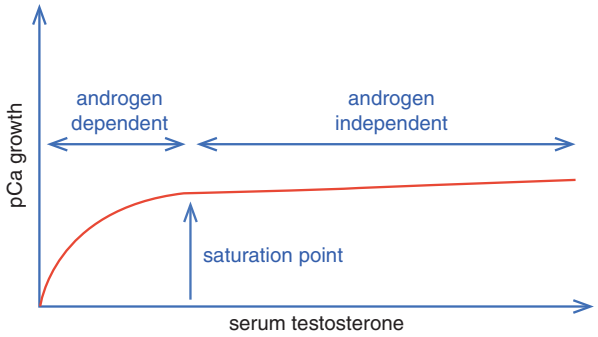

Fig. 15.1 The saturation model. Beyond some very low levels of serum total testosterone, all the androgen receptors are ligated, therefore there is no further ability for testosterone to stimulate prostate growth and PSA elevation

Monath et al. [7] investigated the correlation between serum levels of T 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 T was 458 ng/dL. There was no correlation between serum T and serum PSA concentrations ($r=0.054$, $p=0.515$). Similarly, The Massachusetts Male Aging Study [10] included 1576 men and found no correlation between PSA levels and serum T. Even male exposure to supraphysiological concentrations of T for periods of 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 T at physiologic levels or above do not seem to influence the prostate, reinforcing the finite capacity of androgen receptors to bind to T. However, at serum T concentrations below the saturation point, there is a relative shortage of T or DHT, and in those cases the concentration of androgens becomes a limiting factor to prostatic tissue proliferation, whether malignant or benign. The administration of T to these men will cause a rise in PSA, whereas administration of T to men with baseline T levels above this point will result in minimal or no effect on the prostate (Fig. 15.1) [2, 8].

The saturation model explains the dramatic effect on PSA observed after reduction of T to castrate levels as well as the lack of PSA response to treatment with supraphysiologic 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 [11]. A similar result was obtained in a prospective, placebo-controlled 6-month study using 1.62% T gel [12]. Additionally, in a study of 3156 men presenting to an Italian andrology center, PSA values showed a saturation curve identical to the one shown theoretically in Fig. 15.1, with a saturation point of approximately 250 ng/dl [13].

Other mechanisms that contribute to the saturation effect of androgens on prostate tissue may exist. Marks and colleagues [14] studied the effects of T therapy on prostate tissue of 44 men with androgen deficiency (68–70 years, assessed by

questionnaire and serum total T <300 ng/dL). The individuals were randomized to receive T enanthate (150 mg intramuscularly every 2 weeks) or placebo. Interestingly, it was observed that intra-prostatic T and DHT hao hormones. The prostatic hormonal milieu seems therefore to differ significantly from the serum. In addition, there was no amplification of androgen-sensitive gene expression. The results suggest that 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 with higher rates of PCa with aging, but only 20 % express the disease clinically which demonstrates that T by itself presents 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 years, when undergoing prostate biopsy prior to entering the T replacement therapy program, presented subclinical PCa diagnoses in a surprising 14 %, and the rates increased to 29 %. If only those 60 years and older would have been considered. However, the most interesting observation was that hypogonadal men receiving T therapy had a diagnosis of PCa in only about 1 % when follow during the therapy, and not less interesting is the fact that all other men who potentially had hidden PCa did not develop clinically detectable disease based on the increase of serum levels of T [11–15].

Endogenous Testosterone and the Risk of Prostate Cancer

For years a number of studies had been conducted by various authors in many parts of the world trying to establish a causal relationship between serum T levels and risk of PCa [15].

A study of pooled data from 18 prospective case controlled studies assessed the relationship between serum hormone levels and the risk of PCa. The study included 3886 men with PCa and 6438 men in control groups. The results of the study showed no relationship between androgens, including total T 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 [16].

A meta-analysis of 25 retrospective studies compared serum T levels in men with and without PCa. In four of the studies men with PCa effectively exhibited higher serum levels of T. However, in 15 of them, T levels were similar and in six of them, an inverse relationship was observed [17].

Testosterone Levels and Prognosis of Prostate Cancer

Contrary to long-term 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 [18–22]. 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 T [23].

Unfavorable prognosis of PCa was also observed in a multicenter study published in 2003 by Massengill [24] including men undergoing radical prostatectomy. In that study, men with PCa at advanced stages showed lower mean serum levels of T when compared with men with initial tumors. In addition, multivariate analysis showed subnormal levels of serum T as independent predictors of disease at a more advanced stage (pathologic stage T3 and T4). Positive surgical margins [25] and biochemical recurrence [26] after radical prostatectomy have also been more frequently observed in men with subnormal levels of T.

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 information. Further study is needed to definitively establish the biological relationship between serum T and PCa. However, it is clear that new perspectives are needed. In 1999, Prehn [27] suggested that prostate cells are prone to acquiring characteristics that make them less androgen-sensitive in a low T environment, and that tumors that develop under such hormonal conditions would be less differentiated and more susceptible to oncogenesis.

These data could possibly explain the findings of significant clinical trials such as the Prostate Cancer Prevention Trial, which evaluated the effect of finasteride, inhibitor of the enzyme 5-alpha-reductase, in the prevention of PCa. Although its use was reduced by 25 %, the overall rate of PCa tumors diagnosed in the group treated with this drug had more unfavorable histologic features [28].

Prostate Cancer in Men in Testosterone Therapy Programs

The incidence of PCa in men receiving T therapy has been demonstrated to be similar to that expected in the general population. Mean PSA usually rises into the normal range in the first months of T 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 [20].

In 2004, Rhoden and Morgentaler [15] published data showing that the PCa detection rate in patients undergoing clinical trials of T therapy was no higher than in individuals in a placebo group. Specifically, only 5 cases (1.1 %) of PCa were observed among 461 men treated with hormone replacement T when followed for 6–36 months. The meta-analysis cited above also found no greater increased risk of PCa in men receiving T compared with men who received placebo [17].

A systematic [29] review with strict inclusion criteria selected 11 studies involving men in T therapy placebo-controlled recently showed that only seven of 543 men (1.3 %) in the treatment group developed PCa, while in the control group this event occurred in five of 333 men (1.5 %). These rates are low, considering that the individuals were followed continuously and the study was more detailed than usual in men in screening or routine evaluation programs for early diagnosis of PCa.

Baillargeon et al., using SEER (Surveillance, Epidemiology, and End Results)—Medicare dataset, recently identified 52,579 men diagnosed with incidental PCa who had a minimum of 5 year continuous enrollment in such program before the diagnosis of PCa [30]. During that time period 574 men received T therapy. One was a statistical analysis using logistic regression for demographic and clinical characteristics, previous explosion of T therapy was not associated with increased risk of high grade PCa (odds ration [OR]: 0.84, 95 % confidence interval [CI]: 0.67–1.05). Additionally, high-risk disease did not increase according to the total number of T injections (OR; 1.00, 95 % CI: 0.98–1.01).

Testosterone Therapy After Radical Prostatectomy

There are an increasing number of studies demonstrating that hypogonadal men previously treated for PCa and no signs of recurrence may be candidates for T therapy.

The first study in this category was published in 2004 by Kaufman and Graydon [31] and included seven patients treated for localized prostate neoplasia by radical prostatectomy (RP). Although all the 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 the study was small, but the contribution to the development of understanding the relationship between T and PCa was extremely relevant.

Perhaps the most important contribution to the literature in this category is the article published by Shabsigh et al. [29], where the authors assessed biochemical recurrence in hypogonadal men previously treated for PCa. 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 two cases (1.8 %), a rate below those that are frequently observed in most of the series involving RP results. However, it should be taken into consideration that this selected population is certainly different from most series which assess biochemical recurrence in men undergoing treatment with curative intent for PCa.

Similarly, a study conducted by Agarwal and Oefelein [32] included ten men with TD who had previously undergone retropubic radical prostatectomy and without biochemical recurrence signals. Most patients that were included had PCa with favorable prognosis, although a man with Gleason score 8 was also 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 T levels having a 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 a retrospective study on T therapy after RP. Fifty-seven patients with undetectable PSA and negative surgical margins received T 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 T therapy proved to be safe in patients treated for PCa [33].

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 T who were compared with 49 eugonadal controls. A significant increase in PSA was observed in only individuals from the treatment group, but biochemical recurrence was defined in four patients in the treatment group and eight cases in the control group [34].

An interesting evaluation was performed by Kaplan and Hu [35] 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 T 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 nonsurgical methods with curative intent (radiotherapy and brachytherapy) seems even more complex and its prescription may appear more risky than after RP. Because the gland is not removed, viable prostate tissue might remain biologically responsive to T. 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 begin T therapy.

The first report of T administration after brachytherapy had been published by Sarosdy [36] and comprised a series of 31 men with serum T levels ranging from 30 to 255 ng/dL and symptoms consistent with TD who had received exogenous T for 0.5–4.5 years after brachytherapy. The study aimed to assess the risk of biochemical recurrence of PCa. The median serum PSA level was 5.3 ng/mL prior to 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 T can be safely administrated in men with TD who underwent brachytherapy as a treatment of located PCa, but close monitoring should be provided.

Yet another study was more recently published and included 20 men treated with brachytherapy for PCa who received T therapy for symptomatic T deficiency, showing significant clinical improvement and no cases of rising PSA and cancer recurrence [37].

Morales and colleagues [38] described five men with signs and symptoms of TD after treatment for localized PCa with EBRT who received T after PSA nadir (the lowest PSA level after treatment) had been achieved. All men had histologic 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 T 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 a level greater than 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 T deficiency who received T 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 [39].

A recent multi-institutional study that included 98 men with TD treated for PCa with radiotherapy showed a significant increase in serum T 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) had no influence on endpoints [34].

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; furthermore, 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 with intervention and control groups [40].

Studies involving PCa and T have obvious limitations which certainly limit 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.

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 pathology. This practice is called active surveillance, and the males generally undergo regular PSA testing and follow-up prostate biopsies at regular intervals. Some of the men have symptomatic T deficiency and desire treatment. The use of T therapy in these men is highly controversial.

A study by Morgentaler [41] reported on a 2-year history of T 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 a 2-year period, his PSA declined into the 6 range. He was never biopsied again because of 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 age 90 years. Subsequently, Morgentaler et al. [42]

reported on 13 patients with symptomatic T deficiency who received T 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 those men. All patients underwent at least one additional prostate biopsy, with a mean of two sets of biopsies per individual. No definite progression of cancer was noted in any patient. Cancer was not found in 54 % of follow-up biopsies in those 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 those two groups [43].

Testosterone Therapy in High-Risk Prostate Cancer

In 2013, a retrospective review involving 103 hypogonadal men treated with T after radical prostatectomy between 2007 and 2011 was published. Twenty-six of them presented with 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 in only this high-risk group (0.004–0.14 ng/ml, $p=0.017$), but no patient met the criteria for biochemical recurrence [44].

The authors concluded that T therapy is a viable treatment for patients with PCa, even when the disease has high-risk characteristics, because the incidence of recurrence in the groups proved to be smaller than expected.

Conclusions

T 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. However, the actual risk of developing PCa in men receiving T is unknown. The current scientific evidence comes from clinical studies that included a few hundred men, and the studies were not designed to establish the risk of developing PCa. It is estimated that approximately 6000 randomized males be required to receive T or placebo for 5 years to assess whether T increases the incidence of PCa by 30 %. None of the cited randomized placebo-controlled trials postulated prostate biopsies at the start and end of the study. Thus, the prevalence and incidence of occult prostate carcinoma have not been studied accordingly.

Although the information is coming from small clinical studies, data demonstrate the safety of T therapy after treated PCa, even in men with high-risk PCa, leading to an increase in its prescription, which in turn results in production of even more scientific evidence supporting T replacement as being safe for these patients.

Table 15.1 Studies reporting testosterone replacement therapy 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 [31]	RP	7	24	97–434	<0.1–<0.1	0
Agarwal, 2005 [32]	RP	10	19	197–591	<0.1–<0.1	0
Sarosdy, 2007 [36]	BT	31	54	188–498	5.3–<1.0	0
Khera, 2009 [33]	RP	57	36	255–459	<0.1–<0.1	0
Morales, 2009 [38]	EBRT	5	14.6	150–418	0.3–0.47	0
Morgentaler, 2011 [42]	AS	13	30	238–664	5.5–3.6	0
Pastuszak, 2013 [44]	BT, EBRT	13	45.6	178–368	0.3–0.66	0
Pastuszak, 2013 [44]	RP	103	27.5	261–460.5	0.004–0.007	4
Balbontin, 2014 [37]	BT	20	31	343–587	0.7–0.1	0
Pastuszak, 2015 [34]	BT, EBRT	98	40.8	209–420	0.075–0.09	6

BT brachytherapy, *EBRT* external beam radiation therapy, *RP* radical prostatectomy, *AS* active surveillance

The emergence of publications about active surveillance in PCa brings important contributions to the biological behavior of this neoplasm toward T therapy, because the follow-up protocols include prostate biopsies at regular intervals, in most cases annually. However, individuals who meet the 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 (Table 15.1).

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Testosterone and Cardiovascular Effects

16

Bu B. Yeap

Abbreviations

ARIC	Atherosclerosis Risk in Communities Study
BMI	Body mass index
BHS	Busselton Health Survey
CVD	Cardiovascular disease
CHS	Cardiovascular Health Study
CI	Confidence interval
CHD	Coronary heart disease
DHT	Dihydrotestosterone
E2	Estradiol
EMAS	European Male Aging Study
GC	Gas chromatography
HR	Hazard ratio
HIMS	Health In Men Study
IHD	Ischemic heart disease
LTL	Leucocyte telomere length
LC	Liquid chromatography
LH	Luteinizing hormone
MS	Mass spectrometry
MACE	Major Adverse Cardiovascular Events
MI	Myocardial infarction
MrOS	Osteoporotic fractures in men
Q	Quartile

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RCT	Randomized controlled trial
SHBG	Sex hormone-binding globulin
T	Testosterone

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 aging is a decline in circulating testosterone (T) [3, 4]. The presence of obesity or accumulation of morbidities can be reflected in lower T concentrations [5, 6]. However, even healthy older men have lower circulating T compared with reproductively normal younger men [7, 8]. In 124 healthy, reproductively normal men 21–35 years old, the reference interval for T assayed using mass spectrometry was 10.4–30.1 nmol/L [7]. By contrast, in 394 men 70–89 years old who reported excellent or very good health with no history of smoking, diabetes, CVD, cancer, depression, or dementia, the reference interval for plasma T assayed using mass spectrometry was 6.4–25.6 nmol/L [8]. 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 [9].

T is produced by the testis under the stimulation of pituitary luteinizing hormone (LH) and circulates largely bound to sex hormone-binding globulin (SHBG) and albumin, with a small fraction unbound or free [10]. T undergoes conversion with 5α-reductase to dihydrotestosterone (DHT), a more potent ligand for the androgen receptor, and with aromatase to estradiol (E2), a ligand for estrogen receptors [11]. Therefore, biological actions of T flow on from the function of the hypothalamo-pituitary-testicular axis to the circulation of T and its conversion to bioactive metabolites DHT and E2, to tissue effects that regulate male sexual development, virilization, and body composition in adult men [9]. Of note, circulating concentrations of DHT in men parallel those of T declining with increasing age, while E2 concentrations tend to be more stable [8, 12, 13].

Sex Hormone Assays and Calculation of Free T

Circadian variation affects T concentrations, which are higher in the morning and lower in the evening [14, 15]. The diurnal variation in DHT and E2 is lower compared with T in both middle aged and older men [14]. Therefore, early morning sampling is optimal, and when possible, in the fasting state [5, 16]. Automated immunoassays of T tend to exhibit non-specificity and method-dependent bias [7, 17]. Therefore, mass spectrometry is the preferred assay methodology for accurate measurement of sex

steroids [18]. SHBG increases with age and is lower in the setting of insulin resistance and obesity, thus there are scenarios where consideration of unbound or free T may be informative on an individual level [19]. However, measurement of circulating free T by equilibrium dialysis is labor intensive and not routinely performed, instead, free T is commonly calculated using mass action or empirical equations [20, 21]. Depending on the method of calculation, calculated free T can vary from measured free T representing a potential limitation for its use [20].

Epidemiologic 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 summarized in Table 16.1, part A. Three studies in predominantly middle-aged men did not find any association of T with incidence of CVD events [22, 23, 25]. Of studies measuring E2, one study found that higher E2 was associated with a lower incidence of CVD events [23], but another study in older men associated higher E2 with increased risk of stroke [24]. 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 ischemic attacks [26], while higher LH was associated with incidence of ischemic heart disease (IHD) events [27]. A smaller study of older men found no association of baseline T or E2 with incident CVD events [28], but another study (also in older men) reported T in the lowest and highest quintiles to be associated with CVD events [29] suggesting a U-shaped association.

Recent large cohort studies where sex steroids were measured using mass spectrometry have been informative (Table 16.1, 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). In the Cardiovascular Study (CHS), involved T was not associated with cardiovascular death, or non-fatal MI or stroke, but DHT was with higher risk for concentrations <1.7 or >2.6 nmol/L [32]. DHT (<1.7 or >2.6 nmol/L) was associated with ischemic stroke when that outcome was analyzed separately [33]. In an updated analysis from the Western Australian Health In Men Study (HIMS), T, DHT, and E2 were not associated with incident MI [34]. By contrast, higher T or DHT was associated with lower incidence of stroke. For men with T in Q4 (≥15.8 nmol/L) compared with Q1 (≤9.8 nmol/L), the risk of 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) [34]. 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 [35]. Of note, the initial and extended studies identifying low T as an independent predictor for higher incidence of stroke in older men [26, 34] have recently been confirmed by the Copenhagen Study [30] (Table 16.1, part A).

Table 16.1 Cohort studies examining associations between sex hormones with cardiovascular events in middle-aged and older men

Study author and year	Size (*n* of men)	Follow-up (year)	Age (year)	Results
A				
Smith GD, 2005 [22]	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 but not multivariable adjusted analyses.
Arnlov J, 2006 [23]	2084	10	56	386 had a first cardiovascular event. Higher total E2 at baseline associated with lower incidence of CVD events. T not associated.
Abbott RD, 2007 [24]	2197	≤7	71–93	124 had a first stroke. Baseline E2 in top quintile (≥125 pmol/L) associated with higher risk, total T not associated.
Vikan T, 2009 [25]	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 BB, 2009 [26]	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 Z, 2011 [27]	3637	5.1	70–88	618 men experienced an IHD event. Higher LH associated with incident IHD.
Haring R, 2013 [28]	254	5, 10	75.5	No associations of baseline total T or total E2 with incident CVD events.
Soisson V, 2013 [29]	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 HN, 2016 [30]	4602	20	57	560 stroke events. Total T in lowest decile (0–10th percentile) associated with stroke.
B				
Ohlsson C, 2011 [31]	2416	5	69–81	485 CVD events. Men with a total T[a] in the highest quartile (≥19 mol/L) had a lower risk of CVD event. E2 was not associated.
Shores MM, 2014 [32]	1032	9	76	436 men had a cardiovascular event. Total T[b] was not associated with cardiovascular events, DHT <1.7 or >2.6 nmol/L associated.
Shores MM, 2014 [33]	1032	10	76	114 men had ischaemic stroke. Total T[b] was not associated with stroke, DHT <1.7 or >2.6 nmol/L associated.
Yeap BB, 2014 [34]	3690	6.6	70–89	Incident MI occurred in 344 men, stroke in 300. T[c], DHT, and E2 were not associated with MI. Higher total T (>12.6 nmol/L) or DHT (>1.34 nmol/L) associated with lower incidence of stroke.
Srinath R, 2015 [35]	1558	12.8	63.1	287 men had a CHD event. T[d] was not associated with incidence of CHD events.

IHD = ischemic heart disease, CVD = cardiovascular disease, MI = myocardial infarction, TIA = transient ischemic attack, CHD = coronary heart disease. *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

[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

Several conclusions can be drawn from these studies. Cohort studies based on the use of immunoassays for sex steroids (Table 16.1, part A) provide limited evidence that T is associated with incidence of MI per se, but do provide evidence for an association of low total or free T with incidence of stroke and transient ischemic attack [26, 30]. Considering cohort studies where sex steroids were measured accurately using mass spectrometry-based methods (Table 16.1, part B), neither the CHS nor the ARIC studies found any association of lower T with coronary or cardiovascular events [32, 35]. 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 [31], and stroke in HIMS [34]. Therefore, lower circulating T is a biomarker for CVD risk, particularly 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 levels are associated with adverse cardiovascular risk factors (e.g. [35, 36]) 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 [31–34].

Epidemiologic 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 summarized in Table 16.2, part A. Cohort and case control studies have reported associations of lower T with higher mortality [25, 37, 38, 41, 43–45]. Several studies have reported contrasting or equivocal results, or implicated other anabolic hormones in addition to T [28, 39, 40]. The relationship of E2 to mortality risk in men cannot be clearly defined with contrasting findings reported [42, 43]. Overall, these studies implicate lower T as a biomarker for mortality risk, albeit the studies are heterogeneous and causality remains to be proved [49]. 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.

Cohort studies in which the relationship between baseline sex steroids assayed using mass spectrometry and the outcome of mortality were studied and are summarized in Table 16.2, 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 HR 0.59, 95 % CI = 0.42–0.83) [46]. No significant associations for were seen for T or E2 with cardiovascular mortality. In the mortality analysis from HIMS, comparing 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, 95 % CI 0.65–0.94), with no difference seen in mortality for men with total T in Q4 [47]. 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 with

Table 16.2 Cohort studies examining associations between sex hormones and mortality in middle-aged and older men

Study author and year	Size (*n* of men)	Follow-up (year)	Age (year)	Results
A				
Shores MM, 2006 [37]	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 K-T, 2007 [38]	825 and 1489	≤10	40–79	825 deaths, 1489 controls. Total T was inversely related to mortality from all causes, CVD and cancer.
Araujo AB, 2007 [39]	1686	15.3	40–70	395 deaths. Higher free T associated with higher IHD mortality. Equivocal association of lower DHT with IHD mortality.
Maggio M, 2007 [40]	410	6	≥65	126 deaths. Combination of bioavailable T, insulin-like growth factor-I, and dehydroepiandrosterone sulphate in lowest quartiles were associated with higher mortality.
Laughlin GA, 2008 [41]	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 T, 2009 [25]	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 P, 2009 [42]	782	10	≥50	Higher total E2 predicted increased mortality after the third year.
Menke A, 2010 [43]	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 R, 2010 [44]	1954	7.2	20–79	195 deaths. Total T <8.7 nmol/L was associated with increased all-cause and CVD mortality and cancer death.
Hyde Z, 2012 [45]	3637	5.1	70–88	605 deaths, 207 from CVD. Lower free T (100 pmol/L vs 280 pmol/L) predicted all-cause and CVD mortality.
Haring R, 2013 [28]	254	5, 10	75.5	Higher baseline total T associated with lower 5-year but not 10-year mortality risk. E2 was not associated.
B				
Tivesten A, 2009 [46]	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 BB, 2014a [47]	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.

(continued)

Table 16.2 (continued)

Study author and year	Size (*n* of men)	Follow-up (year)	Age (year)	Results
Pye SR, 2014 [48]	2599	4.3	40–79	147 deaths. Presence of sexual symptoms, total T[c] <8 nmol/L and free T <220 pmol/L were associated with mortality.
Shores MM, 2014b [32]	1032	9	76	777 deaths. Total T[d] was not associated with mortality, DHT <1.0 nmol/L was associated.
Srinath R, 2015 [35]	1558	12.8	63.1	347 deaths, 29 from CHD. Total T[e] not associated with all-cause or CHD mortality.

IHD = ischemic 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

[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

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) [47]. There was no significant association of quintiles of either total or free T with all-cause or CVD-related mortality in the European Male Aging Study (EMAS), instead men with sexual symptoms and T <8 nmol/L had an increased risk of death from any cause [48]. In an analysis from the CHS, neither total nor free T were associated with all-cause or CVD-related mortality, although a curvilinear association of DHT with all-cause mortality was noted [32].

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 [48]. ARIC had only 29 cases of deaths resulting from CHD [35]. Although smaller, the CHS study had longer follow-up and accumulated a large number of outcome events which paralleled the marked attrition of the cohort as a whole (777 deaths in 1032 men, 75 % cumulative mortality) [33]. 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) [46, 47], and in the case of HIMS, for the outcome of IHD mortality ($N = 325$) [47]. 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 [46, 47]. Importantly, the

findings from HIMS indicate that higher DHT is a biomarker for lower risk of death from IHD in older men [47]. Thus, higher circulating DHT may represent a survival or resilience factor following an IHD event.

The T Controversy: Results from Randomized Controlled Trials

These epidemiologic studies are consistent with a beneficial or protective effect of sex hormones particularly T and to an extent DHT on the risk of CVD in men. However, causality, or proof that intervention with T supplementation would protect against CVD events, can only be determined conclusively by means of randomied controlled trials (RCTs). Several small RCTs have shown effects of T supplementation to protect against myocardial ischemia. In 46 men with stable angina, 12 weeks of transdermal T reduced exercise-induced myocardial ischemia [50]. In 15 men with angina, intramuscular long-acting depot T over 12 months reduced time to ischemia on exercise testing [51]. In a study of 87 older men with diabetes, oral T undecanoate over 12 weeks reduced the frequency of angina [52]. Therefore, the publication of the Testosterone in Older Men with Mobility Limitations (TOM) trial sparked intense debate [53] (Table 16.3). 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 randomized 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 randomized, due an excess of cardiovascular adverse events in the T arm [53]. Men were 74 years old on average, and half had preexisting CVD. This contrasts with a comparable study in which 274 men ≥65 years old who were frail or intermediate-frail with total T ≤12 nmol/L or free T ≤250 pmol/L were randomized to 50 mg daily transdermal T or placebo for 6 months [54]. 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, [57]) were designed to examine cardiovascular events as pre-specified outcomes, utilizing reporting of adverse events.

A 3-year RCT of transdermal T in 308 men ≥60 years old 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 [55]. 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 recruited 790 men ≥65 years old, 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 [58]. 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 anemia. The primary result of the T Trials showed a modest benefit of T on sexual function [56]. However, even this RCT was not powered for CVD events as outcomes, with no difference in cardiovascular adverse events noted between the

Table 16.3 Pivotal recently reported randomized controlled trials (RCTs) of T supplementation in middle-aged and older men

Study author and year	Population (men)	Formulation of T	*N* active	*N* placebo	Duration	Result
Basaria S, 2010 [53]	≥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 because of excess cardiovascular events in T arm
Srinivas-Shankar U, 2010 [54]	≥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 S, 2015 [55]	≥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 P, 2016 [56]	≥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

two arms of the trial. The Western Australian Busselton Health Survey (BHS) is a longitudinal cohort study of men living Busselton region of Western Australia [59]. In the BHS 1994–1995 cohort, the observed 5-year risks were 11 % for CVD events, 3 % for stroke events, and 3 % for MI events in men 50–69 years old. Therefore, an RCT with the outcome of CVD events in this age group would require 1634 men in each of placebo and T groups to be treated for 5 years to detect a relative risk reduction of 30 % (Knuiman M, personal communication). 6477 men in each group would be needed for an outcome of MI or stroke. Thus there are considerable barriers to conducting a large scale RCT with CVD events as the primary outcome.

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 16.4). Meta-analyses completed prior to the publication of the Basaria and Srinivas-Shankar studies did not find any significant difference in the risk of cardiovascular adverse events in T compared with placebo recipients [60, 61]. 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 [62]. In conjunction 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 aging men and to mandate a label change warning of the possible risks [66]. 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 16.4). Those include a meta-analysis focusing on larger RCTs [63], a meta-analysis including 75 trials with 5464 men and mean duration of 34 weeks [64], and a meta-analysis including 35 RCTs with 3703 men lasting ≥12 weeks [65]. In those three meta-analyses using random effects models which were more suited to inclusion of heterogeneous studies [67, 68], there was no T association with cardiovascular adverse events (Table 16.4). Therefore, meta-analyses of T RCTs in general have not found T supplementation to be associated with excess cardiovascular adverse effects.

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. Those have major limitations, particularly their observational nature and the absence of randomization with the possibility of selection bias, as well as limited clinical data regarding the indications for T treatment. Furthermore, those studies have reported contrasting results (Table 16.5). Prescription of T has been associated with

Table 16.4 Meta-analyses of cardiovascular adverse events in randomized controlled trials (RCTs) of T supplementation in men

Study characteristics				Results	
Study author and year	*N* of RCTs	*N* active	*N* placebo	Adverse signal	No adverse signal
Haddad RM, 2007 [60]	30	808	834		No significant difference in odds ratio for any cardiovascular adverse event or for MI.
Fernandez-Balsells MM, 2010 [61]	51	2716			No significant difference for all-cause mortality, coronary bypass surgery or MI.
Xu L, 2013 [62]	27	2994		T associated with increased risk cardiovascular-related event (OR 1.54, 95 % CI = 1.09–2.18)[a].	
Ruige JB, 2013 [63]	10 (>100 participants)	1289	848		No significant difference in cardiovascular adverse events.
Corona G, 2014 [64]	75	3016	2448		No association of T supplementation with cardiovascular risk. For MACE OR = 1.01 (95 % CI 0.57–1.77).
Borst SE, 2014 [65]	35	3703			No significant risk for cardiovascular-related adverse events.

MI = myocardial infarction, MACE = major adverse cardiovascular events, OR = odds ratio, 95 % CI = confidence interval. Unless otherwise specified, meta-analyses were conducted using random effects models

[a]Fixed effects model

reduced mortality in male veterans with baseline T ≤8.7 nmol/L [69] and also reduced mortality in men with Type 2 diabetes with baseline T ≤10.4 nmol/L [70]. Those studies suggested a potential benefit of T supplementation, albeit care is needed with their interpretation [76]. 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 [71]. 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 [77]. A published erratum acknowledged the incorrect classification of a number of

Table 16.5 Retrospective studies of men prescribed T that examined associations of T prescriptions with cardiovascular events and mortality in middle-aged and older men

Study characteristics				Results	
Study author and year	Size (*n* of men)	Follow-up (year)	Age (year)	Favors no T	Favors T
Shores MM, 2012 [69]	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 V, 2013 [70]	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 R, 2013 [71]	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.	
Finkle WD, 2014 [72]	55,593	90 days	54.4	Men prescribed T. Higher rate of non-fatal MI in 90 days following prescription compared with preceding 1 year.	
Baillargeon J, 2014 [73]	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 R, 2015 [74]	83,010	6.2; 4.6; 4.7	66		Male veterans with low T. TRT resulting in normalization of circulating T (n=43,931) was associated with lower risk of death, MI and stroke, compared with TRT without normalization of T (n=25,701) or no TRT (n=13,378).
Anderson J, 2016 [75]	4736	≥3	61.2		Men with low T. T therapy achieving normal T (n=2241) was associated with reduced risk of MACE compared with persistent low T (n=801). T therapy achieving either normal T or high T (n=1694) associated with lower all-cause mortality compared with persistent low T.

MI = myocardial infarction, TRT = testosterone replacement therapy, MACE = major cardiovascular adverse event comprising death, non-fatal MI and non-fatal stroke

men and identified 100 women who needed to be excluded [78]. Another retrospective study reported an increased risk of MI within 90 days of T prescription compared with the preceding 12 months in men 65 years old and older, or men younger than 65 years who has a prior history of CVD, based on a relatively small number of incident events and a low absolute event rate [72]. A national sample of Medicare beneficiaries ≥66 years old found that T treatment was not associated with the risk of MI; in fact, in men with worse prognostic scores, T treatment was associated with lower risk of MI [73].

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 of 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 [74]. 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 with men who received no treatment, and also compared with men who received treatment but had subsequent low T despite this [74]. The 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 [75]. Compared with 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 [75]. Men (all receiving T supplementation) who achieved a "high" T also had a lower rate of MACE at 3 years but that result was not statistically significant. Both "normal" T and "high" T groups had a lower risk of all-cause mortality at 3 years compared with the "low" T group [75]. The authors commented on a non-significant trend towards higher risk of stroke in men achieving "high" T. While all of these studies have recognized limitations, particularly the absence of randomization and multiple potential sources of bias and confounding, the contrasting results illustrate the need for definitive prospective randomized controlled trials to clarify whether T supplementation in middle-aged or older men would reduce rather than increase the risk of CVD. The design of such RCTs should ensure that on-treatment T concentrations are normalized but not excessive.

Mendelian Randomization: Bioactive Metabolites of T and Biological Aging

Pending definitive RCTs of T therapy powered for the outcome of cardiovascular events, another method of conducting a randomized study utilizes the conversion of T into its bioactive metabolites DHT and E2 [11]. The involvement of 5α-reductase (SRD5A2), which amplifies the action of T by converting it to DHT and aromatase (CYP19A1), which diversifies its action by metabolizing it to E2 provide a valuable opportunity to explore causation using Mendelian randomization [79]. If a functional polymorphism in either the 5α-reductase (*SRD5A2*) or aromatase (*CYP19A1*)

gene results in lower or higher DHT or E2, the exposure which is effectively allocated in a randomized manner at birth is independent of subsequent lifestyle factors and unaffected by reverse causality. A Mendelian randomization can be conducted using an intermediate endpoint which captures a lifetime exposure to risk and is predictive of cardiovascular disease.

A recent study has utilized that approach [80] using the outcome of leucocyte telomere length (LTL), which is a measure of biological aging [81]. Telomeres are essential DNA-protein complexes at the physical ends of chromosomes comprising TTAGGG repeats, which protect the ends from fusion and degradation [81]. Conventional DNA replicative enzymes cannot fully replicate telomere ends, thus their length is progressively shortened with each cell cycle. The enzyme telomerase counters this process by elongating telomeres [82]. Shortening of telomeres results in cellular dysfunction [81], and shorter LTL has been shown to predict higher risk of age-associated morbidity and mortality [83, 84]. Of note, LTL correlates with telomere length in vascular and other tissues [85, 86], and shorter LTL predicts increased incidence of CVD events [87, 88].

Therefore, if 5α-reductase (*SRD5A2*) or aromatase (*CYP19A1*) gene polymorphisms are associated with differences in DHT or E2 and with shorter or longer LTL, it can be postulated that the genetically determined hormone exposure contributes causally to LTL, and thus that sex hormones regulate telomere length in vivo.

A recent study using both epidemiological and Mendelian randomization approaches has provided novel results [80]. In an analysis of 980 men from the Western Australian BHS there was a progressive decline in LTL (expressed as the ratio of *T*elomeric DNA to the *S*ingle-copy control gene DNA or T/S ratio) with increasing age, from 1.89 ± 0.41 at <30 years to 1.50 ± 0.49 at 70 to <80 years. The estimated linear regression was T/S ratio = 2.13–0.0081 age ($p < 0.0001$). Thus, for an increase of a decade in age, T/S ratio was lower by approximately 0.08. After adjustment for age, serum DHT and E2 were positively correlated with LTL (DHT $r = 0.069$, $p = 0.030$; E2 $r = 0.068$, $p = 0.034$) [80]. Finally, Mendelian randomization analyses identified three dominant alleles of *CYP19A1* which were each associated with both a reduction in circulating E2 (~10 pmol/L) and a difference in LTL corresponding to an increase of a decade of chronological age. Therefore, the results support the concept that higher exposure to the bioactive metabolites of T, particularly E2, may slow biological aging in men [80]. While confirmatory studies are needed, those results support further research into the question of whether T supplementation, by conversion to bioactive metabolites including E2, could slow biological aging in the vasculature and thereby protect against progression and clinical manifestations of CVD.

Conclusions

Lower circulating T and DHT are robust predictors for incidence of CVD, particularly stroke, and in the case of lower DHT for IHD mortality in older men. Multivariate analyses of longitudinal data are consistent with beneficial effects of T or DHT on

cardiovascular risk, but 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 did not. Mendelian randomization analysis implicates E2, a bioactive metabolite of T, as a favorable modulator of biological aging in men, illustrating a possible mechanism by which T supplementation could favorably influence the health of multiple body systems including the vasculature.

Men who have low T who are androgen deficient resulting from 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 [89]. 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 or DHT who do not have such pathology. This would require large-scale RCTs powered to detect effects of T on cardiovascular events, which to our knowledge, to date have not been performed. The Australian Testosterone for the Prevention of Type 2 Diabetes in Men at High Risk (T4DM) trial is a multi-center RCT currently in progress seeking to recruit 1488 men with impaired glucose tolerance or newly diagnosed Type 2 diabetes, with total T $\leq$14 nmol/L (ACTRN12612000287831). T4DM will examine the efficacy of T treatment in conjunction with a lifestyle program in comparison to a lifestyle program alone over a 24-month period to prevent progression to Type 2 diabetes in men with impaired glucose tolerance, or to normalize glucose tolerance in men with newly diagnosed Type 2 diabetes. T4DM will not be powered for the outcome of cardiovascular events. Thus, additional studies are needed to explore mechanistic pathways by which T exerts effects on the cardiovascular system, which will pave the way for large-scale RCTs to clarify whether or not T supplementation could preserve health in the increasing population of older men.

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Testosterone and Women

17

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and Ciciliana Maila Zilio Rech

Testosterone Production in Women

Following very low androgen levels in childhood, kisspeptin triggers pulsatile gonadotropin releasing hormone (GnRH) secretion in the medial basal hypothalamus and preoptic area [1] which in turn stimulates luteinizing hormone (LH) that reaches the ovary through the blood stream, generating testosterone (T) and estradiol (E2). Upon puberty and during the reproductive age, T production in women is shared equally between the theca cells of the ovary and the adrenal cortex, accounting for approximately 300 μg daily [2]. As in men, testosterone secretion maintains a circadian rhythm, with higher levels in the morning and a nadir around midnight [3].

One-third of the circulating testosterone results from direct ovarian and adrenal secretion; the remaining two-thirds results from peripheral conversion of precursors such as delta 4 androstenedione (A4) in non-steroid producing tissues (Fig. 17.1) [4].

LH receptors are the most important effectors of androgen production within ovarian theca cells. Existing insulin and lipoproteins (high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL)) theca receptors act in synergy with LH, amplifying testosterone biosynthesis. While hyperinsulinemia by itself was shown to result in hyperandrogenism in women, conjunct signaling of the LH receptor/cAMP/PKA and insulin receptor/protein tyrosine kinase (PTK) pathways can cause marked increases in androgen biosynthesis. Lipoproteins contribute to the process by increasing intracellular cholesterol, which in turn is transferred to P450C22 via StAR [5].

A small mid-cycle testosterone peak, coincident with the LH surge in ovulatory cycles was confirmed by more sensitive immunoassays [6]; apart from this

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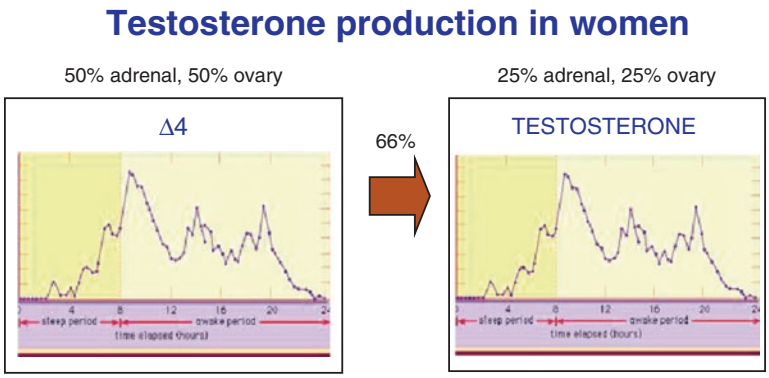

Fig. 17.1 Sources of testosterone production in women during the menacme

mid-cycle increase, testosterone levels in the mid-follicular, late follicular, and luteal phases do not vary substantially [7]. During the late reproductive years, with the increment of anovulatory cycles, the modest mid-cycle rise was observed not to occur, despite preservation of normal free T levels at other phases of the cycle [8].

Aging, but not the menopause transition, promotes a progressive reduction in testosterone serum levels. Percent ratios from production sources also change with age: in view of the decaying testosterone direct ovarian ad adrenal production, peripheral conversion from A4 and DHEA assumes more importance [9].

On the contrary, different authors have described that the menopause transition is accompanied by relative androgen excess. Yasui et al. [4] divided 231 healthy women into seven reproductive stages, from those with regular menstrual cycles and normal follicle-stimulating hormone (FSH) levels to those that were more than 5 years post-menopause. Total testosterone and its fractions did not change significantly. Estradiol levels drastically decreased and the ratio of testosterone to estradiol (T/E) gradually increased during the menopausal transition and through the postmenopausal stages, showing a relative testosterone excess in postmenopausal women. This relative androgen excess in hormonal milieu was associated with increased central abdominal adiposity [10]. Other studies associated baseline total T/E ratio and its rate of change with increased incident metabolic syndrome, independent of ethnicity [11].

Menopause brought on surgically via oophorectomy, however, behaves differently. In an Australian ancillary study, women with bilateral oophorectomy 55 years old or older showed significantly lower total and free T levels than age-matched controls with both ovaries [12]. Various studies have confirmed post-oophorectomy testosterone decline, compared with natural menopause [13]. In regard to aging, the epidemiological cross-sectional survey in 1423 Australian women [12] showed a gradual decrease in ovarian and adrenal functions, beginning in the early reproductive years, as early as 25 years of age: total testosterone decreased 55 % from 18 to 75 years of age, free T decreased 49 % and DHEA-S decreased 77 % (Table 17.1).

Table 17.1 Androgen levels change across lifespan in a reference group of Australian women [12]

Age group	18–24	25–34	35–44	45–54	55–64	65–75
Total testosterone (25–83 ng/dl)	45,53	31,98	26,9	23,34	19,0	20,46
Free calculated testosterone (370–1110 pg/dl)	680,4	497,1	393,9	340,6	311,5	281,2
DHEA–S (150–400 μg/dl)	227,4	174,8	159,6	126,6	87,4	65,18
Androstenedione (140–395 ng/dl)	243,8	185,5	148,4	120,1	90,4	88,4

Testosterone Transport

Approximately 60–65 % of testosterone is carried in peripheral blood tightly bound to sex hormone binding globulin (SHBG), forming a circulating reservoir; 35–40 % is loosely bound to albumin, <5 % is bound to corticosteroid-binding globulin (CBG), and less than 2 % circulates as free testosterone. As albumin and CBG binding are relatively weak, T can easily disassociate from those proteins to interact with the testosterone receptor [4]. The sum of free and non-SHBG bound T is called bioavailable testosterone, because it can be utilized whenever necessary. In many situations, such as pregnancy and estrogen therapy, SHBG production is increased resulting in lower levels of free T; by contrast, insulin, obesity, and menopause decrease SHBG levels, resulting in slightly increased concentrations of free testosterone.

Reproductive levels of SHBG are higher in women, and after menopause do not increase so markedly as in aging men [4] Also differing from men, higher SHBG in women translates into a favorable metabolic profile, with lower total, LDL-cholesterol and triglycerides, and higher HDL-cholesterol [14], lower insulin levels, and reduced risk of metabolic syndrome [15]. Accordingly, lower SHBG in women was associated with the occurrence of cardiovascular disease [16].

Testosterone Measurement

Radioimmunoassay kits currently being used are adequate to measure higher male total T levels, while women's low total testosterone level measurements using radio immuno assay (RIA) are not so precise, and still need validation. The gold standard for measuring total T in women is an ultrasensitive method such as mass spectrometry. Free testosterone may be measured by equilibrium dialysis. Free and bioavailable testosterone can be calculated from total T and SHBG concentrations using published algorithms for men.

Testosterone Metabolism

Testosterone is converted to estradiol through aromatase and to dihydrotestosterone (DHT) by the 5 alpha reductase enzymes, in target tissues and also at the periphery, mainly at the adipose tissue. Thus, testosterone actions manifest as a result of estradiol biosynthesis activating the estrogen receptor (ER) or through DHT-specific binding to the androgen receptor (AR, Fig. 17.2).

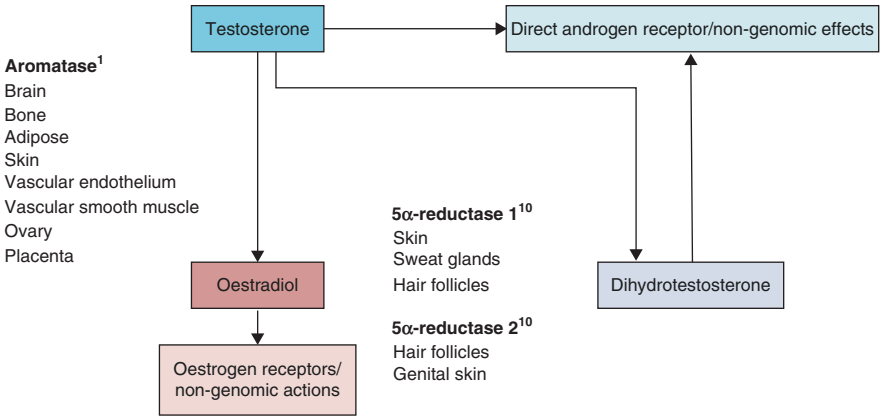

Fig. 17.2 Testosterone transformation into Estradiol (through aromatase) and to DHT (through 5 alpha reductase) [16]

Testosterone Actions in Women

Testosterone is an essential hormone for women with direct physiological actions on body systems or via aromatization to estrogens. ARs are present in a variety of tissues such as the skeletal muscles, skin, gastrointestinal tract, genitourinary tract, bone, brain, cardiovascular system, placenta, and adipose tissues [16] (Fig. 17.2).

AR mediates the peripheral effects of testosterone. The main mechanism of action for AR is direct regulation of gene transcription. After the binding of an androgen to its receptor, a conformational change occurs, causing the dissociation of heat shock proteins, translocation into the nucleus, and dimerization. The AR dimer binds to a specific sequence of DNA, known as a hormone response element, thereby up- or down-regulating specific gene transcription. Furthermore, AR may also act via a nongenomic pathway that entails the rapid activation of kinase-signaling cascades and the modulation of intracellular calcium levels. As far as the direct action is concerned, testosterone effect occurs via AR, both directly and indirectly through its metabolite, that is, dihydrotestosterone, in which it is converted by 5α-reductase [17].

In spite of the important role of testosterone and its high circulating concentrations, relative to estradiol in women, studies of its actions are still scarce.

Sexuality

Over the past decades, large observational studies have demonstrated associations between androgens and sexual function, suggesting that androgen levels were independent determinants of sexual behavior in women, and androgen therapy would therefore be beneficial for treatment of low libido. Surveys in postmenopausal

women using transdermal testosterone, leading to supra-physiological serum T levels (average of 110 mg/dl) reported improvement of desire in women with a hypoactive sexual disorder compared with placebo [18–21]. However, an important point is that sexual function was not consistently related to changes in physiological circulating concentrations of androgens at menopausal transition or early postmenopause. Instead, associations were found sometimes—and sometimes not—between specific androgens and self-reported measures of sexual function, both in pre- and postmenopausal women. Turna et al. [22] found lower levels of total and free T in 20 postmenopausal women who were 45–70 years old complaining of low libido, compared with 20 matched for decade controls with normal libido (48–60 years old), all classified by female sexual function index (FSFI) questionnaire and treated with 0.625 mg conjugated estrogens, associated or not with 2.5 mg medroxyprogesterone acetate. Nonetheless, Davis et al. [23] found no association between sexual function scores, studied by the Profile of Female Sexual Function (PFSF) questionnaire, and low testosterone levels in 1021 women 18–75 years old.

Although the primary indication of testosterone prescription for women is low sexual desire, the multifactorial nature of women's sexual function, the different questionnaires used to measure female sexual dysfunction, and the difficulty of measuring low levels of testosterone, its precursors, and its metabolites by the methods available until recently makes an "androgen deficiency syndrome" diagnosis a real challenge for physicians. Meanwhile, we see the widespread use of testosterone by women−either off-label or compounded [16].

Cardiovascular System

Over the past years, a large number of studies in men have demonstrated that normal testosterone levels are associated with cardiovascular health. Appropriate testosterone therapy in men with hypogonadism improved obesity, myocardial ischemia, exercise capacity, and heart-rate-corrected (QTc) length [24]. In women, cardiovascular T actions remain uncertain.

The beneficial endothelial effect of E2 in women, as a result of non-classical action in type α estrogen receptors, stimulates endothelial vascular endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) production [25] is well established. Synthesis and activity of NO are responsible for the modulation of vascular tone, regulation of prostaglandin production and endothelin gene expression, inhibition of endothelin-induced vasoconstriction and sympathetic activity, added to an antiproliferative effect on the vascular smooth muscle.

At physiological concentrations, testosterone was shown to have favorable effects on vasomotor tone, endothelial function, and peripheral vascular resistance through direct effects on the blood vessel wall [26]. Testosterone improves arterial function in women by enhancing endothelium-dependent (flow-mediated) and endothelium-independent brachial artery vasodilation, as shown by Montalcini et al. [9] who described a positive correlation between serum T levels and flow-mediated brachial artery vasodilatation assessed by Doppler ultrasound in 60

postmenopausal women. Additionally, Worboys et al. [27] found increased endothelium-dependent and independent vasodilatation after 50 mg subcutaneous testosterone implants for 6 weeks, associated with estrogen-progestin therapy.

An optimal range of endogenous testosterone seems to be required for protective effects on cardiovascular disease. In the Rancho Bernardo cohort (1984–2004) [28], both the lowest and the highest bioavailable testosterone (BT) quintiles were associated with increased age-adjusted risk of incident coronary heart disease (CHD), with a 79 % ($p = 0.046$) increased risk for women with low BT and a 96 % ($p = 0.022$) increased risk for those with high BT. This association persisted after additional adjustment for adiposity and lifestyle characteristics.

Observational studies indicate that testosterone therapy (see later in this chapter) has favorable cardiovascular effects measured by surrogate outcomes; however, associations between endogenous testosterone and the risk of cardiovascular disease and total mortality, particularly in older women, are still unknown.

Brain

Testosterone modulates serotonergic transmission, and serotonin plays a key role in the development of depression [29]. This could be a reason why depressive disorders are more prevalent in women compared with men [30], as well as why depression in males increases with age [31], becasue plasma testosterone drops. Furthermore, testosterone levels are lower in depressive patients compared with healthy controls [32] and exogenous testosterone appeared to improve depressive symptoms in both sexes [33].

Mchenry et al. [34] reviewed recent studies describing higher anxiety in women compared with men. Added to the antidepressant effects described, testosterone presented a dose-dependent anxiolytic effect in a study performed with male mice, very likely mediated by 5-alpha reductase. This effect could be also seen in healthy women, who presented reduced fear after a single testosterone administration.

In relation to memory, it is known that women have better verbal memory, while men have an advantage in visual-spatial memory. But this action seems to be curvilinear and sex-dependent: in women, higher testosterone is associated with better mental rotation; in men, lower testosterone is associated with better spatial abilities. Various clinical studies in postmenopausal women showed improvements in learning and memory after testosterone supplementation [35]. The mechanism of action, however, could be a result of testosterone or estradiol, mediated by aromatase conversion. The controversies continued after other evidence showed no effect on memory or other analyzed behavioral measures in postmenopausal women who received high doses of testosterone [36], on the contrary, verbal memory was impaired in another study conducted with oophorectomized women who received testosterone or placebo in addition to estrogen supplementation [37].

This gender peculiarity might be mediated by the organizational effect of prenatal T on brain structures such as the amygdala or the hippocampus. In studies using Functional magnetic resonance imaging, participants with complete androgen

insensitivity syndrome presented with a female-like neural activation pattern in the parietal lobe, indicating an association with gonadal hormone exposure rather than genetic sex itself with brain functions [38].

Overall, the association of endogenous or exogenous testosterone with cognitive function and behavior is still controversial [39]. Dosing, timing, and route considerably affect brain outcomes, probably because of T reduction to DHT or conversion to E2, increasing androgen or estrogen activity, respectively. Outcomes are subjective and the assessment of psychometric parameters not as precise as in other fields.

Bone

Estrogen deficiency is a major risk factor for osteoporosis in women, but androgen also plays an important role in bone mass physiology, particularly bone formation [40]. High androgen levels in premenopausal, but not in postmenopausal, women were predictors of increased bone mineral density (BMD) [41]. Other studies, including menopausal hormone therapy, described a positive correlation with testosterone concentrations and BMD [42]. Endogenous low free T and high SHBG levels in untreated women 65 years or older, followed for 2 years, increased the relative risk of hip fracture [43]. Again, the androgen contribution of endogenous testosterone or its transformation into E2 regarding BMD is still debated. Data about the effects of exogenous testosterone on women's bone are limited and, until now, no study evaluated fracture as endpoint [44].

Body Composition

Higher free T levels in postmenopausal women are associated with greater lean body mass, consistent with an anabolic effect of T. However, women with higher free T levels showed to have higher body fat than their controls [45]. The accepted mechanism is that hyperinsulinemia, due to insulin resistance, could then lead to insulin-stimulated T production by the postmenopausal ovary.

Gynecological Cancer

Estrogen estimulation is considered a central mechanism in the evolution of receptor positive breast cancer; however, the relation between androgens and the risk of breast cancer is uncertain. Polycystic ovary syndrome, a cause of hyperandrogenism in women, does not increase the risk of breast cancer. Androgen receptors could exert a growth inhibitory effect in estrogen receptor α positive tumors and impact prognosis because of the antiproliferative and pro-apoptotic nature [46].

Although some epidemiological studies have shown positive associations between endogenous concentrations of testosterone and the risk of breast cancer, preclinical studies suggest protective effects for some subtypes of cancer, and

observational studies have consistently presented no increase of risk. No clinical trial has been of sufficient duration to provide any conclusion about testosterone security. The conversion of testosterone to estrogen in breast tissue, promoted by aromatase, which increases with age, also can provide biased results [16].

In a study with female-to-male transsexuals, the use of exogenous testosterone for 3 months was not associated with risk of endometrial cancer, probably due to the antiproliferative effect in endometrium [47] but longer studies are not available.

No association between androgens and risk of invasive epithelial ovarian cancer was found in a large nested case–control study of the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, that compared 565 cases with 1097 paired controls [48].

Skin and Hair

Androgens stimulate overgrowth of follicular epithelium, and increase sebum production. DHT, the most potent androgen, is produced by conversion of T by the enzyme 5-α-reductase present in pilosebaceous follicles. Hyperinsulinemia, due to peripheral insulin resistance present in women with PCOS, promotes hyperandrogenemia through the binding of insulin to the insulin-like growth factor-1 (IGF-1) receptor amplifying androgen production by the theca cell in response to LH. Because insulin decreases levels of SHBG, the circulating levels of free testosterone are also increased.

Acne is commonly provoked by androgen excess, affecting about 5–10 % of all women of reproductive age, either by overproduction, androgen peripheral metabolism disorders, or by induction and activation of androgen metabolism in the skin. The levels of circulating androgens do not relate to the severity of acne, indicating that there may be overproduction in the skin. The SAHA syndrome, an association of seborrhea, acne, hirsutism and androgenetic alopecia, is a complete manifestation of androgen effects on the pilossebaceous units.

Androgenetic alopecia is a common genetic condition of hair loss caused by androgens. It affects 20–53 % of women in the sixth decade of life and is determined by a single autosomal dominant gene with variable penetrance. The female baldness pattern can begin at puberty and progress for many years. The hairline implant is preserved and there is a progressive thinning at the top of the scalp [49]. It consists of a progressive reduction of the time of the growth phase, cycle after cycle and miniaturization of the hair follicle involved. The follicles present in greater numbers during the telogen phase and are shortened during the anagen phase [50].

Hirsutism is characterized by the presence of an excessive amount of terminal hair in places where they are not normally found, following a typical male distribution. The explanation is a change in the hair growth cycle induced by androgens, with an extension of the anagen phase [51].

Immunity and Hematology

Over the past years, various experimental and clinical studies have provided evidence for a possible immunosuppressive role for testosterone: there is a higher incidence of autoimmune diseases in women; men's castration aided in the development and exacerbated the consequences of experimental autoimmune diseases such as autoimmune encephalomyelitis, multiple sclerosis, diabetes, thyroiditis, and adjuvant arthritis; the provision of testosterone to females was shown to ameliorate symptoms in a variety of autoimmune disease models.

Testosterone supplementation reduces the expression of interferon (IFN)-γ and increases the expression of the anti-inflammatory cytokine interleukin (IL)-10 by autoantigen-specific T lymphocytes [52]. Complementary in vitro studies have found that $CD4^+$ T lymphocytes treated with 5α-dihydrotestosterone also respond with increased IL-10 production [53].

Testosterone decreased the secretion of the Th1 cytokines IFN-γ and IL-2 in a study with experimental autoimmune orchitis in rats. In shifting the balance of Th1/Th2 cytokines, a T cell-driven autoimmune response was inhibited, thus preventing an excessive proinflammatory response and the consequent tissue damage [54].

The mechanism through which testosterone stimulates erythropoiesis is unclear. Testosterone enhances the proliferation of erythroid burst and colony-forming units by stimulating specific nuclear receptors, and the effect is completely abolished by pretreating marrow cells with cyproterone and flutamide. Moreover, androgens cause a sustained expansion of human female, but not male, erythroid progenitors. This could explain why testosterone-anemia association is stronger in women than in men. The increase in erythropoietic activity by stimulating erythropoietin (EPO) has being questioned in the study by Ferrucci et al. [55], in which a low testosterone level remained a strong risk factor for anemia even with adjustment for EPO levels. That is consistent with previous data showing that the erythropoietic activity of androgens is relatively erythropoietin independent. The same study suggests that the increased hemoglobin levels could mediate potential targets of T administration as muscle strength, bone mineral density, physical performance, and cognition.

Eyes

Low androgen levels contribute to tear film instability and evaporative dry eye that are characteristic of primary and secondary Sjögren's syndromes. Androgens regulate meibomian gland function, control the quality and/or quantity of lipids produced by the tissue, and promote the formation of the tear film's lipid layer [56].

Testosterone Excess in Women

Polycystic Ovary Syndrome (PCOS)

PCOS is a heterogeneous and complex disorder that has both adverse reproductive and metabolic implications for affected women. However, there is generally poor understanding of its etiology [57].

A recent Endocrine Society-appointed Task Force of experts developed an evidence-based guideline using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system to describe both the strength of recommendations and the quality of evidence to define, diagnose, and treat PCOS. According to the guideline:

The diagnosis of PCOS should be made according to National Institutes of Health/Rotterdam/AE-PCOS Society diagnostic criteria, namely, if two of the three following diagnostic criteria are met: (1) androgen excess, (2) ovulatory dysfunction, (3) ultrasound appearance of polycystic ovaries, whereas disorders that mimic the clinical features of PCOS are excluded. Those include, in all women: thyroid disease, hperprolactinemia, and nonclassic congenital adrenal hyperplasia (primarily 21-hydroxylase deficiency characterized by serum 17-hydroxyprogesterone above defined cut-off levels). In selected women with amenorrhea and more severe phenotypes, more extensive evaluation excluding other causes must be done [57–59].

The diagnosis in adolescents must be based on the presence of clinical and/or biochemical evidence of hyperandrogenism (after exclusion of other pathologies) associated with persistent oligomenorrhea and typical PCO morphology (three of three diagnostic criteria; anovulation and ultrasound appearance are not sufficient to make a diagnosis in adolescents). Although there is some uncertainty about diagnostic criteria of PCOS in adolescents, it is important to recognize the disorder at that time, because an earlier intervention can be made, planning potential therapeutic strategies in an affected subject [58].

A presumptive diagnosis of PCOS can be based upon a well-documented long-term history of oligomenorrhea and hyperandrogenism during the reproductive years regarding diagnosis in perimenopause and menopause. In fact, there are currently no diagnostic criteria for PCOS in perimenopausal and menopausal women [57–59].

The evaluation of the features that compose PCOS diagnostic criteria are described below:

Androgen Excess

Clinical manifestations include hirsutism, acne, and androgenic alopecia. In rare instances, male pattern balding, increased muscle mass, deepening of the voice, or clitoromegaly may occur, suggesting virilizing androgen levels and a possible underlying ovarian or adrenal neoplasm or severe insulin-resistant states [57].

Hirsutism is defined as excessive terminal (coarse) hair that appears in a male body hair pattern [60]. Racial factors affecting the initial density of pilosebaceous units and their responsiveness to androgens may result in differences in hair distribution and density in women with PCOS. Scales of hirsutism assessment such as the modified Ferriman-Gallwey scale will need to deal with those differences, therefore, varying cutoffs [57, 58].

Acne is common in women with PCOS, particularly in the teenage years; the prevalence varies (14–25 %), with some difference in relation to ethnicity and age. Androgenic alopecia is less frequent and presents later, but it remains a distressing complaint with significant psychopathological comorbidities.

There is a poor correlation between clinical features and biochemical hyperandrogenism [59].

Polycystic Ovaries Morphology (Visualized by Ultrasound)

In 2004, Rotterdam PCOS criteria updated the definition of typical PCO morphology as the presence of 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or an increased ovarian volume (10 mL) in at least one ovary. As a result of recent improved ovarian imaging, many believe this follicle number threshold value should be increased, and an expert panel recommended using a cutoff of >25 follicles per ovary to diagnose a polycystic ovary. The follicle number per ovary correlates well and thus is a good surrogate marker for ovarian hyperandrogenism [61, 62].

Serum Anti-Müllerian Hormone (AMH), originating mostly from granulosa cells of large preantral/small antral follicles, has recently emerged as a possible surrogate marker because it can be used to measure ovarian reserve but also reflects the greater number of follicules present in ovaries with polycystic morphology. The strong association between AMH and ultrasound-performed follicle count has led some authors to compare them in the diagnosis of PCOS, and the results in current literature are not homogenous. Future research is required to explore AMH predictive value for treatment outcomes, in particular for ovulation induction. It is impossible at present to propose a consensual and universal diagnostic threshold for serum AMH predictive of ovarian dysfunction in PCOS [57–59].

Chronic Anovulation

According to the Androgen Excess/PCOS Society Task Force Report, as many as 85 % of women with PCOS have presented with clinical evidence of menstrual irregularities. Clinicians diagnose oligomenorrhea when menstrual cycles last longer than 35 days or occur less than eight times a year, although women with regular menstrual cycles may nonetheless have chronic anovulation [63].

Obesity is associated with reduced SHBG levels, leading to higher free androgen levels and prolonged follicular phases (without anovulation) leading to a longer menstrual cycle which could be confused with a PCOS diagnosis. However, PCOS has been strongly associated with insulin resistance (IR) and obesity, at least in patients seen in referral populations. Although increased BMI has been associated with increased IR and risk of developing metabolic syndrome, type 2 diabetes

mellitus and cardiovascular disease, the relationships between BMI and IR in women with PCOS are heterogeneous. Some obese PCOS women demonstrate no measurable metabolic abnormalities, while IR occurs in obese and lean PCOS women alike. Approximately 70 % of women with PCOS demonstrate IR and compensatory hyperinsulinemia, beyond that because of their degree of obesity. While the prevalence of overall adiposity or obesity appears to be as common among women with PCOS as in the general population, approximately 60 % of such patients seen in the clinical setting are obese. In those patients there is adipose tissue dysfunction, in the form of impaired glucose transport, exaggerated inflammatory markers, impaired adiponectin secretion, and reduced GLUT-4 expression, implying that factors other than adiposity per se account for the IR observed in PCOS. Prior studies of body composition in PCOS focused on lean or fat mass alone and did not explore how whole body fat deposition interacts with lean mass to affect IR. Ezeh et al. hypothesized that the fat:lean ratio, which integrates the antagonistic effects of both fat and lean mass depots, differs among PCOS patients and controls and accounts, at least in part, for the greater degrees of IR in the former. Compared with controls of similar BMI, women with PCOS demonstrate a higher amount of total body fat in relation to lean mass, which may in part be responsible for the observed metabolic dysfunction in women with PCOS, and may provide potential body composition correlates of metabolic features in PCOS [57, 64].

Congenital Adrenal Hyperplasia

The diagnosis and prevalence of nonclassic adrenal hyperplasia (NC-CAH) continues to be debated. Other terms that have been used to describe this syndrome include late-onset, adult-onset, attenuated, incomplete, and cryptic adrenal hyperplasia. This form of adrenal hyperplasia is caused by a partial deficiency in the 21-hydroxylase activity. Although deficiencies in 11-hydroxylase and 3β-HSD may result in NC-CAH, defects in 21-hydroxylase account for more than 90% of CAH cases (or patients). The clinical presentation is almost identical to that of patients with PCOS. The prevalence of this disorder varies according to ethnic background, and reports by different investigators have varied widely.

Basal levels of 17-hydroxyprogesterone (17 OHP) lower than 2 ng/mL (6 nmol/l) effectively rules out NC-CAH. If the level is above 2 ng/ml, initial screening with the adrenocorticotropic hormone (ACTH) stimulation test should be done to distinguish NC-CAH from PCOS. Values above 10 ng/ml have been considered diagnostic. Others cutoff levels have been proposed, but the one proposed by Azziz et al. is the most frequently used [60, 65, 66].

During the menopausal transition, mild signs of hyperandrogenism such as hirsutism may appear as part of expected hormonal changes, but the development of frank virilization suggests a specific source of androgen excess that can be a result of benign conditions (such as hyperthecosis) or to adrenal or ovarian androgen-secreting tumors. Long-term history of oligomenorrhea and hyperandrogenism during the reproductive years is critical to differentiate the progressive development of

virilization that characterizes benign causes from the rapid progression that characterizes malignant tumors. Imaging techniques do not always reveal the cause of hyperandrogenism and may even be misleading [67].

Hyperthecosis

Hyperthecosis is the presence of severe hyperandrogenism and insulin resistance seen primarily in postmenopausal women, and the main differential diagnosis is from androgen secreting tumors.

Androgen-Secreting Tumors of the Ovary and Adrenal

Most androgen-secreting tumors arise from the ovary. These ovarian tumors secrete large quantities of testosterone or its precursor, androstenedione. They include Sertoli-Leydig, hilus, or lipoid cell tumors, and infrequently, granulosa-theca tumors.

Sertoli-Leydig cell tumors, which account for fewer than 1 % of all solid ovarian tumors, tend to occur during the second to fourth decades of life, whereas hilus cell tumors occur more frequently in postmenopausal women. By the time the signs and symptoms of androgen excess cause the patient to seek medical assistance, Sertoli-Leydig cell tumors are usually so large that they are readily palpable on pelvic examination, whereas hilus cell tumors are still small. In women with either type of tumor, the serum testosterone level is markedly elevated. Granulosa-theca tumors primarily produce estradiol but may occasionally produce testosterone. Rapidly progressing symptoms of androgen excess are typical of both ovarian and adrenal androgen-producing tumors, typically associated with defeminizing signs, such as loss of female body contour and decreased breast size. As the tumor continues to grow, more and more testosterone is produced, resulting in rapidly worsening hirsutism and progressive virilization. Elevated serum testosterone levels are characteristically associated with ovarian tumors [67].

Evaluation of Androgen Excess (Table 17.2)

Conditions such as androgen excess, obesity, acromegaly, hypothyroidism, and liver disease decrease SHBG binding, therefore increase bioavailable testosterone, augmenting the effect of testosterone. The normal serum levels of androgens, particularly free testosterone determined by radioimmunoassay (RIA), vary from laboratory to laboratory. The clinician should be aware of the normal ranges of the clinical laboratory used and the limitations of RIAs performed without rigorous quality control. A total testosterone value of three times the upper normal range (or >200 ng/dL) suggests a neoplasm, particularly if the clinical history supports this diagnosis, but serum testosterone levels that are not so high may occasionally be

Table 17.2 Recommended tests to exclude causes of hyperandrogenism other than PCOS [68]

Initial testing	– Total testosterone – Prolactin – Thyroid-stimulating hormone
Further testing based on clinical presentation	– 17-OHP (8:00 a.m.) – 17-OHP 60 min after IV ACTH – Cortisol (8:00 a.m.) after 1 mg dexamethasone at midnight – DHEAS – Androstenedione – Imaging of ovaries (transvaginal ultrasonography) – Imaging of adrenals (abdominal ultrasonography, CT, MRI) – Adrenal scintilography after intravenous radiolabeled cholesterol

associated with virilizing ovarian tumors. If an androgen-secreting tumor is suspected, measurement of androstenedione is clinically useful. A severely elevated level of androstenedione is consistent with an ovarian or adrenal tumor. When an elevated level of testosterone is found and confirmed by clinical history, meticulously performed transvaginal ultrasonography should be able to detect the ovarian tumor.

In contrast to testosterone-secreting tumors of the ovary, if dehydroepiandrosterone sulphate (DHEAS) levels exceed 800 μg/dL, adrenal imaging by computed tomography (CT) or magnetic resonance imaging (MRI) should be ordered. Virilization of recent onset and short duration warrants immediate investigation, even if testosterone and DHEAS levels are mildly elevated. With improvements in scanning techniques—vaginal ultrasonography for the ovary; abdominal ultrasonography, CT, and MRI for the adrenal—the diagnosis of even a small ovarian or adrenal tumor can be made.

Adrenal imaging after intravenous administration of radiolabeled iodomethylnorcholesterol (NP-59) is a noninvasive method for determining the adrenal cortical functionality. Unlike CT or MRI which provide only an anatomical image, the scintigraphic image gives a functional characterization of the adrenal glands in vivo. Therefore, the recruitment of a cortical nodule is virtually diagnostic, while the decrease or absence of an adrenal mass uptake (discordant image) suggests a hypofunctional mass, destroying or occupying space. If no neoplasm can be localized, imaging of the ovary or adrenal after intravenous administration of radiolabeled iodomethylnorcholesterol (NP-59), which detects active steroid-producing tumors, has proved useful but has limitations depending on tumor size. Although technically difficult, combined adrenal and ovarian venous sampling may be required to confirm the source of androgen excess before the best surgical approach is determined [67].

Recently, very small ovarian or adrenal tumors, not visible through other methods, were identified by means of positron emission tomography-scan technique.

Treatment of Hyperandrogenism in Women

Therapy for androgen excess should be directed toward its specific cause and suppression of abnormal androgen secretion. Specific treatments for hirsutism and virilization are indicated for ovarian and adrenal tumors, hyperthecosis, Cushing's syndrome, and adrenal hyperplasia. Neoplasms warrant surgical intervention. Suppression with a GnRH analog may be tried initially for ovarian hyperthecosis. For nonclassic adrenal hyperplasia, glucocorticoid replacement should be implemented, as it is for adrenal insufficiency. When treating androgen excess associated with NC-CAH, an antiandrogen (e.g., spironolactone) in combination with an oral contraceptive or a glucocorticoid may be used. The doses of glucocorticoids needed to suppress the adrenal can often cause signs and symptoms of glucocorticoid excess during long-term treatment. A combination of an oral contraceptive plus spironolactone is favored to treat androgen excess in NC-CAH if the patient responds to the treatment with decreased hirsutism.

Oral Contraceptives

Oral contraceptives reduce circulating testosterone and androgen precursors by LH suppression and SHBG stimulation, thereby reducing hirsutism in hyperandrogenic patients. It is advisable to use an oral contraceptive containing 30 or 35 μg of ethinyl estradiol to achieve effective suppression of LH. A meta-analysis showed that treatment with oral contraceptives for 6 months reduced Ferriman-Gallwey scores of hirsutism by an average of 27%.

Spironolactone

Because spironolactone acts through mechanisms different from those of oral contraceptives, overall effectiveness is improved by combining these two medications in patients with hirsutism, including those with PCOS, idiopathic hirsutism, or NC-CAH. Spironolactone is an androgen receptor antagonist but also directly inhibits 5α-reductase activity. Doses used in clinical studies have varied from 50 to 400 mg daily; although 100-mg/day are usually effective for the treatment of hirsutism, higher doses (200–300 mg/day) may be preferable in extremely hirsute or markedly obese women. Thus, the initial recommended dosage is 100 mg/day, gradually increasing it by increments of 25 mg/day every 3 months up to 200 mg/day on the basis of the response. This approach may be helpful to minimize side effects such as arterial hypotension, menstrual irregularity, gastritis, dry skin, and anovulation. In patients with normal renal function, hyperkalemia is almost never seen.

Cyproterone Acetate (CPA)

CPA is a 17-hydroxyprogesterone-acetate derivative with strong progestagenic properties. CPA acts as an antiandrogen by competing with DHT and T for binding to the androgen receptor. There is also some evidence that CPA and ethinyl estradiol in combination can inhibit 5α-reductase activity in skin. Described side effects include liver toxicity, headache, weight gain, breast tenderness, loss of libido, edema, and mood changes. Because of its slow metabolism, the drug is usually administered in doses of 50–100 mg on days 5 through 15 of the cycle; when ethinyl estradiol is added, it is typically administered in 50-μg doses on the first 10 days of the monthly administration. This regimen is needed for menstrual control and is referred to as the *reverse sequential regimen*. CPA in doses of 50–100 mg/day, combined with ethinyl estradiol at 30–35 μg/day, is as effective as the combination of spironolactone (100 mg/day) and an oral contraceptive for the treatment of hirsutism. In smaller doses (2 mg), CPA has been administered as an oral contraceptive in daily combination with 35 μg of ethinyl estradiol. This regimen is primarily suited for individuals with a milder form of hyperandrogenism.

Finasteride

Finasteride is not actually an antiandrogen but a 5α-reductase inhibitor. At a dose of 5 mg/day, a significant improvement in hirsutism is observed after 6 months of therapy, without significant side effects. In hirsute women, the decline in circulating DHT levels is small and cannot be used to monitor therapy. Although the treatment regimen increases testosterone levels, SHBG levels remain unaffected. A meta-analysis showed that finasteride treatment for 6 months reduced Ferriman-Gallwey scores of hirsutism by an average of 20.3 %.

Flutamide

Flutamide is a potent antiandrogen used in the treatment of prostate cancer and has been shown to be effective in the treatment of hirsutism. The mean Ferriman-Gallwey score is reduced by 41.3 %. Nevertheless, occasional severe hepatotoxicity makes the drug unsuitable for the indication of hirsutism.

Metformin and Thiazolidinediones

Most studies conducted during the past decade have suggested that treatment with metformin (1500–2700 mg/day) for 6 months significantly reduces hirsutism as assessed by the Ferriman-Gallwey scoring system, however modestly (on average 19.1 %). In obese adolescent women with PCOS, metformin in combination with lifestyle modification (i.e., diet with a 500 kcal/day deficit and exercise 30 min/day)

and oral contraceptives reduced the total testosterone level and waist circumference. Thiazolidinediones (4 mg/day of rosiglitazone or 30 mg/day of pioglitazone) also reduced Ferriman-Gallwey scores significantly, suggesting that insulin-sensitizing agents might be used in the treatment of hirsutism, especially for women who do not wish to use other oral agents.

Patients with the most common form of hirsutism (i.e., PCOS) are often initially treated with a combination of two agents, one that suppresses the ovary (e.g., an oral contraceptive) and another that suppresses the extraovarian (peripheral) action of androgens (e.g., spironolactone). An oral contraceptive containing 30–35 μg of ethinyl estradiol combined with spironolactone (100 mg/day) is the initial treatment of choice. Even in women with idiopathic hirsutism, the addition of an oral contraceptive to the antiandrogen spironolactone can improve efficacy and prevent abnormal bleeding. For women with only minor complaints of hirsutism, the use of an oral contraceptive alone may be an appropriate first approach. Moderate lifestyle modification (i.e., diet with a 500 kcal/day deficit and 30 min/day of exercise) should be a part of hirsutism management in obese patients. Because the growth phase of body hair lasts 3–6 months, a response should not be expected before 6 months after treatment onset [69].

Low Testosterone in Women

Turner's Syndrome

Turner's syndrome (TS) is the most common cause of hypogonadism in women, occurring in 1:2500–1:3000 live-born girls. Approximately half have X monosomy (45,X), 5–10 % have a duplication of the long arm of one X (46,X,i(Xq)), and most of the rest have mosaicism for 45,X [70]. The risk of gonadal failure in children with mosaicism is unknown. Some cases are diagnosed in childhood because of dysmorphisms and cardiac or renal abnormalities. In about 40 % of cases, diagnosis may only occur at puberty due to short stature and delayed puberty. With the exception of familial short stature or constitutional delay, TS is the most common cause of short stature in otherwise healthy girls. Apart from short stature, the condition is diagnosed when adolescents fail to enter puberty or because of menstrual irregularities or recurrent pregnancy loss in adulthood. The diagnosis should be excluded in cases of primary or secondary amenorrhea.

Both the short arm and the long arm of the X chromosome contain important genes for ovarian function, and aneuploidy alone may lead to a reduction in the number and survival of oocytes [71]; 90 % of patients with TS will require hormone-replacement therapy to initiate puberty and complete growth. The uteri are generally small but structural uterine abnormalities are rare. In many girls with TS, pubic and axillary hair will develop spontaneously, resulting from adrenarche. Some girls have enough residual ovarian function for breast budding or vaginal spotting to occur, but secondary amenorrhea will develop. A small number will maintain ovulatory cycles for a time. Despite spontaneous menarche, premature ovarian failure typically

occurs. If the status of ovarian function in adolescence is unclear, measurement of FSH, LH, and E2 levels can help determine the need for hormone-replacement therapy. Psychosocial issues and the patient's maturity and wishes must also be considered. Girls who have received recombinant human growth hormone and who have completed most of their growth, as judged on the basis of bone age or growth velocity, may start hormone-replacement therapy at the age of 12 years if desired. There is no single formula for the use of hormone-replacement therapy. Beyond the usual sex hormone replacement, oxandrolone (Ox), a synthetic anabolic steroid derived from DHT, when combined with growth hormone (GH), has been shown to increase height velocity and to improve final height [72] but the paucity and small studies with oxandrolone, the absence of placebo-controlled ones and reports of virilization with clitoromegaly and voice deepening braked its use. A recent review of three randomized clinical trial (RCT) reported that the addition of Ox to GH treatment leads to an increase of on average 2.3–4.6 cm in adult height. If Ox dosages <0.06 mg/kg/day are used, side effects are modest. Monitoring signs of virilization, breast development, and possibly blood lipids during Ox treatment is recommended. In girls with TS who are severely short for their age, in whom very short adult stature is anticipated, or in whom the growth rate is modest despite good compliance with GH, adjunctive treatment with Ox at a dosage of 0.03–0.05 mg/kg/day starting from the age of 8–10 years onward can be considered [69].

Premature Ovarian Failure

Premature ovarian failure (POF) occurs as a result of dysfunctional ovarian follicles or decline in primordial follicles. The diagnosis of POF is generally accepted in women who are younger than 40 years. Early menopause is a term used to describe women who enter menopause in ages older than 40 years but younger than 45 years [73]. POF occurs in 1 in 100 in women younger than 40 years, 1 in 1000 in women younger than 30 years, and 1 in 10,000 in women younger than 20 years. Primary POF can present as primary or secondary amenorrhoea; the etiology will be undetermined in 90 % of women. Secondary POF is at increased prevalence, as survival following treatment for malignancies through surgery, chemotherapy, and radiotherapy improves [74].

Most women with POF will have a normal karyotype. Despite this, in 6 % percent there will be mutations in the FMR1 gene responsible for the Fragile X Syndrome, which can be detected in genetic testing. However, those with primary amenorrhoea present an abnormal karyotype in more than 50 % of cases. Pre-POF is most commonly associated with TS and gonadotoxic treatment for childhood cancer. POF can also be associated with hypothyroidism (25 %), Addison's disease (3 %), and diabetes mellitus type 1 (2.5 %).

The most commonly applied diagnostic criterium is 4 months of amenorrhoea coupled with serum levels of FSH greater than 40 IU/l on two occasions. Initial investigations should include, beyond sexual hormones, thyroid stimulating

hormone (TSH) and prolactin. AMH may be considered for the assessment of follicular reserve.

Women with POF have an increased risk of premature death, mainly from cardiovascular disease, osteoporosis, and increased risk of fracture. They are also at higher risk of dementia and Parkinsonism [75].

Women with 46,XX spontaneous POF have impaired sexual function compared with controls, despite usual hormone therapy (HT) promoting physiological E2 levels. It is possible that T plays a role in this complaint because young women with POF have significantly lower free and total T levels than regularly menstruating women of similar age, both during an interval off of HT and, more importantly, during physiological replacement with transdermal E2 [76]. In a randomized controlled trial conducted to investigate the efficacy of 12 months 150 μg T patch, added or not to usual HT, in 128 women with POF, physiological hormone levels were achieved but T neither aggravated nor improved baseline reports of quality of life or self-esteem and had minimal effect on mood [77].

Bilateral Oophorectomy

Every year, approximately 300,000 women in the United States undergo "prophylactic" bilateral oophorectomy for a benign condition, during hysterectomy [78, 79]. This practice has increased over the past several decades [80] based on the theory that postmenopausal ovaries are not major hormone producing glands and there are concerns about risk of ovarian cancer, a type of malignancy usually diagnosed in advanced stages, famed by difficult screening [81] and low survival rates [82]. Other reasons include avoidance of further gynecological surgical interventions related to retained ovaries; reduction of symptoms associated with advanced endometriosis, not responsive to other medical or surgical therapies, and to solve intractable and severe premenstrual syndrome [83]. For women who do not have genetic variants that increase the risk of ovarian cancer, the risk-benefit balance of a preventive surgery remains uncertain and controversial [84].

As discussed earlier, in natural menopause, T levels show a less pronounced descent compared with estrogens because postmenopausal ovaries continue to produce testosterone [12]. This phenomenon elicits a relative hyperandrogenism in those women. In this sense, significant production of ovarian testosterone occurs even 10 years after natural menopause, possibly mediated by high levels of LH in ovarian theca cells [85]. On the other hand, after bilateral oophorectomy both estradiol and testosterone declines occur in an abrupt way.

An US cohort study with long-term follow-up of 1091 women with bilateral oophorectomy before the onset of menopause, matched with 2383 referent women in natural menopause, showed that those who underwent surgery when younger than 45 years had an increased mortality associated with cardiovascular disease compared with referent women. Additionally, mortality was significantly elevated in women not treated with estrogen through age 45 years or older, but not in those treated [86]. Accordingly, populational studies including menopausal women up to

69 years reported higher cardiovascular risk in those who underwent bilateral oophorectomy before 50 years of age [87].

In the Women's Health Initiative (WHI) study, oophorectomy and hysterectomy were associated with a twofold increased risk of coronary artery calcification compared with those women whose ovaries were retained [88].

A meta-analysis of pooled data from 18 observational studies of post-menopausal status and cardiovascular disease (CVD) concluded that oophorectomy in post-menopausal women adversely affected the incidence of CVD (RR: 2.62, 95 % CI: 2.05–3.35) compared with natural menopause (RR: 1.14, 95 % CI: 0.86–1.51) [80].

As one possible mechanistic explanation, in a study by our group we found that even in recent menopause and under appropriate estrogen replacement, oophorectomized women presented consistent signs of endothelial dysfunction associated with lower levels of endogenous testosterone, compared with women with both ovaries [89].

Some studies compared women who underwent bilateral oophorectomy before menopause with referent women and also consistently showed an increased risk of cognitive decline and dementia, as well as in the risk of metabolic syndrome [90].

Testosterone Therapy

Testosterone replacement is controversial because a testosterone deficiency syndrome is not well characterized; there are limited data supporting improvement in signs and symptoms with therapy and no long-term studies of risk, particularly cardiovascular studies. The Endocrine Society recommends against the routine prescription of T or dehydroepiandrosterone for the treatment of women with low androgen levels due to hypopituitarism, adrenal insufficiency, surgical menopause, pharmacological glucocorticoid administration, or other conditions associated with low androgen levels [8]. Evidence supports only the short-term efficacy and safety of slightly high (above physiological) doses of T treatment for postmenopausal women with sexual dysfunction due to hypoactive sexual desire disorder, remembering that endogenous T levels did not predict response to therapy [91]. However, testosterone is being increasingly used in pre- and postmenopausal women, with or without concomitant estrogen/progestin therapy, although doses and types of androgen formulations are not consensual. Most available testosterone medications are designed for men; the few androgens licensed for women have not shown resounding efficacy and long-term safety and recently some were withdrawn from the market by their own pharmaceutical companies, after approval from regulatory agencies and years of commercialization, alleging economic issues. The main types of testosterone therapy licensed for women are described below.

Oral Methyltestosterone

Early attempts at oral testosterone were not successful due to irregular absorption, hepatic first-pass conversion, and variable increases in serum testosterone levels.

In contrast, methyl-testosterone (MT) showed a more predictable profile and in low doses, 1.25–2.5 mg/day, was not associated with hepatic dysfunction. The medication was commercialized in 1965 in the USA in combination with esterified estrogens, indicated for severe postmenopausal hot flushes. A double-blind trial randomized 20 postmenopausal women, already using 1.25 mg oral esterified estrogens, to associate or not oral methyltestosterone 2.5 mg. At 8 weeks, women receiving MT reported significant improvements in sexual desire and satisfaction compared with the estrogen alone group. A subsequent trial included 218 postmenopausal women with hypoactive sexual desire disorder (HSDD), receiving baseline 0.625 mg oral esterified estrogen therapy, randomized to continue on estrogens alone or estrogens associated with 1.25 mg MT during a 4-month period. MT increased the concentration of bioavailable T and suppressed SHBG, enhanced sexual desire and interest, but no improvements in sexual function scores [92] were observed. Treatment with the combination was well tolerated. Many other studies were performed; one of the longest studies [93] randomized 311 surgically menopausal women to conjugated esterified estrogens vs. conjugated esterified estrogens plus MT over 2 years. Women on MT plus estrogen showed increased spine and hip bone mineral density (BMD), equal sexual interest and well being, lower HDL-C and apolipoprotein B and A1 compared with conjugated esterified estrogens alone. In other studies, triglycerides and apolipoprotein III C also decreased [94]. There is a trend for oral androgens to induce an adverse shift in lipoprotein profile compared with estrogen and to subcutaneous T that did not show adversely altered levels of lipids, C-reactive protein, or glucose metabolism markers. Regarding body composition, a randomized controlled trial of 16 weeks has demonstrated greater increases in lean body mass and strength and greater reductions in fat in postmenopausal women given combined esterified estrogen and MT compared with esterified estrogen alone [95].

At the lowest 1.25 mg/day dose, MT was shown to be lipid neutral when given in conjunction with oral estrogen, but one third of women reported one or more symptoms of virilization after 2 years. The combined estrogen-MT reference drug was on the American market until 2009, when the manufacturer discontinued its supply, alleging commercial reasons. In fact, this drug was approved under an older regulation and currently the Food and Drug Administration is adopting a new regulation system—Drug Efficacy Study and Implementation (DESI), which requires additional studies to prove drug efficacy. The cost-benefit of such studies may be the reason behind discontinuation.

Testosterone Subcutaneous Implants

Parenteral testosterone was also studied in the 1980s, alone and with or without estradiol, in women with bilateral oophorectomy, suggesting improvements in well being, sexual function, and energy levels [96, 97]. Subsequently in the early 1990s, implants containing 50 or 100 m of testosterone were approved in the United Kingdom and parts of Europe. A simple procedure, through a subcutaneous pellet

insertion in the lower abdomen, provided acute testosterone elevation above the normal range, short after placement and gradual decrease during 6 months. Due to considerable interindividual variation, it was advised to check testosterone levels before the advised interval for a new implant insertion. When combined with estrogen implants, T implant therapy was shown to significantly enhance sexual activity, satisfaction, pleasure, fantasy, and orgasm in postmenopausal women. Davis [98] randomized 32 postmenopausal women to 50 mg estradiol implants, alone or associated to testosterone 50 mg, administered 3 times monthly for 2 years. Women with an intact uterus took cyclical oral progestins. BMD increased more rapidly in the testosterone-treated group for total body ($p<0.008$), vertebral L1-L4 ($p<0.001$), and trochanteric ($p<0.005$) measurements. All sexual parameters improved in both groups, but addition of testosterone resulted in a significantly greater improvement for sexual activity ($p<0.03$), satisfaction ($p<0.03$), pleasure ($p<0.01$), orgasm ($p<0.035$), and relevancy ($p<0.05$). Total cholesterol and LDL-cholesterol fell in both groups, as did total body fat. Total body fat-free mass (DEXA, anthropometry, impedance) increased in the estrogen& testosterone (E&T) group only. Worboys et al. [27] investigated endothelial function in 36 postmenopausal women who were previous estrogen plus progestin users, at baseline and 6 weeks after receiving a 50 mg testosterone implant, compared with a control group not using any kind of hormone therapy. Mean blood pressure, heart rate, circulating E2, SHBG, and lipids were unchanged. Endothelial function showed a 42 % mean increase in brachial artery flow mediated dilation while no change was seen at the control group. Long-term studies of testosterone implants lasting more than 2 years have not been performed. In 2012 the main producer (which is different from the American MT producer) announced the withdrawal of the implants "based on the fact that significant and extended process enhancements would be needed to sustain future manufacturing". Currently, testosterone implants are only available in some UK clinics able to obtain a source from outside the country. In the last few days minor companies began to announce the entrance of a similar 100 mg implant into the UK market.

The decision to withdraw testosterone implants was close to a third pharmaceutical company spontaneous withdrawal of testosterone patches, described below. Currently there are no licensed female testosterone replacement preparations available in Europe or in the United States.

Transdermal Testosterone Patch

Transdermal delivery avoids first-pass hepatic metabolism and consistent levels of T [99] can be achieved. Earlier testosterone patch studies included 75 surgically induced menopausal women 31–56 years of age using oral estrogen therapy, with a previously satisfying sex life who reported impaired sexual function following surgery. The participants were randomized to 150 or 300 μg/day testosterone patch or placebo twice weekly for 12 weeks and the higher dose testosterone group reported significantly higher scores for frequency of sexual activity, sexual fantasies, masturbation and orgasm, improvement in well-being and depressed mood, although total,

Table 17.3 Hormone levels obtained after 12 weeks of 150 or 300 μg/day testosterone patches compared with placebo [19]

Hormone	Baseline	Placebo	Testosterone 150 mcg	Testosterone 300 mcg	Normal range
Free T (pg/ml)	1.1 ± 0.7	1.2 ± 0.8	3.9 ± 2.4	5.9 ± 4.8	1.3–6.8
Biovailable T (ng/dl)	2.0 ± 1.4	2.2 ± 1.3	7.1 ± 4.1	11.4 ± 9.5	1.6 ± 12.7
Total T (ng/dl)	21 ± 10	22 ± 12	64 ± 25	102 ± 39	14 ± 54

bioavailable, free testosterone, DHT, SHBG, and estrone serum levels were slightly supra-physiological (Table 17.3) [19].

A subsequent trial with 318 surgically induced postmenopausal women already on estrogen, with persistent HSDD using 150, 300, or 450 μg/day testosterone patches or placebo for 24 weeks found significant increases in sexual desire (67 %) and activity (two more per month) only in the 300 μg/day group compared with placebo, which improved sexual desire in 48 %. The 300-μg/day patches were then adopted for following approval procedures for "replacement" therapy, in spite of confirmed higher testosterone and estrogen levels achieved [18]. However, frequency of adverse events was similar among all groups and no severe events were reported during short-term studies.

Later, phase III multicenter Investigation of Natural Testosterone In Menopausal Women Also Taking Estrogen in Surgically Menopausal Women (INTIMATE SM) was performed. The INTIMATE SM 1 included 562 women with HSDD and lasted for 24 weeks. Questionnaires used to quantify sexual function revealed an increase of 2.1 satisfying sexual episodes (74 %) in the 300 μg/day testosterone patch group versus an increase of 0.98 episodes (33 %) per month in the placebo group [21]. The INTIMATE SM 2 randomly assigned 532 women with HSDD to 300 μg/day testosterone patch or placebo with oral or transdermal estrogen and the results were similar to INTIMATE 1 [100].

Although discreet, the clinical relevance of sexual improvement for the studied women was assessed by Kingsberg et al. [101] in 132 postmenopausal women from the INTIMATE SM studies (approximately 12 % of the total participant pool). Women were asked: "Overall…would you say that you experienced a meaningful benefit from the study patches?" Women who labeled themselves as receiving a meaningful benefit in testosterone and placebo groups were those whose satisfying sexual experiences increased to 4.4 times in 4 weeks, desire score changed from "seldom" to "sometimes" feeling desire, distress rating moved from "often" to "seldom" being distressed. DeRogatis et al. [102] conducted a similar study establishing as thresholds to delineate responders from non-responders an increase in frequency of satisfying sexual activity greater than one episode in 4 weeks, that may seem small, however, was meaningful and clinically significant for the women sampled, reducing distress.

In December 2004, the Food and Drug Administration (FDA) Advisory Committee reviewed the data from the INTIMATE SM studies and other clinic trials exploring transdermal testosterone for HSDD in postmenopausal women.

Although the committee accepted the efficacy of the medication, the FDA declined to approve the female testosterone patch due to a lack of long-term safety data. But the 28-cm^2-testosterone patch containing 8.4 mg testosterone and providing 300 μg of testosterone per 24 h was approved by the european medicines agency (EMA) in Europe in July 2006 for the treatment of HSSD in women who suffered surgical menopause, receiving concomitant estrogen therapy. Simultaneously, the use of testosterone patches without estrogen was tested through the APHRODITE study [103] a double-blinded, placebo-controlled trial of 814 women with HSDD not currently taking estrogen that were randomly assigned to either a 150 or 300-μg testosterone patches or placebo over a 24-week trial period, and extended for safety for almost 2 years, optionally. Again, an increase of satisfying sexual episodes was seen in the women receiving 300 μg/day of testosterone (more 2.1 satisfying sexual episodes/4 weeks versus 0.7 episodes in the placebo group), higher scores for sexual desire and decreased scores for personal distress, comparable with the INTIMATE SM studies. Adverse events (hair growth) were higher in the 300-μg/day-testosterone group. Breast cancer was diagnosed in three women in the testosterone groups.

Other authors reported an improvement of peak oxygen consumption, distance walked over the 6-min walking test, muscle strength, and insulin resistance in women with congestive cardiac failure treated with 300 μg testosterone patch, compared with those receiving placebo [104] Venous thromboembolic events also did not differ in short-term trials that compared transdermal testosterone patches (TTP) to placebo [8].

As cited mentioned, pharmaceutical producer companies withdrew testosterone patches from Europe in October 2012 for commercial, not safety reasons.

Testosterone Cream

Testosterone therapy for women is approved and available in Australia in the form of testosterone 1 % cream. It is measured out on a plastic spatula and approximately 1–2 cm is applied daily to the skin of the lower body. Recommendations are to monitor blood levels initially after 3 weeks, and then every 6 months. Short-term studies have shown efficacy in both premenopausal and postmenopausal women. Goldstat et al. [105] performed a randomized, placebo-controlled, crossover, efficacy study of testosterone cream with two double-blind, 12-week, treatment periods separated by a single-blind, 4-week, washout period. Application of the testosterone cream (10 mg/day) improved well being, mood, and sexual function without any adverse effects in premenopausal women (mean age 39.7 ± 4.2 years) with low libido. Again, mean total testosterone levels during treatment reached the high end of the normal range.

Testosterone Gel for Women

Pharmacological studies compared 10 mg, 20 or 30 mg of testosterone as a 1 % hydroalcoholic gel/day, applied in a thin layer on the outside of the thigh each

morning, over an area of approximately 15 cm. Baseline serum T level was 1.1 ± 0.9 nmol/l and after 10 mg and 20 mg treatment rose to 3.2 nmol/l and 7.2 nmol/l, respectively. Serum levels of testosterone achieved with 10 mg testosterone applied transdermally [106] was considered acceptable and from 2005 on, double-blind, randomized, placebo-controlled crossover studies were conducted with daily application of this dose over 3 months. Improvements in arousal, excitement and fantasies, anxiety, and well being were described in 53 postmenopausal women with low libido already taking hormone replacement therapy. Side effects did not differ between the testosterone and placebo groups and no changes in lipid profile were seen. A similar study in hysterectomized women already using transdermal estrogen yielded similar results [107], comparable with the approved testosterone patch.

Another pharmacokinetics study [2] analyzed the time course and profile of serum testosterone concentrations during treatment with lower doses of testosterone gel in postmenopausal women with both ovaries, 51 years or older, with total testosterone levels less than 33 ng/dl. After baseline women were treated with 4.4, 8.8, or 13.2 mg of 1 % testosterone gel daily (each actuation of the pump dispensed 0.44 g gel containing 4.4 mg testosterone) for 7 days each in random order, with a 7-day washout period between doses. Two groups were studied: women who had not received estrogen therapy (group I) and those who had received stable estrogen therapy for 3 months or more (group II). Total and free testosterone concentrations were measured for 48 h on the seventh day of each dose administration through sensitive liquid chromatography tandem mass spectrometry, for the measurement of total testosterone levels in women. The average steady-state concentrations (Cav) of serum total and free testosterone increased with increasing testosterone dose andere not affected by estrogen therapy. In both groups, the 4.4-mg dose increased Cav total and free testosterone into the mid- to high-normal range of menstruating women, whereas 8.8- and 13.2-mg doses raised total and free testosterone above the physiological range. Each milligram of testosterone applied transdermally raised Cav by approximately 5.5–6.7 ng/dl. Estradiol concentrations were not significantly increased.

Unapproved Medications

Sublingual Testosterone

An experimental study with a single dose of 0.5 mg testosterone undecanoate and placebo administered sublingually to eight healthy sexually functional women described that testosterone intake promoted a sharp increase in serum levels within 15 min, in genital responsiveness after 3–4 h and in genital arousal and sensations 4–24 h later [108].

Transdermal Testosterone Gel (for Men)

Testosterone gels licensed for men are available in 50-mg/5 mL or 25 mg/2.5 ml sachets. A testosterone axillary solution is available at a 30-mg/pump dosage. Reduced dosage of 0.5–1.0 ml/day or 1⁄4 sachet on alternate days, applied to the lower abdomen (each sachet lasting for approximately 1 week) represents an off label prescription, and responsibility lies with the health professional rather than the manufacturer/supplier. Compounded testosterone 1 % gel produced by local pharmacies at a dose of 0.5–1.0 ml/day represents the same approach. Although no studies are available with such dosages, androgenic side effects and risks were shown to be minimal and reversible if testosterone levels were maintained within the female physiological range. Some studies have shown benefits to the skeleton, cognition, well being, and the vagina; these data require confirmation [109].

Balancing Benefits and Risks of Testosterone Therapy in Women

At this point it is clear that the available testosterone drugs for women represent a pharmacological therapy, not replacement, because they are provided independent of a diagnosis of androgen deficiency syndrome (which is controversial), basically for low libido and sexual difficulties [106]. Furthermore, most published studies used supraphysiological testosterone doses, which achieved high or normal high serum levels. A recent Cochrane meta-analysis identified 35 randomized trials with 5053 participants. Overall testosterone pharmacological therapy, with or without concurrent HRT, in postmenopausal women with normal adrenal function improved various domains of sexual function [110] including libido, reducing personal distress; depending on the dosage, preparation and route, reduced total and HDL cholesterol, triglycerides and increased LDL, acne, and hirsutism. No consistent effects were noted on body composition or bone density. Quality of the evidence was low and long-term safety data were sparse.

Cosmetic androgenic side effects of testosterone therapy are rare when blood levels are kept in the normal range for women. However, when dealing with higher levels, potential effects include acne, increased body hair, balding, and deepening of the voice. Thus women who suffer or suffered from these conditions should not use testosterone therapy. Monitoring with blood tests during treatment is essential. Application site reactions and mild hair growth were the most common observed adverse effects.

As previously discussed, another concern is testosterone conversion to estrogen by the aromatase enzyme or direct stimulation of the androgen receptor on the breast or endometrium and potential increased cancer risk. A prospective cohort study in the Nurses' Health Study showed [111] an increased relative risk for breast cancer appearance or recurrence in women using estrogen plus T in comparison with estrogen alone or no replacement therapy. On the contrary, recent data support a role for testosterone in breast cancer prevention and recurrence when associated

Table 17.4 The BLISS criteria for inclusion and exclusion [114]

BLISS inclusion criteria	BLISS exclusion criteria
• Postmenopausal women older than 50 years with a diagnosis of HSSD	• Treatment with antiandrogens/SERMs • Use of androgen therapy (<2 months before) • Hystory of gynecological cancer • Hystory of melanoma or any cancer • Mammogram BI RADS 3 or more • Acute CV event (< 6 months before) • Major psychiatric illness • Life expectancy <3 years • Use of investigational drug (<30 days)
• CV risk score ≥2 points where: – Age 60–70 years = 1 point and >70 = 2 points – Diabetes mellitus = 2 points – Documented CV disease = 2 points – Smoker ≥10 cigarettes/day = 1 point – Hypertension = 1 point – Dyslipidemia = 1 point	

with anastrazole [112]. In breast cancer survivors, this combination of treated symptoms of hormone deficiency was not associated with recurrent disease. Testosterone + anastrozole implants placed in breast tissue around malignant tumors reduced tumou size, supporting a antiproliferative effect. However, these data are not conclusive and presently, there is no support to influence on the risk of breast cancer by testosterone therapy, while endometrial safety lacks data.

FDA safety concerns about T therapy in women focused primarily on cardiovascular risk. On the contrary, flow-mediated dilation, expressing the endothelium-dependent artery vasodilation, was increased by administration of parenteral testosterone in postmenopausal women taking estrogen [27]. Some studies have shown that testosterone may increase insulin sensitivity [113] in selected groups of women such as those with hypopituitarism and subnormal testosterone levels. Lipid changes may depend on the route of testosterone administration; insulin sensitivity/resistance may depend on endogenous profile as well as ideal serum concentrations of testosterone, neither high (as in PCOS) nor low (as in hypopituitarism or oophorectomy). Testosterone actions are thus patient-, route-, dose-, and gender-specific, different from men.

More information may be provided by a large multicenter long-term study of the safety of low-dose 1 % testosterone hydroalcoholic gel 0.22 g/day (BLISS) in postmenopausal (surgically and naturally) women with HSDD that is being conducted, using a composite of cardiovascular (CV) events including death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, hospitalized unstable angina, and venous thromboembolic events, as well as breast cancer. Inclusion criteria contemplate women with moderately elevated CV risk (Table 17.4) who will be followed up for up to 5 years postrandomization [114].

In conclusion, testosterone administration for women resulted in limited adverse events when physiologic levels of circulating testosterone are achieved but sexual benefits (even if discreet) were obtained when slightly supraphysiological doses were used, making this practice a therapy, not a replacement. In this sense, low androgen levels should not guide testosterone therapy; in carefully selected women with sexual dysfunction, particularly HSDD, testosterone therapy is being investigated and may be justifiable if an individual risk-benefit profile is demonstrated. The current recommendation is to restrict testosterone in women to a short-term

therapy. Long-term risks and benefits of pharmacological testosterone therapy in women remain unknown.

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Testosterone and Endocrine Disruptors: Influence of Endocrine Disruptors on Male Reproductive Tract

18

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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. 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. Those substances are able to accumulate in adipose tissue over 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. Data obtained thus far has mainly been observed in the contamination of animals and confirms the hypothesis that many of those compounds can alter the normal balance of the endocrine system, and that group of chemicals has been given the name 'endocrine disruptors' [1].

An endocrine-disrupting compound has been defined by the U.S. Environmental Protection Agency 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

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or antagonists. Organs at particular risk for developmental abnormalities are those with receptors for steroid hormones: external genitalia, mammary glands, fallopian tubes, uterus, cervix, vagina, prostate, seminal vesicles, epididymis, testes, brain, skeleton, thyroid, liver, kidney, and immune system [2].

In addition to the binding of EDCs on steroid hormone receptors, recent evidence suggests that they could disrupt the endocrine system through various pathways. The EDCs can act through the recruitment of coactivators or correpressors in the enzymatic activities altering hormone synthesis and/or metabolism or act directly on gene expression through epigenetic modifications. Epigenetic changes have greater importance because they produce effects in the exposed individual as well as in subsequent generations (a transgenerational effect) [3].

Some chemicals considered to be endocrine disruptors are important in the manufacturing industry and there has been controversy about whether low doses can produce deleterious effects in humans. For many years toxicologists have believed that the effects of toxic substances were directly proportional to the dose ingested, thus, larger doses could cause more side effects than smaller doses based on a monotonic dose–response curve. However, several studies of EDCs contradict that concept, similar to hormones, EDCs could have 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, 2]. It is worth mentioning 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 EDC exposure to adult populations is a great concern, the exposure to fetuses and infants is more worrisome because their susceptibility to the EDC adverse effects is greater. Adverse effects during development can occur with smaller doses than those considered deleterious for adults [1, 2]. The reasons for the 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 in the fetal programming of diseases in adulthood, particularly those involving the reproductive tract.

It is generally assumed that chronic and low-level EDC exposure after maturity does not permanently alter the functioning of hormone-responsive tissues; however, the possibility does exist. The 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 increases in the incidence of those disorders may 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 testosterone synthesis or action, and in the development of the male reproductive system (Table 18.1).

Table 18.1 History, route of exposure, sources, half-life, and effects of commons 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, neurologic 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, weight loss, 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

(continued)

Table 18.1 (continued)

EDC	Introduction date	Banned/restricted date	Route of exposure	Sources	Half-life	Effects
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, 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 neurologic effects, transgenerational reproductive effects, potential carcinogen

BPA bisphenol A, *DDT* p,p′-Dichlorodiphenyltrichloroethane, *DES* diethylstilbestrol, *EE2* ethinyl estradiol, *MXC* methoxychlor, *PVC* polyvinyl chloride, *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 has been recognized for environmental control agencies worldwide, including among them pesticides, pollutants, substances used in the production of plastics, etc. The EDCs can be classified according to their use, for example pesticides, or its structural property, like dioxins, steroids, polyaromatic compounds, etc.

Industrial Materials

Bisphenol A

Bisphenol-A (BPA) is a monomer used in the manufacture of thermal paper, polycarbonate, 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 [5]. 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 and pacifier nipples, medical equipment, dental products, electronic devices, CDs and DVDs. Thermal paper is produced in massive quantities because it is used in register receipts, books, faxes, and labels and it is also utilized (after recycling) to produce brochures, tickets, mailing envelopes, newspapers, paper towels, toilet paper, and food cartons. Populations around the world are exposed to BPA mainly through food and drinking water and by dust inhalation and dermal contact [6], although food is the highest source of BPA exposure.

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 [4, 7–12]. BPA binds to several receptors including estrogen and androgen receptors as well as to aryl hydrocarbon receptor and peroxisome proliferator-activated receptor [13–15].

It has been proved that BPA behaves similarly to natural estrogen 17-β estradiol, inducing estrogen receptors, but in the concentrations about one thousand times higher (10^{-6}–10^{-4} M) than estradiol [14]. Initially, BPA was described as a weak environmental estrogen whose activity toward classic nuclear estrogen receptor (ER) α and β was over 1000–10,000 times lower in comparison with 17β-estradiol. Nevertheless, further investigations have shown that even in very low concentrations (pico/nanomolar) BPA exerted multidirectional effects on physiological functions of cells and tissues by binding with receptors present out of the nucleus [16].

Estrogenic activity of BPA has been well documented in animal studies [13]. Male mice exposed to BPA during gestational days 16–18 showed increased anogenital distance and prostate size and decreased epididymal weight; those changes persisted during adulthood, and decreased sperm production was also observed [17–19]. The prostate enlargement is mediated through ER present in the stroma, and the effect was blocked by antiestrogens [17]. BPA has been shown to increase

the expression of androgen receptors in the prostate stroma of mice [20]. A recent study also revealed that the increase in the number and size of dorsolateral prostate ducts and an overall increase in prostate duct volume observed in male mouse fetuses is due to an increased proliferation of basal epithelial cells [18]. In conjunction, the results indicate that prenatal BPA exposure results in permanent alterations of the morphology, histoarchitecture, and cell proliferation control in the prostate and other androgen-target tissues, which could be a predisposition to diseases in adult life.

BPA is able to bind and activate both ERα and ERβ and has been shown to suppress androgen production through in vivo and in vitro rat Leydig cells [21]. BPA exposure of male rats with a high fat diet impaired antioxidant capacity in the testis [22]. Exposure of Swiss albino mice to a BPA concentration in the range 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. Those results suggest that BPA induces oxidative stress by altering the expression of inducible nitric oxide synthase, which consequently leads to down regulation of steroidogenic acute regulatory protein expression in the testis [23].

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 testes. Due to the current uncertainty regarding the effects of BPA fetal exposure on human testis, and due to the insufficient number of epidemiologic studies on BPA endocrine disruptive effects, caution should be used in extrapolating these results to human reproductive health [24].

It has been documented in rodents and other animal models that testosterone is converted into estradiol by aromatase that is present in specific brain regions during perinatal development. The conversion of testosterone to estradiol plays an important role in the sexual differentiation of the rodent brain [25]. It is likely that perinatal exposure to environmental estrogen might affect this developmental process. To date, anatomic evidence of alterations in the sexual dimorphism of two brain regions has been reported in rats exposed perinatally to BPA [26–28].

In addition to 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 [29–32]. Recent data suggest that human BPA exposure increases the risk for obesity, diabetes, and heart disease [33–35].

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 and in 2011, The European Commission restricted the use of BPA in plastic infant feeding bottles. In 2013, the Food and Drug Administration banned the use of BPA in baby formula packaging.

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, 2].

Many adverse effects such as abnormal testicular morphology, decreased spermatogenesis, impairment of testicular steroidogenesis, and adverse effects on reproductive performance on the male reproductive system have been demonstrated when overtly toxic doses of TCDD were administered to post-pubescent rats. However, it was reported that TCDD doses even as low as 0.16 pg/kg in pregnant rats resulted in significant reduction in serum testosterone levels shortly after birth, as well as a significant delay in testicular descent, decreased seminal vesicle, and ventral prostate weights which are androgen-dependent parameters. The results demonstrated that perinatal TCDD exposure could affect androgenic status without causing overt toxicity. The male reproductive system in rats appears to be more sensitive to the toxic effects of in utero and lactational TCDD exposure than any other organ [36].

Polychorinated Byphenyls

Polychlorinated biphenyls (PCBs) are a class of industrial chemicals that were globally mass-produced from the late 1920s until they were banned in 1979. The 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 [37]. The general population is exposed primarily through ingestion of contaminated foods (e.g., fish, meat, and dairy products) because PCBs can bioaccumulate up the food chain [1, 2].

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 the human prostate cancer cell line LNCaP found that some compounds reduced cell proliferation, prostate-specific antigen (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 men occupationally exposed to PCBs [38].

Studies in fish demonstrated effects of PCBs on the gonadotropin-releasing hormone receptor (GnRH) system, decreasing preoptic-hypothalamic GnRH content, pituitary GnRH receptors, and the luteinizing hormone (LH) response to GnRH stimulus. The effect was mimicked by an inhibitor of serotonin synthesis, suggesting the possible mediation of PCB effects on the serotonergic pathway [39].

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 perturbation of normal developmental apoptosis. Gene expression of brain derived neurotrophic factor, γ-aminobutyric acid (GABAβ) receptors 1 and 2, insulin-like growth factor (IGF-1), kisspeptin receptor, N-methyl-D-aspartate (NMDA) receptor subunits (NR2b and NR2c), prodynorphin and transforming growth factor (TGFα), known to play important roles in differentiation and migration of hypothalamic neurons, were also changed by PCB exposure [40].

Epidemiologic 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 [41, 42].

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 [43, 44]. Phthalates and phthalate esters are used as liquid plasticizers in a wide range of products including plastics, coatings, cosmetics, and medical tubing. The 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 even detectable in human fluids such as urine, serum and milk [1, 2].

The 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 also been 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 pubertal animals, 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 with permanent changes (feminization) in the retention of nipples/areolae (sexually dimorphic structures in rodents) and demasculinization of the growth of the perineum, resulting in a reduced anogenital distance [45].

Perfluorinated Compounds

Perfluorinated compounds (PFC) are synthetic chemicals with lipophobic and hydrophobic properties. The 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 [46].

Perfluorooctane sulfonate (PFOS) is a neurotoxic agent. The effects of PFOS exposure on the hypothalamic-pituitary-testicular axis were evaluated in adult male

rats 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 follicle-stimulating hormone (FSH) gene expressions also increased. Thus, PFOS exposure in adult male rats can modify the physiological activity of the reproductive system, which could in part be explained by structural alterations in the hypothalamus and gonadotrophic cells [47].

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 has made it a major environmental contaminant. The United States banned DDT in 1972 due to its effects on the environment and potential human 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 [48].

Methoxychlor

The organochlorine pesticide methoxychlor (MXC) was introduced in 1948 and was widely used as replacement for DDT. In 2003, its use was banned as a pesticide in the USA [2]. MXC and its major metabolite 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl)ethane (HPTE) have endocrine disruptor effects. Both MXC and HPTE have estrogenic activities via binding to estrogen receptors and also have antiandrogen effects that are mediated via direct 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 3β-hydroxysteroid dehydrogenase (HSD3B) activity in a non-competitive model [49].

Organotins

Organotins have been widely used as antifouling biocides for fishing nets and ships, agricultural fungicides, and rodent repellents [50]. Organotins tributyltin and triphenyltin have been shown to have antiandrogen action (Fig. 18.1) [49]. 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 vertebrates and invertebrates animals

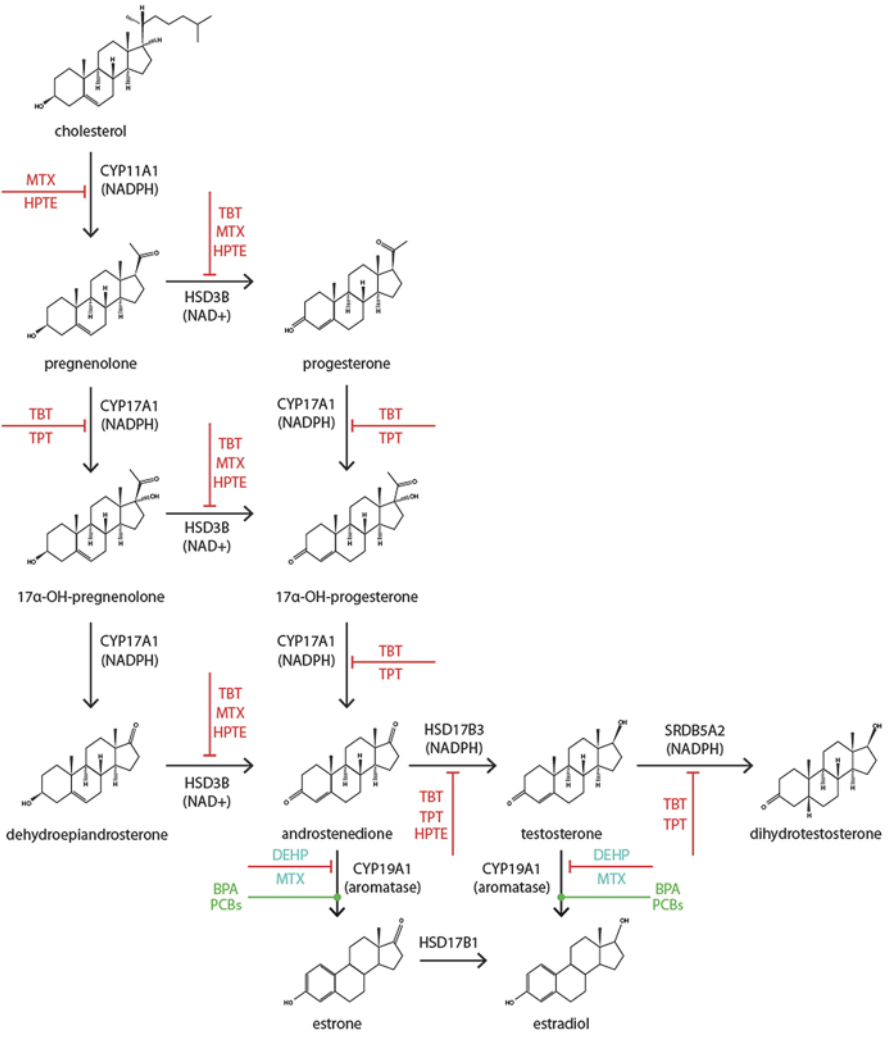

Fig. 18.1 Endocrine Disruptors acting on steroid biosynthetic and metabolic pathways. Adapted from: Ye L et al., Molecules 2011 [49]. *BPA* bisphenol A, *PCBs* polychlorinated biphenyl, *DEHP* diethylphthalate, *MTX* methoxychlor, *TBT* Tributyltin, *TPT* triphenyltin, *HPTE* 1,1,1-trichloro-2,2-bis(4-hydroxyphenyl)ethane

have shown that they interact with steroid synthesis and clearance, inhibiting testosterone biosynthetic and metabolizing enzymes [51].

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 [52]. The in vitro effects of TPT on human testosterone biosynthetic and metabolizing enzymes include the 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 [51]. 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 [53].

Another study investigated the effects of organotins on testosterone conjugation activities, microssomal acyltransferases, and cytosolic sulfotransferases in various invertebrate phyla (molluscs, crustaceans an echinoderms). It was observed that organotins compounds altered both testosterone esterification and sulfation, but with significant differences among species [54].

Vinclozolin

Vinclozolin (VIN) is a fungicide that is commonly used on turf grass, ornamental plants, grapes, and other fruits and vegetables. VIN and its major metabolites (enanilide and butenoic acid) act as anti-androgens by inhibiting androgen receptor (AR) activity. AR activity is required for male reproductive development, and expression of AR influences sexual differentiation, gonadal formation, and reproductive functions [55].

Transient intrauterine exposure to VIN during embrionic gonadal sex determination in male rats induced decreased spermatogenic capacity and increased incidence of male infertility in adults from the F1 generation. Those effects were transferred through the male germ line to nearly all males of all subsequent generations examined (F1 to F4). The effects on reproduction correlate with altered DNA methylation patterns in the germ line [56]. 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 [55].

Although EDC 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 anti-androgenic, 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 discussed below.

Human Disorders Related to EDC Exposure: Evidence from Association Studies

Humans are exposed to at hundreds of environmental chemicals and a major limitation of epidemiological studies is that they usually measure 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 various 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 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 [21].

Animal studies have shown that hypospadia is a common outcome in male pups that have been exposed to antiandrogens in utero. Anogenital distance is sexually dimorphic and androgen dependent, and in males is typically twice that of females (this holds true for humans and rodents). Anogenital distance is considered a sensitive marker of antiandrogen action during development according to toxicologic studies in rodents. Decreased anogenital distance among male infants with prenatal phthalate exposure was demonstrated for the first time in 2005. The authors observed an inverse correlation between prenatal exposure to phthalates and anogenital distance in infant boys and a higher risk of impaired testicular descent [57].

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 recent 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. Epidemiologic data suggested that lifestyle and environmental factors are important contributors to the increasing incidence of TDS symptoms, and genetic factors may be important for the individual susceptibility to endocrine disrupters (Fig. 18.2) [58].

'Idiopathic' partial androgen insensitivity syndrome (PAIS), PAIS-like phenotype, may in some cases be related to EDC contamination during fetal life. Gaspari et al. demonstrated that 11 out of 28 (39.3%) newborn/infant males with 46,XY disorders of sex differentiation presented with normal androgen production, and no AR, SRD5A2 and steroidogenic factor 1 gene mutations. Parental fetal exposure to

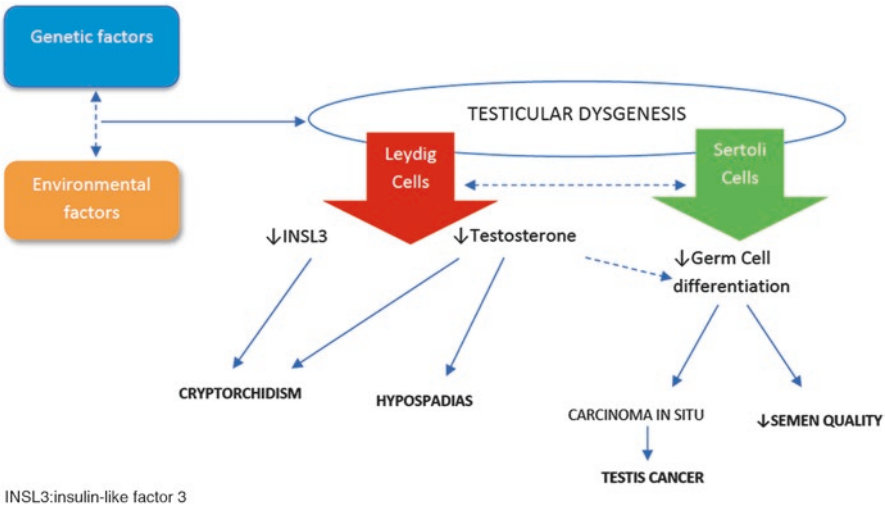

Fig. 18.2 Etiology and pathogenesis of the testicular dysgenesis syndrome. Adapted from: Bay K et al., Best Practice & Research Clinical Endocrinology & Metabolism 2006 [58]

EDCs before or during the patients' fetal life could be involved. The mean estrogenic bioactivity, analyzed by ultrasensitive bioassay, in the 11 patients with fetal EDC exposure was significantly increased (6.65 ± 8.07 pg/ml) in comparison to the remaining 17 cases (1.27 ± 0.34 pg/ml) and to controls (1.06 ± 0.44 pg/ml; $P < 0.05$) [59].

Impaired androgen production associated with EDC exposure was observed in a study with a significant sample size. In the cross-sectional study, serum and semen samples of 247 healthy men were evaluated to investigate whether PFC exposure influences testicular function. Serum testosterone, E_2, 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 [46].

In a recent 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 toxicologic and low epidemiologic evidence for male infertility and low testosterone concentration to phthalate exposure; and low epidemiologic and weak toxicologic evidence for testicular cancer and prenatal PBDE exposure. The authors concluded that EDCs may contribute to male reproductive disorders, which may result in annual treatment cost of €15 billion in the European Union [60].

In primates, male sexual brain differentiation is believed to result from a combination of estrogen receptor and androgen receptor-mediated processes [61]. Low estrogen levels are required for the development of the female brain phenotype and

effects on the developing brain could therefore be expected from estrogenic, as well as antiandrogenic EDCs [62]. Lichtensteiger et al. recently evaluated the effect of mixtures of predominantly antiandrogenic or estrogenic EDCs and a complex EDC mixture containing the neuroactive drug paracetamol (which has an antiandrogenic activity) on the developing rat brain during a critical period, a sex hormone-sensitive period. All mixtures had a strong and mixture-specific impact on gene encoding for components of excitatory glutamatergic synapses and on genes controlling migration and pathway of glutamatergic and GABAergic neurons, as well as on genes linked with increased risk of autism spectrum disorders. Because the development of the glutamatergic synapses is regulated by sex steroids also in hippocampus, this may represent a general target of ECD mixtures [63].

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. The reawakening of the HPG axis stimulates gonadal sex steroid production leading to the development of secondary sexual characteristics, accelerated growth, and achievement of fertility. Onset of puberty is characterized by pulsatile GnRH release from the hypothalamus that stimulates pituitary LH secretion, which in turn drives testosterone production in boys through testicular Leydig cells. GnRH also increases FSH secretion which promotes maturation of the seminiferous tubules and spermatogonia. Serum concentrations of LH, testosterone, and inhibin B are positively associated with age and pubertal progression in males (Fig. 18.3) [64].

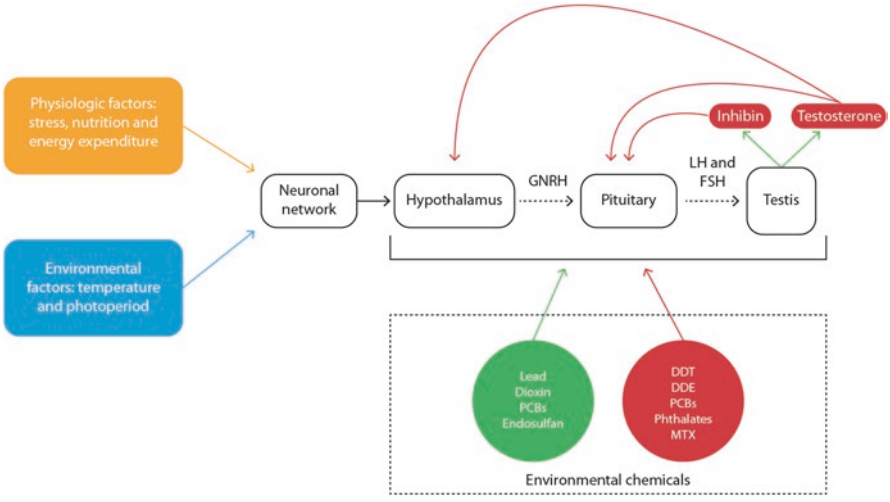

Fig. 18.3 Schematic of Hypothalamic–Pituitary–Gonadal (HPG) axis and the potential endocrine targets for EDC action on puberty. Adapted from: Zawatski W & Lee MM, Journal of Endocrinology 2013 [65]

In girls, FSH levels rise during the early stages of puberty, and LH levels tend to rise in the later stages; from beginning to late puberty, the LH concentration rises more than 100-fold [64]. While earlier onset of thelarche and pubarche in females has been consistently observed in recent decades, 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 puberty development [64].

For instance, the exposure of developing rodents to high doses of estrogenic EDCs advances puberty and alters their reproductive function. Low environmental doses of those 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 the incidence of genital abnormality in boys and earlier sexual maturation in girls [65, 66].

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 males 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 [67].

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 epidemiologic 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 [68].

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 those 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 with controls [69]. However, this association was not confirmed in

a large cohort comprising 555 healthy boys [70]. These data show the need to perform longitudinal studies to verify the role of DEHP modulation in pubertal gynecomastia.

Fertility

The decline in semen quality throughout men's lifetimes has been the subject of international debate with some studies suggesting that the quality of human semen decreases before 50 years of age, while others did not report a decline [71, 72].

Despite the relevance of EDC exposure, particularly PCBs, pesticides, and phthalates, the epidemiologic evidence of its relationship with 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 [73]. In contrast, a Swedish study found no association of MBP or monobenzyl phthalate with any of the semen parameters [74]. 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. Those relationships have been consistently observed in countries such as India, the Netherlands, Taiwan, Sweden, and the United States. In those series, serum PCB levels ranged from low to high values, and were related to the consumption of fish or contaminated rice oil [75, 76]. Regarding exposure to dioxins, it is suggested that the exposure period can also play a critical role in semen quality [77]. The explosion of a chemical plant in 1976 in Seveso, Italy, led to environmental contamination with high levels of TCDD. The men who were exposed 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, men exposed between 18 and 26 years of age had no association with serum TCDD levels and semen quality.

Additionally, it was observed in another study that men exposed to PCBs under the age of 20 years were found to have a lower chance of fathering a male offspring compared with non-exposed age-matched men. The sperm of men born to those women exposed to PCBs had abnormal morphology, reduced motility and strength [69]. Those 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 lower than that observed in the changes in semen quality. However, it is important to mention 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 [78].

It is observed that there is geographic variability in the increases of the incidence of TGCT, while the increase of the incidence occurred in a relatively short period, those data indicated that genetic factors alone cannot explain the phenomenon. Therefore, environmental factors and lifestyle may play a causative role in the process. Those hypotheses are supported by migration studies in which the first generation of immigrants have incidence rates similar to those of their country of origin, but their descendants have tumor rates similar to those of the country in which they reside [79].

At present, the evidence of the relationship between EDCs and the risk of TGCTs are limited. Interestingly, in a case-controlled 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 [80]. The measured organochlorine include 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.

Much of the results found in population studies are in accordance with experimental studies in animals regarding the studies of EDC effects on male reproductive system, but there are some pitfalls. A mixture of various 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, therefore it is not always clear whether to look for in utero, perinatal, pubertal, or life-long exposure. Additionally, epidemiologic research in general may be influenced by many factors such as selection of the study area, sample size, gender, age, and body fat so that adjustment for confounders must 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 epidemiologic 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 [1, 2] and principles for public health protection [81]. Those statements were recently revised, summarized, and released [82]. 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 public, the media, politicians, and governmental agencies should be educated on ways to avoid EDC exposure and to specifically protect developing children.

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Testosterone Misuse and Abuse

19

Rakesh Iyer and David J. Handelsman

Background

The pivotal role of the testis as the source of virility and fertility has been known for centuries. In ancient times, castration of men and animals was practiced to generate obedient slaves or harem guardians, as punishment, religious self-mutilation, or revenge for sexual misdemeanor, 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 such 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 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 Carribean in search of the fabled Fountain of Youth and other imagined schemes for life extension [3]. Undoubtedly, however, the greatest flowering of rejuvenation quackery occurred at the turn of the twentieth century and was associated with the names of Brown-Sequard, Steinach, and Voronoff [4]. Organotherapy had garnered scientific credence in the late nineteenth century when Berthold experimentally demonstrated the androgen dependence of male secondary sexual characteristics by finding that castration-induced

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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 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 and heightened his intellectual capacity and sexual potency for prolonged periods. This was derided by contemporary peers as fantasy [5] and subsequently proved to be a placebo effect because his well-documented aqueous extraction procedure obtained only trivial amounts of hydrophobic testosterone [6]. Nevertheless, treatment using 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 vast 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 onset 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 characterized 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 term testosterone, and was 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 of 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 unrecognized 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, body builders, and others for recreational, cosmetic, or occupational reasons.

Testosterone Use

The testis has two distinct but interrelated physiologic 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 lutenising

hormone (LH)-dependent testosterone secretion, whereas defects of the hypothalamus-pituitary unit reduces pituitary LH secretion that impairs the LH drive to Leydig cell testosterone secretion. Physiologic testosterone replacement remains the appropriate treatment for men with pathological hypothalamo-pituitary or primary testicular disorders that are usually life-long 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 human chorionic gonadotropin (hCG) and Follicle-stimulating hormone (FSH) treatment [9].

Any hormone deficiency condition resulting from a pathological disorder of the endocrine organ and/or its neuroendocrine or other regulation warrants replacement of the deficient hormone with the goal to rectify the deficiency by restoring physiologic exposure to the deficient hormone. In that process the aim is 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 pharmacologic 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.

Testosterone can be used in two distinct clinical modes. In one, testosterone is used for physiologic androgen replacement therapy (ART) for men with an 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 conditions or, analogously, replacement of non-endocrine organs by transplantation for organ failure. The other mode of testosterone use is as part of pharmacologic androgen therapy (PAT) whereby testosterone, or more usually a synthetic androgen analog based on testosterone structure, is used with the goal of improving the natural history of an underlying non-endocrine systemic disorder rather than rectifying a hormonal deficiency condition. Synthetic androgens have the advantage of enhanced potency and other pharmacologic properties (e.g. oral bioavailability, prolonged duration of action) so as to be more useful therapeutically in non-endocrine disorders. One key difference between physiologic and pharmacologic 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 estrogen. By contrast, PAT 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 physiologic 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 classic 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 underdiagnosed as exemplified by Klinefelter's syndrome, the most frequent single cause of classic AD, in which only ~25 % are diagnosed during their lifetime [11] even in countries with national health systems which ensure access to medical care regardless of financial limitation. Classic AD is a clinical diagnosis with a pathologic 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 fetal life, to incomplete sexual maturation during adolescence and to regression of virilization 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 conditions as well as most chronic diseases. Hence, while such non-specific symptoms are typical of classic AD, they occur highly consistently and at reproducible serum testosterone concentrations within individuals [12] but vary greatly between individuals. As a result, individual non-specific symptoms would have extremely low positive predictive value for pathologic 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 overdiagnose apparent AD.

Knowledge of testosterone physiology and influences on it is required to appropriately interpret serum testosterone measurements. As serum testosterone shows diurnal and intra-individual variation [13], multiple morning blood samples to measure serum testosterone along with serum LH, FSH, and sex hormone-binding globulin (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 life-long classic 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 normalization of physical and somatic symptoms and by maintaining its positive effects on somatic tissues.

Pharmacologic Androgen Therapy (PAT)

Use of testosterone, or more often its synthetic androgen analogs, as treatment for non-reproductive disease is 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 anemia and anemia 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 remain a cost-effective, affordable alternative.

Testosterone Misuse

Misuse of testosterone is defined as prescribing of testosterone without valid medical indications, and includes prescribing androgens for male sexual dysfunction (in the absence of proven AD), male infertility, and as a tonic for nonspecific symptoms associated with aging, particularly sexual dysfunction and reduced vitality. Despite absence of any valid new indications, off-label testosterone prescribing has increased markedly in more than 40 countries over first decade of the new century and in its second half in particular [16], most dramatically in Canada where per capita testosterone sales increased by 40-fold. In the US, testosterone sales increased tenfold [16] during that decade whereas prescriptions among health-insured men 40 years or older increased by only threefold [17] indicating that the greatest increases in testosterone prescribing were 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 those 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 overuse/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 differences and differences among countries [20–23] with the most dramatic escalation in Canada [16] where many internet pharmacies are physically based and not subjected to US Food and Drug Administration (FDA) regulatory controls. In addition to the direct-to-consumer advertising in North America, and single-issue and anti-aging 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).

Major additional drivers complicit in this greatly expanded “market” for testosterone have 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-aging 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 prefer mostly long acting rather than daily administration products for long-term therapy [28]. Although newer testosterone products were licensed for the stated indication of pathological hypogonadism thereby avoiding any need to prove safety and efficacy, after licensing 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 conditions other than pathological hypogonadism.

Testosterone and Male Aging

The most prominent misuse of testosterone is currently as an unproved treatment for male aging. The search for means of an ageless existence remains a resilient social fantasy which recurs whenever sufficient social stability and affluence allows for indulgence in the wishful fantasy of eternal youth or its revival contra-aging. In that setting the prospect of ameliorating male aging 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 past two decades when such wishful thinking reemerged to regain public and professional attention as neorejuvenation therapy for middle-aged and older men [18, 29]. In that revival it was alleged to alleviate symptoms that accompany aging using testosterone to treat newly minted conditions under various misnomers such as “andropause”, “LowT”, and “late-onset hypogonadism”.

Modern medicine aims to not only prolong life but also to improve its quality and foster healthy aging in the process. Increasing life expectancy alone brings more evident age related comorbidities based on the physical and mental changes as part of diminishing capacities and organ functions with aging. Broadly, endocrine organs are also subject to changes that accompany aging with declining serum testosterone like that of thyroxine or insulin like growth factor-1 (IGF-I). However, there is growing evidence that the gradual, inconsistent and modest decline in circulating testosterone associated with progressive aging may be a result of the comorbidities of aging rather than aging itself. Hence, the extension of testosterone treatment to men with partial, subclinical, and/or compensated androgen deficiency states remains not only unproved, but also increasingly implausible in the face of the evidence that the decline in testosterone is the consequence of comorbidities of aging rather than their cause or a deficiency state.

Among unselected men in the community, serum testosterone levels consistently decline with aging [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 showing lower rates [31, 35] or no decline at all among men who remain in excellent health, free from major comorbidities [36]. Similarly, reproductive function is also maintained even after the sixth decade of life in sexually active men [37, 38] with well-known examples of men in their 80s 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 aging has been misleadingly equated with menopause or pathological hypogonadism. However, the functional decline in circulating testosterone accompanying male aging or its comorbidities 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 nonspecific. Such nonspecific 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 aging. Hence, characterizing "andropause" based on arbitrary blood testosterone thresholds levels together with non-specific symptoms [26, 27] remains fundamentally misguided. Such nonspecific symptoms have minimal positive predictive value for authentic androgen deficiency when applied to unselected middle-aged or older men. Coupling of symptoms with a coincident low serum testosterone only selects for men who have comorbidities of aging. Hence, in essence, this "definition" of andropause is merely a surrogate for aging and its comorbidities, not a novel medical condition in itself.

It is increasingly recognized from studies that age itself does not determine androgen status in aging men but that acquired comorbidities that accumulate during aging determine the perceived impact of aging 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 with the impact of an extra decade of aging [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 comorbidities 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

Genuine, pathologically-based androgen deficiency at any age 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 recognized 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 disorder, which is a sentinel feature of underlying cardiovascular disease and 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]. Phosphodiesterase inhibitor (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 proof that a modest decline in circulating morbidities that accumulate during aging. As such it represents functional hypothalamic-mediated reaction to chronic illness rather than a rectifiable deficiency state with the potential to improve any somatic features of male aging. However, definitive proof requires adequately powered, placebo-controlled clinical studies which should be directed at the comorbidities of aging 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 practiced 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 hemoglobin. In unison, these effects produce major competitive advantage and represent the most potent ergogenic class of drugs known to power sports. Androgens are also widely 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. Recently however, the growing market demand has fostered the development of never-marketed, so-called “designer” and “nutraceutical” androgens. They are synthetic androgens with the distinction between these designations based simply on the ephemeral circumstances in which they are marketed and identified. Those 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 represent a vestige of the unsuccessful quest by the pharmaceutical industry to dissociate the virilizing 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 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 hemoglobin gave athletes a competitive advantage [68]. With the fall of the Berlin Wall, state-sponsored systematic androgen doping by East Germany was unconvered [68] and other comparable Eastern European national doping programs likely remain undisclosed. Toward the end of the Cold War androgen abuse spread from elite power sports to the larger market of the recreational user for cosmetic and image enhancing effects in countries with sustainable illicit drug subculture networks. By the 1990s it 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 fueled by entrepreneurial drug dealers fostering an underground folklore, epitomized by the infamous Underground Steroid Handbook of the early 1980s, culminating to where the majority of current androgen abusers aim to achieve cosmetic goals such as body building and/or image sculpting for recreational or occupational reasons rather than sports doping [69, 70]. In recent times the alarming practice of systematic state-sponsored doping as exemplified by the East German state-sponsored doping program [68] may have resurfaced and involves 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 proved that supra-physiologic 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 synthesized 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. Those problems led to the progressive withdrawal of such 17α alkylated androgens from

the legitimate clinical market due to their toxicity as safer androgens became 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]. Those compounds, with well-known chemical structures, are readily detected by mass spectrometry-based detection methods [80], deterring androgen doping using those compounds because they are highly likely to be detected, a fact widely publicized by the 1988 disqualification of Ben Johnson's Olympic gold medal winning 100 m sprint performance, because of 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 synthesized 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 the 1960s was identified [86]. A recent review notes at least six designer androgens available for purchase over the Internet [79]. Nevertheless once identified, these designer androgens have never again been detected in regular doping tests reflecting effective deterrence. However, similar never-marketed androgens are also commonly found in unregulated, over-the-counter, and Internet marketed food supplements, which often don't 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 synthesize an androgen with purely anabolic but devoid of virilizing 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 a new class of drugs called specific androgen receptor modulator (SARM) [88, 89], directly analogous to the class of estrogen mixed partial agonists/antagonists specific known under the marketing term, specific estrogen receptor modulators (SERM). In theory, SARMs are tissue selective androgen receptor ligands synthesized with the goal 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 the development and pre-marketing phase have become available over the Internet with one such compound, Andarine, prohibited in sport since 2008, identified in the urine of athletes [92].

Epidemiology of Androgen Abuse

Androgens have been prohibited in elite sports since the 1970s and world anti-doping agency (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 test results recorded in WADA's 32 approved national anti-doping laboratories. For example, in 2014, among the total of 283,304 samples analyzed, 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 19.1) 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 organizational 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 to sanctions on the Russian athletics team, national anti-doping laboratory, and agency.

Table 19.1 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-estrogen (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

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 centers. 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 the general population as 3.3 % with the prevalence four times higher in men (6.4 %) than in women (1.6 %) [95]. The prevalence of community-based androgen abuse varies according to geographic location, with the highest prevalence reported in the Middle East and South America, as well as among immigrants and minorities within a country [96], findings suggesting the influence of psycho-social 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-reporting 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 during the 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 the year 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 seven gay men had used androgens over 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 using androgens compared with their heterosexual counterparts [102]. There is also evidence that ethnic minorities are more prone to abuse of androgens [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 as direct or indirect. Direct androgen doping involves administration of marketed and non-marketed synthetic androgens or exogenous natural androgens such as testosterone, dihydrotestosterone (DHT), as well as pro-androgens such as dehydroepiandrosterone (DHEA) and androstenedione. Initially, marketed

synthetic androgens were the commonly abused androgens but they 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 utilizing non-androgenic drugs (e.g. hCG, LH, estrogen 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 to 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 the 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 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 particularly as pituitary gonadotrophins (mainly FSH) extracted from cadaveric pituitaries were reported in 1985 to cause deaths from Creutzfeld-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) doses [104]. This lack of efficacy coupled with the need for multiple daily injections and high cost render it 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 provide no performance enhancement and hCG testing can detect early pregnancies which unreasonably breaches 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 desensitization and down regulation ensues as expected as a result of sustained, unphysiologic GnRH stimulation [107]. The prevalence of such GnRH analog use is unknown.

Estrogen 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-estrogens (e.g. clomiphene, tamoxifen, raloxifene, newer SERMs) [109] or by the use of aromatase inhibitors, which inhibit estradiol synthesis. Estrogen blockers are commonly used as part of 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 defering its recovery [110]. By contrast, estrogen blockade by either class of drugs has negligible effects on blood testosterone concentrations in women [103]. This leads to the requirement that female athletes with breast cancer treated with adjuvant anti-estrogen treatment require a therapeutic use exemption to permit their use of banned anti-estrogens.

Patterns of Androgen Abuse

There is wide variation in androgen abuse pattern. It is influenced by the type of sports involved, geography, and drug availability. Commonly used patterns are cycling, stacking, or pyramiding.

Cycling is a common practice characterized by an "on" cycle when users administer steroids for a period of time followed by a rest period called an "off" cycle. The duration of a cycle can vary among users and the rest period is used to recover androgen sensitivity, avoid detection, and/or minimize the side effects. Cycling is used more by competitive athletes to avoid detection and intermittently restore sensitivity to androgens whereas non-competitive body builders, lacking concern for detection, more often use androgen continuously [111]. Pyramiding involves increasing the androgen dose to the 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 pharmacologic profiles to gain synergistic benefit; however, the various androgens often differ mainly or even solely in their trade names.

Androgen abusers also combine use of other banned drugs such as growth hormone (GH), GH secretagogs, and erythropoietin for their ergogenic effects which are claimed to synergize 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 offense 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 non-feasible. 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. That created 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 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 populations [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 are 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 the 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 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 synthesized 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. Therefore, 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 mass spectrometry (MS) further refines the ability to distinguish between endogenous and exogenous steroids included in such cases [120, 121]. As noted, suppression of urine LH may provide corroborative evidence for the administration of any exogenous 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 align 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. On the other hand, they gain the

advantage of also identifying pro-androgens, chemicals with or without 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]. A limitation of in vitro androgen bioassays is their susceptibility to non-specific matrix interference effects from biological fluids such as urine as well as the difficulty in establishing suitably rigorous standardized 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 tumors [129, 130]. Currently, more accurate, sensitive and specific immunoextraction-mass spectrometry proteomic methods are becoming available [131] with some proof-of-principle clinical studies [132]. 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 tumors of the testis or extra-gonadal midline germ cell tumors which require prompt, expert medical management [133] but which are rare, possibly due to the protective effect of exercise on testis cancer [134, 135]. Estrogen blockade by anti-estrogens or aromatase inhibitors are 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 the 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] and are reviewed only in outline form in this chapter. They fall into several categories including cardiovascular, psychological, reproductive, and other effects.

Cardiovascular

A wide variety of cardiovascular ill effects have been associated with androgen abuse. Pathological findings including lethal arrhythmias, cardiomyopathy, left ventricular hypertrophy, myocarditis, myocardial infarction, cardiac tamponade, cardiac failure, thrombotic and hemorrhagic 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 randomized, 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 the clitoris. Recovery of reproductive function after stopping androgen use hinges on duration and intensity of abuse. With prolonged, high dose use, such as professional body builders often using massive doses continuously for years without interruption, it may take more than a year or in some extreme cases, may never recover. Underground and internet doping folklore claims that using hCG or antiestrogens may hasten the recovery of the 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 tumors (adenoma, carcinoma, cholangiosarcoma, angiosarcoma) as well as peliosis hepatis and drug hepatotoxicity (usually cholestatic). Most hepatic tumors 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 effect 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 judgment, 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 idiosyncractic 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 behavior 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 aging. 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-aging 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 aging.

Androgen abuse in elite sports and within the community remains and ongoing concern. Effective deterrence of banned androgens in elite sports has been achieved by 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 the efficacy of androgen for doping in power sports means that such 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, 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 behavior [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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