REVIEW ARTICLE

Drugs 2004; 64 (17): 1861-1891
0012-6667/04/0017-1861/$34.00/0

Androgen Replacement Therapy
Present and Future

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Contents

Abstract

The major goal of androgen substitution is to replace testosterone at levels as close to physiological levels as is possible. For some androgen-dependent functions testosterone is a pro-hormone, peripherally converted to 5α-dihydrotestosterone (DHT) and 17β-estradiol (E_2), of which the levels preferably should be within normal physiological ranges. Furthermore, androgens should have a good safety profile without adverse effects on the prostate, serum lipids, liver or respiratory function, and they must be convenient to use and patient-friendly, with a relative independence from medical services.

Natural testosterone is viewed as the best androgen for substitution in hypogonadal men. The reason behind the selection is that testosterone can be converted to DHT and E_2, thus developing the full spectrum of testosterone activities in long-term substitution.

The mainstays of testosterone substitution are parenteral testosterone esters (testosterone enantate and testosterone cipionate) administered every 2–3 weeks. A major disadvantage is the strongly fluctuating levels of plasma testosterone, which are not in the physiological range at least 50% of the time. Also, the generated plasma E_2 is usually supraphysiological. A major improvement is parenteral testosterone undecanoate producing normal plasma levels of testosterone for 12 weeks, with normal plasma levels of DHT and E_2 also. Subcutaneous testosterone implants provide the patient, depending on the dose of implants, with normal plasma testosterone for 3–6 months. However, their use is not widespread. Oral testosterone undecanoate dissolved in castor oil bypasses the liver via its lymphatic absorption. At a dosage of 80mg twice daily, plasma testosterone levels are largely in the normal range, but plasma DHT tends to be elevated. For two decades transdermal testosterone preparations have been available and have an attractive pharmacokinetic profile. Scrotal testosterone patches generate supraphysiological plasma DHT levels, which is not the case with the nonscrotal testosterone patches. Transdermal testosterone gel produces fewer skin irritations than the patches and offers greater flexibility in dosage. Oromucosal testosterone preparations have recently become available.

Testosterone replacement is usually of long duration and so patient compliance is of utmost importance. Therefore, the patient must be involved in the selection of type of testosterone preparation.

Administration of testosterone to young individuals has almost no adverse effects. With increasing age the risk of adverse effects on the prostate, the cardiovascular system and erythropoiesis increases. Consequently, short-acting testosterone preparations are better suited for aging androgen-deficient men.

1. Androgen Replacement Therapy: General Considerations

1.1 Testosterone

Androgen replacement therapy (ART) aims to replace physiological actions of endogenous testosterone by steadily maintaining physiological blood levels of testosterone.[1] The underlying conditions that render androgen replacement necessary are usually irreversible. The consequence is that life-long androgen replacement is required; however, patient compliance with life-long androgen replacement depends on convenient pharmaceutical formulations ensuring continuity of androgen replacement. The traditional association is that androgens mainly subserve male sexual functioning, but studies over the last two decades have convincingly shown that the actions of androgens are much broader, including those on bone, muscle, cardiovascular functions and

brain and, consequently, androgens have a profound impact on quality of life.[2,3]

The benefits of ART are clear, but the delivery of testosterone to hypogonadal men in a way that approximates normal levels and patterns still poses a therapeutic challenge. Developing an ideal form of ART requires much effort. Among experts, there is consensus that the major goal of testosterone substitution is "to replace testosterone levels at as close to physiological levels as is possible".[1] General agreements about such an ART are: (i) a delivery of the physiological amount of testosterone (3–10 mg/day); (ii) consistent levels of testosterone, 5α-dihydrotestosterone (DHT) and 17β-estradiol (E_2) within normal physiological ranges; (iii) similar circadian patterns of hormone levels as in healthy young men; (iv) a good safety profile without adverse effects on the prostate, serum lipids, liver or respiratory function; and (v) convenience in usage, patient-friendly, with a relative independence from medical services.

It is also generally accepted that testosterone, naturally produced by the testis, is the best androgen for substitution in hypogonadal men. The reason behind the selection is that the biological action of testosterone is partially mediated by testosterone as such, while for some of its actions it must be converted to DHT or E_2. Natural testosterone thus restores the deficiency and develops the full spectrum of testosterone activities in ART. However, the use of natural testosterone for some treatment indications needs to be reconsidered,[4] because the purpose and potential risks of ART may vary with age or with treatment indication. In aging men, for instance, androgen replacement aims to produce physiological anabolic effects, but a serious concern is prostate safety. It is possible that a synthetic androgen could be developed, for example, which is safer for the prostate than natural testosterone. Further studies are required to decide if there are drugs with androgenic action which provide beneficial androgenic effects while still controlling the risks of replacement. This subject is addressed later in this review (see section 8).

Testosterone is a naturally occurring steroid, for the major part (95%) synthesised in the testis. A small amount is produced by the adrenal gland and in women in the ovary. In many target organs testosterone is the biologically active androgen; in other target organs the actions of testosterone depend on its local enzymatic conversion to DHT and/or E_2.

DHT is the most potent natural androgen, and it is formed exclusively through 5α-reduction of testosterone by the enzyme 5α-reductase. This enzyme has two forms produced by distinct, homologous genes: type 1 5α-reductase expressed in liver, skin and brain; type 2 5α-reductase which is characteristically expressed strongly in the prostate and at lower levels in skin and liver.[5] The functional predominance of prostatic expression of type 2 5α-reductase made it feasible to develop a prostate targeted 5α-reductase inhibitor, finasteride,[6] with the organ specificity. Recently, a new 5α-reductase inhibitor, dutasteride, has been introduced. Dutasteride inhibits type 1 and 2 5α-reductase. Its clinical efficacy has been demonstrated by O'Leary et al.[7] Circulating DHT levels are approximately 10% of the blood testosterone levels (1.7–2.1 nmol/L), mostly arising from non-gonadal tissues which express 5α-reductase. The daily DHT production is approximately 5–10% of the daily testosterone production.[8]

The testis expresses type 1 5α-reductase,[9] but at relatively low levels so that the testis secretes quantitatively minimal DHT into the bloodstream. As the classical androgen target organ, the prostate expresses not only high levels of the androgen receptor[10] but also type 2 5α-reductase so that >95% of testosterone entering the prostate is converted to DHT.[11]

The receptors for testosterone and DHT are identical, but the affinity of DHT for the androgen receptor is about four times stronger and the dissolution rate about five times slower, and therewith the conversion of testosterone to DHT is a biological mechanism to locally amplify testosterone action.[12]

Prenatal conversion of testosterone to DHT is a requirement for the formation of the prostate and male external genitalia, as evidenced by the syndrome of 5α-reductase deficiency characterised by genital malformations.[13]

Beyond this stage of development this conversion of testosterone to DHT is still a necessity. In individuals with 5α-reductase deficiency, development and function of the prostate are subnormal, but approximately normal growth of the phallus, ruga-

tion and pigmentation of the scrotum and deepening of the voice are observed. Postpubertally, the actions of testosterone may be more efficacious when testosterone is converted to DHT. However, reports on long-term use of the type 2 5α-reductase inhibitor finasteride (in men generally over the age of 50 years) does not provide strong indications that this is vital. Finasteride treatment reduces circulating DHT by 60%,[14] and <5% of the patients report decreased libido, impotence and decreased volume of the ejaculate.[15,16] No effects on lipids and erythropoiesis were observed. Therefore, it seems that limitation of the reduction of testosterone to DHT produces no major adverse effects in adulthood.

1.2 Estrogens

Similar to DHT, estrogens in men are largely a product of peripheral aromatisation of androgens. In men, testosterone (both endogenous and exogenous) and adrenal androgens (androstenedione, dehydroepiandrosterone), serve as precursors for chemical conversion to estrone (E_1) and E_2 via the enzyme aromatase. The testis itself produces approximately 20% of the total E_2 and approximately 20% of the total amount of E_1 is produced by the adrenal from androstenedione.[17] The blood production rate of E_2 is about 30–40 μg/24h. This amounts to a secretion rate by the Leydig cells of ±5–10 μg/day, 20μg originating from peripheral conversion of plasma testosterone and about 10–15μg from peripheral conversion of androstenedione.[18] The total quantity of E_2 which is formed in peripheral tissues may, however, be significantly higher since part of the peripherally formed E_2 is further metabolised *in situ* to E_1, estriol (E_3) or 2-hydroxy-estradiol and, hence, does not enter the peripheral circulation.

The adipose tissue is the most important source of estrogens in men, but muscles, brain, mammary tissue, skin, liver and bone are also capable of synthesising estrogens from precursors. Circulating E_2 levels in young men are about 70 ± 15 pmol/L, which are equivalent to the levels in the early follicular phase in women.

Several studies have indicated that while plasma testosterone levels show an age-related decline, plasma estrogen levels remain relatively constant with aging in men, resulting in an increased estrogen **:** androgen ratio.[18-20] Factors accounting for the relatively stable levels of plasma E_2 in old age are the very common relative increase in fat mass with aging and the increase in aromatase activity with aging. The increased estrogen **:** androgen ratio corresponds to the clinical observation of the occurrence of gynaecomastia in aging men.

E_2 also binds to sex hormone-binding globulin (SHBG), though less strongly than testosterone. SHBG levels increase generally with aging in men and this may explain why bioavailable and free E_2 levels fall with aging, as is also the case with free testosterone, of which the decline is more extensive than that of total testosterone.

Traditionally conceptualised as 'female hormones', estrogens appear to have unexpected but important effects on the male reproductive system (for review, see Couse and Korach[21]). It is becoming increasingly clear that estrogens have an important effect on the final phases of skeletal maturation and bone mineralisation in puberty. In addition, some studies in aging men show that estrogen levels have a higher correlation with bone mineral density (BMD) than androgen levels (for review, see Riggs et al.[22]). Age-related decreases of E_2, particularly when levels fall below 40 pmol/L, may be a major cause of bone loss in elderly men.[23,24] Although E_2 is required for the attainment of maximal peak bone mass in both sexes, the additional action of testosterone on stimulating periosteal apposition accounts for the larger size and thicker cortices of the adult male skeleton.

Severely impaired estrogen action in men leads to dyslipidaemia and to impaired flow-dependent vasodilatation in peripheral arteries in response to an ischaemic stimulus probably resulting from endothelial dysfunction.[25] Evidence suggests that the effects of estrogen on the vascular system are not exclusively receptor mediated; some actions are nongenomic.[26] Estrogen effects on the brain are also increasingly recognised.[27] Estrogens exert effects on cognitive function, coordination of movement, pain and affective state. In view of the effects of estrogen on many important organ systems in the (aging) male, further research into the role of estrogens is necessary. This may also be significant for the potentially negative effect of estrogens on prostate disease in old age (addressed in section 8.2.1).

In summary, estrogens seem to fulfil essential biological functions in men.

1.3 Nongenomic Actions of Androgens and Estrogens

It is of note that gonadal steroids have almost exclusively been investigated for their slow and prolonged action involving their passage through the cell membrane to cytosolic or nuclear receptors, where they produce effects on DNA-related protein synthesis. In recent years it has become clear that (some) steroid hormones may also have a direct rapid action (seconds to minutes) that does not involve gene expression and, therefore, are termed nongenomic action. Among the nongenomic actions of steroids that have been described are the interactions with a variety of membrane receptors, ion channels and transporters. Steroids may change the biophysical properties of membranes. The nature of a steroid-induced signal (genomic vs nongenomic) may depend on the type of target cell, the receptor location within cells, as well as the ligand itself.

1.4 Transport of Androgens

Circulating testosterone is largely bound to proteins, primarily albumin and SHBG. The affinity of testosterone for SHBG is about 1000-fold higher than its affinity for albumin. About 60% is bound to SHBG and 38% to albumin and only 1–2% is unbound and designated as free testosterone, which is the physiologically active fraction. Binding proteins as SHBG and albumin can act as a storage for steroids.[28-30] The binding to albumin is rather loose and testosterone bound to albumin is readily available for biological action on target tissues. Therefore, the combined fraction of free testosterone and albumin-bound testosterone are designated as bioavailable testosterone. The earlier held belief that testosterone bound to SHBG is not available for biological action needs modification. The dissociation of protein-bound hormone can occur within the capillary bed with delivery of testosterone to target cells.[31]

With aging, plasma testosterone levels decline while levels of SHBG increase. In one study total testosterone declined cross-sectionally at 0.8% per year of age, whereas both free and albumin-bound testosterone declined at about 2% per year and SHBG increased cross-sectionally at 1.6% per year.[32]

From these data it appears that plasma levels of SHBG have an impact on free and bioavailable testosterone.

1.5 Quantitative Aspects of Androgen Action

One of the problems of ART is the vast amount of androgen molecules to be administered for replacement. The daily testosterone production in the eugonadal adult man lies in the range of 4–7 mg/day, as opposed to daily production of E_2 in women, which lies at its peak in the late follicular phase of the menstrual cycle and amounts to 0.5–1.0 mg/day. This problem can be demonstrated by comparing transdermal delivery of testosterone with that of estradiol. The latter has been relatively easy (two patches per week containing estradiol 0.05–0.1mg), while with transdermal testosterone treatment this is relatively difficult (daily scrotal or large nonscrotal patch containing 8–14mg of testosterone). The large quantities of testosterone needed for their biological actions, as compared with E_2, cannot easily be explained. It is conceivable that an androgen can be designed that retains (most of) its properties, but which has a hormone-receptor interaction in a way comparable with estrogen action where relatively few estrogen molecules achieve powerful biological effects. A selective androgen 7-α-methyl-19-nortestosterone (MENT) is claimed to be ten times more biopotent than normal testosterone[33] and MENT or similar compounds may offer advantages in this regard. MENT will be discussed in section 6.

1.6 Plasma Testosterone Levels Required for Androgen-Related Biological Functions

Androgens exert a spectrum of biological actions, such as effects on libido, spatial cognition and mood, prostate and seminal vesicles, muscle/bone and erythropoiesis. The critical plasma levels of testosterone for actions on the single androgen targets are beginning to be established. If this information is available, the question arises as to what levels of plasma testosterone are attained with the various treatment modalities and do these levels suffice to

restore all androgen-dependent biological functions?

Approximately 50–70% of normal testosterone levels are enough to sustain androgen effects on libido and sexual functions.[34-36] For other biological actions of testosterone, the critical plasma levels are beginning to be established. Bhasin et al.,[35,37] varying the dose of testosterone in eugonadal men whose endogenous testosterone production was suppressed with luteinising hormone-releasing hormone (LHRH) analogues, have in a series of studies convincingly demonstrated that changes in thigh and quadriceps muscle volume, leg power and leg press strength are in conformity with a single linear dose-response relationship. The anabolic effect continued with administration of doses of testosterone producing plasma levels well above the normal range. This is also true for haemoglobin levels. Changes in fat-free mass were correlated with log testosterone levels. Remarkably, maximal prostate antigen levels were attained with relatively low testosterone administration (testosterone enantate 50 mg/week, resulting in plasma levels of testosterone in the range of 12–15 nmol/L). The decline in high-density lipoprotein (HDL)-cholesterol was linearly negatively correlated with plasma testosterone levels.[35,37]

Snyder et al.,[38] investigating the effect of testosterone administration on BMD of the lumbar spine in aging men, found that the effect of testosterone administration was most pronounced in men with low testosterone levels; there was a significant negative relationship between pre-treatment serum testosterone and effect of testosterone treatment on lumbar spine BMD. These findings demonstrated that there is little effect of testosterone on lumbar spine BMD if plasma testosterone levels are raised above the mean. However, in another study of younger men it could be demonstrated that a 100 mg/day dosage of transdermal testosterone gel resulting in high-normal plasma testosterone levels (27 nmol/L) was more effective in decreasing bone resorption markers and increasing osteoblastic activity markers than a 50 mg/day dosage of transdermal testosterone gel resulting in plasma testosterone levels of 19 nmol/L.[39]

Therefore, in summary, anabolic actions of testosterone on bone require at least normal plasma levels of testosterone. The same applies to the rise of serum insulin-like growth factor (IGF)-1 levels. Anabolic effects on muscle and strength require at least normal plasma testosterone levels, but the anabolic action continues when plasma levels are above normal. Prostate stimulation, as far as can be judged from the rise of prostate-specific antigen (PSA) levels, levels off with low-normal levels of plasma testosterone. For restoration of androgen effects on libido, lower than normal levels of plasma testosterone suffice. Testosterone stimulates erythropoiesis and an adverse effect (of too high doses) of androgen administration may be polycythaemia, which is linearly related to the dose of testosterone administered. HDL-cholesterol levels decrease upon testosterone administration and this decline is negatively associated with the dose of testosterone administered.[35]

2. Available Preparations for Testosterone Replacement

Various available preparations of testosterone and their properties are listed in table I. Three approaches are used to make testosterone therapeutically effective: routes of administration, esterification in the 17β-position and chemical modification of the molecule, or a combination of approaches. In clinical practice, particularly in the perception of the patient, the route of administration is most relevant and is used to categorise the preparations described here.

3. Oral and Sublingual Administration

Free unesterified testosterone is absorbed well from the gut but is effectively metabolised and inactivated in the liver before it reaches the target organs. Only when a high dose, such as 200mg of free testosterone, which involves a heavy hepatic load, is ingested, serum testosterone levels become measurable and clinical effects are observed.[40] The capacity of the liver to metabolise testosterone is dependent on age and sex as well as on the function of the liver itself. An oral dose of free testosterone 60mg does not affect peripheral testosterone levels in normal adult men, but produces a significant rise in prepubertal boys and women and in men with liver cirrhosis.[41]

Table I. Properties of different testosterone (T) preparations.

	Normal serum levels of			Convenience	Dose flexibility
	T over 24 hours	E_2	DHT		
Injectable T esters	–	±	±	±	–
Oral TU	±	+	±	+	+
Scrotal T patch	+	+	–	–	–
Nonscrotal T patch	+	+	+	±	–
T gel	+	+	+	+	+
T implants	+	+	+	+	±
Injectable TU	+	+	+	+	–

DHT = 5α-dehydrotestosterone; **E_2** = 17β-estradiol; **TU** = testosterone undecanoate; **+** indicates favourable; ± indicates reasonable; **–** indicates faulty.

Pharmacological changes of the testosterone molecule in the 17α-position render the molecule orally effective. Alkylated derivatives of testosterone including methyltestosterone and fluoxymesterone are administered orally or sublingually. They are metabolised by the liver, like natural testosterone, but more slowly and, like testosterone, interact directly with androgen receptors. Clinical responses are variable[42] and plasma levels cannot be determined because alkylated androgens are not recognised by most testosterone assays. They may increase levels of low-density lipoprotein-cholesterol and profoundly suppress HDL-cholesterol levels because of their route of absorption and metabolism,[43] with resultant possible increased cardiovascular risks.[44] Besides the fact that such high testosterone levels are uneconomical, the prolonged use (especially the 17α-alkylated androgens) has been associated with hepatotoxicity, including hepatocellular adenoma, cholestatic jaundice and haemorrhagic liver cysts.[45]

Another oral androgen, mesterolone, resembling DHT, is resistant to rapid hepatic metabolism and is orally active. It cannot be aromatised to E_2 and may, therefore, lack some of the effects of natural testosterone. Its gonadotropin suppressive capacity is limited and is, therefore, regarded as a moderately potent androgen.

Testosterone undecanoate is testosterone esterified in the 17β-position with a long aliphatic sidechain, undecanoic acid, dissolved in oil and encapsulated in soft gelatin. Of the 40mg capsules 63% (25mg) is testosterone.

After ingestion, its route of absorption from the gastrointestinal tract is shifted from the portal vein to the thoracic duct. Because of its aliphatic chain it travels with lipids in the lymph and reaches the general circulation via the subclavian vein, thus avoiding a first pass through the liver and subsequent metabolism.[46,47]

For its adequate absorption from the gastrointestinal tract it is essential that oral testosterone undecanoate is taken with a meal that contains dietary fat.[48,49] Without dietary fat the resorption and the resulting serum levels of testosterone are minimal.

Maximum serum levels are reached 2–6 hours after ingestion (figure 1) and result in fluctuating serum testosterone levels (for review, see Nieschlag and Behre[41]). To increase shelf life the preparation was recently reformulated and the oil in the capsule is now castor oil instead of oleic acid. Recent studies show that there is dose proportionality between serum testosterone levels and the dose range of 20–80mg.[50] With a dosage of testosterone undecanoate 120–240 mg/day over 80% of hypogonadal men showed plasma testosterone levels in the normal range over 24 hours.[51,52]

Testosterone undecanoate, also on the basis of its flexible dose administration, is probably best suited to supplement the reduced, but still present, endogenous testicular testosterone production in the aging male with lower than normal, but not deeply hypogonadal levels, of testosterone.[53] Long-term use of testosterone undecanoate has been proven to be well tolerated, as demonstrated in a 10-year observation.[54] On the basis of its flexible dose administration scheme testosterone undecanoate has been used in low dose in the management of delayed puberty in boys. It appeared that testosterone undecanoate at a dosage of 20–40 mg/day accelerated the gain in

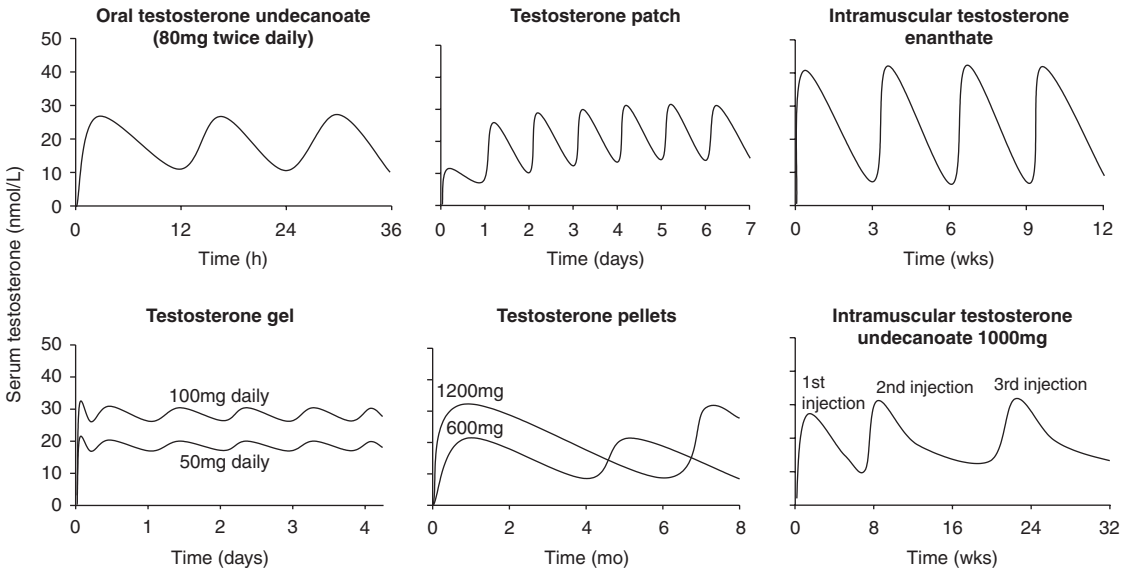

Fig. 1. Serum testosterone levels in different forms of testosterone application (time scales are adjusted per preparation).

height without a significant greater rise in bone age.[55-58] In spite of acceleration of growth testosterone undecanoate did not accelerate the initiation or advancement of puberty itself.[59]

Transbuccal administration of testosterone provides a means of oral administration of testosterone. The resorption of testosterone through the oral mucosa avoids intestinal absorption and subsequent hepatic inactivation of testosterone. In a study by Kim et al.,[60] peak levels of testosterone were reached after less than 30 minutes with an approximately 5-fold increase in plasma levels from baseline. The elimination half-life for this buccal preparation was 1.75 hours. An informative study is the report of Dobs et al.[61] The design was randomised, controlled and double blind. Thirteen men with testosterone levels below 250 ng/dL (8.7 nmol/L) were randomly assigned to take a buccal preparation containing testosterone 10mg or a placebo. Groups were matched for age and nature of hypogonadism. Serum levels of testosterone peaked at 30 minutes at 2688 ± 147 ng/dL (range 1820–3770 ng/dL). Serum levels returned to baseline in 4–6 hours, resulting in an area under the concentration-time curve (AUC) level of 3865 ng/dL that is less than with use of other available testosterone preparations. Profiles of bioavailable testosterone, free testosterone and the metabolites of testosterone, DHT and E_2 were similar to those of testosterone. The effects of buccal testosterone on sexual functioning were comparable with those of parenteral testosterone enantate.

A similar pharmacokinetic profile was observed in women receiving testosterone propionate 1mg buccal lozenges.[62] Peak levels reached were in the earlier mentioned male range. The pharmacokinetics, safety and tolerability of buccal adhesive testosterone tablets were evaluated in a randomised, double-blind crossover design in healthy men made hypogonadal with an LHRH agonist (leuprorelin).[63] The tablets contained testosterone 10mg, 20mg and 30mg. With administration in the early morning a pattern of testosterone levels similar to the circadian variation was observed, with peak values reached after 8–9 hours. Average serum testosterone levels were in the normal range for eugonadal men with each of the dose: testosterone 11.67–14.57 nmol/L, free testosterone 0.026–0.033 nmol/L and DHT 1.66–2.03 nmol/L. Hormone levels increased with dose, but were less than dose proportional. The preparation was well tolerated.

Buccal testosterone administration has recently been extensively tested.[64-66] In one study patients receiving 30mg of testosterone in a buccal mucoadhesive system, applied twice daily, were compared

with patients with a nonscrotal testosterone patch applied once daily. With the buccal system 97% of the patients attained plasma testosterone levels within the normal range (56% with the testosterone patch) and in 84.9% of the time plasma testosterone levels were maintained in the normal range (54.9% with the patch). Plasma DHT levels were in the physiological range.[64] Another study compared plasma levels of testosterone of men with administration of buccal mucoadhesive testosterone twice daily and of testosterone gel. Plasma testosterone levels were similar with both modes of treatment, but plasma DHT levels were lower with the buccal administration but in the physiologial range while these levels were slightly elevated with the gel.[66] The third, smaller-scale study was in agreement with the results of the two other studies.[65]

Sublingual application of testosterone has been tested with the inclusion of the hydrophobic testosterone molecule with 2-hydroxypropyl-β-cyclodextrin (HPBCD). HPBCD enhances testosterone solubility and absorption, but HPBCD itself is not absorbed. In the study of Stuenkel et al.[67] five hypogonadal men received 2.5mg or 5.0mg tablets of sublingual testosterone-HPBCD complex. Maximal levels (a 63 ± 24-fold increase above baseline) were reached after 20 minutes followed by a rapid decline to the normal range after 2 hours with an estimated half-life of decline of 1.87 ± 0.19 hour. The integrated DHT **:** testosterone ratio was normal. Serum E_2 remained in the normal range. There were no accumulations of steroid hormones over the 7-day test period. No clear differences were observed between the test dose of 2.5mg and 5.0mg, suggesting that there is an upper limit to the absorption. Similar results with identical doses were observed in the study of Salehian et al.,[68] but the return to baseline testosterone was a bit slower: 6 hours. Effects on sexual behaviour were comparable with those of parenteral administration of testosterone enantate 200mg every 20 days. A later study investigated the effects of administration of testosterone-HPBCD 5mg three times daily. With this dosage regimen adequate serum testosterone levels were maintained and resulted in an increase of lean body mass and muscle strength. No increase in BMD was observed, but there were significant increases in serum osteocalcin and type 1 procollagen, markers of bone turnover.[69] This drug is not undergoing any further development.

3.1 Transdermal Delivery

Testosterone can be delivered to the circulation through the intact skin, both genital and nongenital.[53] Transdermal administration delivers testosterone at a controlled rate into the systemic circulation avoiding hepatic first pass and reproducing the diurnal rhythm of testosterone secretion, without the peak and through levels observed in long-acting testosterone injections.

3.1.1 Scrotal Testosterone Patch

Scrotal patches were first designed to deliver testosterone through the scrotal skin, where the permeability is five times greater than for other skin sites. It required weekly scrotal shaving, and was difficult for some patients to apply and maintain in position for 24 hours (for review, see Atkinson et al.[70]).

The transdermal scrotal patch (Testoderm™ **1**, Alza Corporation, Mountain View, CA, USA) is a thin film containing 10–15mg of unmodified testosterone and delivers testosterone 4–6 mg/day. Patients using the transscrotal testosterone delivery have reported significant improvements in sexual function, sense of well-being, mood and energy. The patch is worn for 22–24 hours and the scrotum must be hair-free for adhesion. After application plasma testosterone levels rise to a maximum 2–4 hours later and remain within the midnormal range for the next 22–24 hours. After removal, plasma testosterone levels fall very rapidly.

A study in 11 hypogonadal men for 7–10 years showed that plasma testosterone levels remained within the normal range for the full treatment period with transscrotal testosterone.[71] Prostate volumes slightly increased but did not become larger than in a comparison group and PSA levels remained within the normal range. The pharmacokinetic profile met specifications as formulated in the WHO requirements of testosterone substitution. Transdermal scrotal testosterone administration is associated with

1 The use of trade names is for product identification purposes only and does not imply endorsement.

high levels of DHT as a result of high levels of 5α-reductase in the scrotal skin.[70] The patch may be irritating and use is not feasible if the scrotal surface is not adequate. To overcome these limitations, nonscrotal skin patches have been developed.

3.1.2 Nonscrotal Testosterone Patch

Nonscrotal testosterone patches (Androderm™, Watson Pharmaceuticals, Corona, CA, USA) have a reservoir containing testosterone with a permeation-enhancing vehicle and gelling agents.[72] Patches that deliver natural testosterone in the amounts of 5 mg/day (2.5mg for teenagers and 7.5mg for adults) are applied at night on rotating sites on the back, abdomen, upper arms and thighs. The cumulative transfer from the patch to the circulation was 5.48 ± 2.48mg, with 60% delivered during the first 12 hours. The delivery system produces serum testosterone levels with a normal diurnal variation and normal plasma levels of DHT and E_2[73] (figure 1). Improvements have been reported in sexual function, libido, energy level and mood.[72,74]

The most common adverse effects are local skin reactions. Transient, mild-to-moderate erythema at some time during therapy was reported by 50% of men participating in a clinical trial.[73] Generalised allergic dermatitis requiring discontinuation of therapy occurred occasionally. Burn-like blister reactions occurred in 12% of the men.[75]

However, most of these reactions were associated with application of the patch over a bony prominence or on parts of the body that could have been subjected to prolonged pressure during sleep or sitting. Pre-treatment of the application site with triamcinolone cream decreases the skin reactions.[76] Clinical efficacy was as good as with conventional testosterone ester injections.

With regard to drug safety of the testosterone transdermal system, values of PSA and prostate volume by transrectal ultrasound were preserved within normal ranges and no clinically significant changes in lipids or results of serum chemistry studies.[73,74]

3.1.3 Testosterone Gel

Testosterone gel is also used for replacement therapy. Testosterone gel is hydro-alcoholic, 1% (testosterone 10 mg/g of gel) and administered between 5g and 10g of gel a day, amounting to testosterone between 50mg and 100mg. The pharmacokinetics of testosterone gel has been extensively studied. Serum testosterone levels rose 2- to 3-fold 2 hours after application and rose further to 4- to 5-fold after 24 hours.[77] Thereafter, serum testosterone remained steadily in the upper range of normal and returned to baseline within 4 days after termination of application of testosterone gel (figure 1). Mean DHT levels followed the same pattern as testosterone and were at or above the normal adult male range. Serum E_2 levels rose and followed the same patterns as testosterone. The application of the testosterone gel at one site or four sites did not have a substantial impact on the pharmacokinetic profile.[77] Later studies showed that 9–14% of the testosterone administered is bioavailable. Steady-state testosterone levels are achieved 48–72 hours after the first application. Serum testosterone and free testosterone are similar on days 30, 90 and 180 after start of the administration. Serum luteinising hormone (LH) and follicle-stimulating hormone (FSH) were suppressed proportionally to serum testosterone. Only with testosterone gel 100mg dose, and not the 50mg dose, a suppression of SHBG was noted. The formulation of the testosterone gel allows easy dose adjustments (50–75–100mg testosterone).[78]

The clinical efficacy of transdermal testosterone gel on various androgen-dependent target organ systems has been very well documented. The safety profile showed that PSA levels rose in proportion to the increase of testosterone levels but did not exceed normal values. Skin irritation was noted in 5.5% of patients.[39,79] Later studies with a 2.5% testosterone gel showed that 5g of this gel achieved physiological serum testosterone levels in men whose endogenous testosterone production was pharmacologically suppressed. These levels were reached after approximately 10 days. Serum DHT and E_2 did not exceed normal levels. Remarkably, washing of the site of application 10 minutes after application of the gel did not affect pharmacokinetic profiles.[80] Transfer from one person to another was found to be insignificant. No increase of serum testosterone was found after intense rubbing of skins with persons whose endogenous testosterone levels had been suppressed.[80]

The commercially available testosterone gel is AndroGel™ (Besins-Iscovesco, Paris, France; supplied in USA by Unimed Pharmaceuticals, Inc., Buffalo Grove, IL). A recent follow-up study of androgen replacement with the testosterone gel showed that the gel is at least equally as efficacious as treatment with injectable testosterone or testosterone patches.[81] In this study 163 hypogonadal men could be included and evaluable results were available for 124 men. Throughout the observation period plasma levels of total testosterone and free testosterone were in the normal range. Plasma levels of DHT were slightly elevated and the plasma E_2 : testosterone ratio doubled. The initial improvements in libido and mood were maintained and the increases in lean body mass and BMD and the decrease in fat mass progressed or were maintained. Skin irritations were noted in 12 subjects resulting in discontinuation of the gel in one subject. Prostatic events were observed during this observation period but the study was not placebo-controlled, so it is not possible to assess the risks of testosterone replacement on the development of prostate disease;[81] however, similar results were also found in another uncontrolled study.[82]

Recently, a new testosterone gel preparation has been developed: Testim™ (Auxilium Pharmaceuticals Inc., Norristown, PA, USA). Its clinical efficacy was recently demonstrated.[83] Similar to Androgel™, Testim™ is a 1% testosterone gel preparation. One study claims that in 29 hypogonadal patients receiving testosterone 50mg in the Testim™ the maximal levels of testosterone, DHT and free testosterone were respectively 30%, 19%, and 38% greater than with AndroGel™. Similarly, the AUC_{24} for total testosterone, DHT and free testosterone were, respectively, 30%, 11% and 47% larger with Testim™ than with AndroGel™.[83] In a clinical study the mean increases in serum testosterone after 90 days of Testim™ administration were 12.41 nmol/L and 6.54 nmol/L with testosterone gel 100mg and 50mg, respectively. A positive effect was noted on mood, libido and erections. Lean body mass increased and fat mass decreased with both dosages.[84]

3.2 Intramuscular Administration

3.2.1 Testosterone Esters

The most commonly used forms of ART include 17β-hydroxyl esters of testosterone administered with slow-release, oil-based vehicles. Commonly used intramuscular injectable testosterone esters are short-acting testosterone propionate and longer-acting testosterone enantate and testosterone cipionate.

Testosterone enantate is one of the most widely used intramuscular testosterone esters. After a single injection of 250mg maximal testosterone levels are reached after 10 hours, the terminal half-life is 4.5 days.[41] At a dose of 200–250mg of this preparation the optimal injection interval is 2–3 weeks, but peak and trough values are clearly above and below the normal range (figure 1).

Testosterone propionate has a terminal half-life of only 19 hours. After a single injection of 50mg the maximum level is reached after approximately 14 hours.[85] On the basis of this profile, injection intervals are only 2–3 days with peak and trough values above and below the normal range and it is therefore not suitable for monotherapy of testosterone deficiency.

Other testosterone esters are testosterone cipionate and testosterone cyclohexanocarboxylate. The pharmacokinetics of these testosterone esters are very similar to those of testosterone enantate.[86,87] Administration of 200mg of these testosterone esters every 2 weeks provides an acceptable form of testosterone replacement.

Several commercially available testosterone preparations contain a number of short- and longer-acting testosterone esters aiming to deliver more even serum testosterone levels. Pharmacokinetic studies of these preparations show that this goal is not achieved. The peak values are higher than in single testosterone ester preparations and resulting plasma testosterone levels show even larger fluctuations.[41] Therefore, most intramuscular preparations of testosterone are not ideal. With the most commonly used testosterone esters a maximum level follows approximately 72 hours after injection. Testosterone levels slowly diminish during the following 10–14 days, showing an exponential decline of serum testosterone levels reaching baseline at approximately day 21.[88] As a result, the testosterone

levels before the next injection are low.[89,90] The normal pattern of circadian rhythm of testosterone is not provided and the injections are painful.[91] Although levels of DHT are normal, androgen metabolites are frequently not physiological and serum levels of E_2 may become excessive in some men. The profile of testosterone levels may be accompanied by disturbing fluctuations in sexual function, energy level and mood.[92] High post-injection levels of testosterone predispose the patient to acne and polycythaemia, and E_2 causing gynaecomastia. In some patients, injections may be associated with bleeding or bruising.[91] However, these longer-acting testosterone preparations have long time been the mainstay of testosterone treatment and they are the most cost-effective methods, with administration of 200–400mg every 2–4 weeks. The 200mg injection will maintain normal testosterone for approximately 2 weeks while 300mg doses are required for eugonadal ranges for approximately 3 weeks.[89]

3.2.2 Testosterone Bucyclate

Testosterone bucyclate is a long-acting, slow-release preparation. The half-life is about 30 days with maximum levels of 13.1 ± 1.8 nmol/L. An injection of testosterone bucyclate 1000mg maintained serum testosterone levels within the normal range for approximately 16 weeks.[93] With a 600mg testosterone bucyclate injection this was about 12 weeks.[94,95] This preparation is not undergoing further development.

3.2.3 Testosterone Microspheres

Biodegradable microspheres containing testosterone may be a potential new method for slow release of testosterone.[96] Microencapsulated testosterone 630mg in hypogonadal men maintained serum testosterone levels in the eugonadal range for approximately 75 days.[97] Testosterone release from the microcapsule formulation over the first 10 weeks approximated zero order kinetics. Serum DHT levels were in the normal range with a normal testosterone : DHT ratio. Serum LH and FSH declined significantly which was also the case with SHBG. There have been no follow-up studies since 1992.

3.2.4 Testosterone Undecanoate

Testosterone undecanoate dissolved in oil and encapsulated has been available for oral use since the early 1970s. A study from China[98] revealed that that this compound administered parenterally had a significantly longer half-life than the conventional parenteral testosterone esters. After experimentation comparing different concentrations and solutions, testosterone undecanoate 1000mg in 4mL of castor oil proved to deliver an attractive pharmacokinetic profile: the terminal half-life was 33.9 ± 4.9 days with maximal levels of 19.3 ± 2.1 nmol/L reached after 11.4 ± 1.5 days.[99] Later studies with four injections of testosterone undecanoate 1000mg in 4mL of castor oil at 6-week intervals in hypogonadal men showed that intervals between two injections can be as long as 13 weeks. Serum testosterone levels were never found to lie below the lower limit of normal while only short-lived peaks above normal (40.8 ± 3.8 nmol/L) were observed after the third and fourth injection interval.[100] In a study of testosterone undecanoate given to seven hypogonadal men with intervals of 12 weeks, serum testosterone, DHT and E_2 levels were mostly within the normal range and showed a tendency to decrease with longer intervals. Safety parameters, such as bodyweight, haemoglobin, serum lipids and PSA and prostate volume did not change significantly during 3.2 years of observation. Maximum testosterone levels during steady state were measured after 1 week and amounted to 32 ± 11.7 nmol/L. The investigators concluded that this preparation had significant advantages over the conventional parenteral testosterone esters, such as considerably longer intervals between injections and significantly less severe and frequent supraphysiological plasma testosterone levels (figure 1).[101,102]

3.3 Testosterone Implants

Subdermal pellet implantation was among the earliest effective treatment modalities for clinical use of testosterone and became an established form of androgen replacement by 1940 (for review, see Handelsman et al.[103]). Several reports have outlined its desirable pharmacological properties,[103-109] but its use and merits and its complications have been best documented by the group of Handelsman.[103,109-112]

The original implants were not very well standardised, resulting in uneven release, but this problem has been overcome. There are now pellet implants

available in two strengths: 100mg (length 6mm, diameter 4.5mm) and 200mg (length 12mm, diameter 4.5mm).

Pellets are implanted under sterile conditions, usually under the skin of the lower abdominal wall. Implantation at the hip has a higher extrusion rate, but 'track geometry' (two vs four tracks) had no influence.[110] Antibacterial impregnation of the pellets did not decrease the extrusion rate.[109]

Absorption of testosterone from the subdermal pellets occurs via uniform erosion of the pellet's surface from which testosterone leeches out according to the solubility of testosterone in the extracellular fluid. Deviations from the simple surface erosion model may occur late in the time course of absorption if the surface area becomes irregular. Other factors are pellet smoothness/hardness, the site of implantation with its local blood flow and reaction of the surrounding tissue that may lead to encasing of the pellet. The effective testosterone release rate from a 200mg pellet is 1.3 mg/day (95% CI 1.22, 0.137).[112] The number of pellets has no effect on the testosterone absorption rate. With treatment with pellets it is possible to replicate the daily testosterone production rate of 3–9mg in eugonadal men. A single implant of three to six pellets of 200mg will provide the patient with a physiological daily dosage of testosterone for 4–6 months.[112] Pellets constitute a flexible dosage form by using various combinations of pellets of 100mg and 200mg with a delivery of testosterone between 0.65 and 7.8 mg/day in increments of 0.65mg. On the basis of clinical pharmacology and clinical experience the routine dose is four 200mg implants. The time course of plasma testosterone levels is predictable and it is usually sufficient to review a patient after the third month of an uncomplicated implant. It can be calculated that virtually all testosterone will be absorbed from a 200mg pellet within 6 months.

Like other depot testosterone formulations, testosterone implants demonstrate a minor and transient accelerated initial release. This lasts for 1–2 days and involves only 1.5% of total testosterone. The mean plasma testosterone levels reached during these 'bursts' are <50 nmol/L (upper levels of reference values is 35 nmol/L).[112] This compares favourably with injectable testosterone esters which peak to levels between 40 and 80 nmol/L with every administration once every 2–4 weeks (figure 1).

The bioavailability of testosterone (defined by appearance in the blood stream) from testosterone pellets is virtually complete. There is no first pass hepatic inactivation effect and virtually all released testosterone is absorbed into the systemic circulation.

The clinical pharmacology of testosterone implants has been reported in several studies.[103,105,106] The most comprehensive study involved a random sequence crossover design clinical study of 43 androgen-deficient men.[103] These men were treated with consecutively three testosterone pellet regimens (6 × 100mg, 6 × 200mg and 3 × 200mg) at intervals of at least 6 months. Regimen change occurred when blood testosterone levels returned to baseline. Implantation of testosterone pellets provided highly reproducible dose-dependent time course-related plasma levels of total and free testosterone. Plasma testosterone levels on the 6 × 200mg dose were higher but no differences were observed between the 6 × 100mg and the 3 × 200mg pellets implantations which produced similar plasma testosterone levels and a similar time course. Plasma testosterone levels peaked at the first month and gradually declined to return to baseline by 6 months after either 6 × 100mg or the 3 × 200mg pellets implantations. However, the plasma levels remained significantly higher following the 6 × 200mg pellet implantation.

The maintenance of libido, potency and well-being with the testosterone 3 × 200mg implants for 4–5 months appeared to be consistent with the 6 × 200mg implant over 6 months.

In the studies of Handelsman et al.,[103] Jockenhovel et al.[106] and Zacharin and Warne,[108] using a crossover design of testosterone implants and injectable testosterone esters, most men preferred testosterone pellets rather than injectable testosterone esters. The latter were disliked because of the wide swings in plasma testosterone levels, experienced subjectively as swings in mood and energy, and the frequent administrations as compared with the implantation of pellets. Pellet implants are particularly suitable for androgen-deficient men who dislike or are unable to have regular injections. It is best used in men in whom the beneficial effects of testoster-

one replacement and tolerance for androgens have already been established with treatment of shorter-acting testosterone preparations. In the rare event that rapid discontinuation of androgen administration is necessary (such as in the case of a prostate carcinoma) minor surgery to remove the implants may be needed.

Suppression of LH and FSH in hypergonadotropic hypogonadal men occurred in a dose-dependent fashion in all three implant regimens. The 6 × 200mg implants produced a significantly greater suppression than the regimens of 6 × 100mg and 3 × 200mg.[103] With the 6 × 200mg implants the suppression of LH showed nadir levels between 1 and 4 months, with return to baseline only occurring at 6 months. With the 6 × 100mg and 3 × 200mg regimens, nadir levels of LH were observed between 1 and 3 months with an increase beginning by 4 months and returning to baseline by 5 months. Interestingly, the suppression of elevated gonadotropin levels in men with primary hypogonadism mirrored closely the time course of clinical androgen effects and the maintenance of physiological testosterone levels. This suggests that the adequacy of testosterone replacement can be monitored not only by measuring plasma testosterone levels but also the degree of suppression of gonadotropins.

Plasma SHBG levels were not altered by implantation of pellets amounting to 400–1200mg in the study of Conway et al.[105] but a small decrease was seen in the study of Jockenhovel et al.[106] This contrasts with the marked decrease of plasma SHBG after administration of injectable testosterone esters or oral testosterone undecanoate.[105] In the view of Conway et al.,[113] a decline of plasma SHBG with testosterone replacements is an indication of an overload of replaced androgen on liver metabolism.

During the first 4 months after implantation of six 100mg pellets in androgen-deficient men haemoglobulin levels rose. There were no other significant changes in other biochemical variables.[103,112]

In experienced hands pellet implantation has few adverse effects and is generally well tolerated. In a review of 973 consecutive implantations prospectively studied in 221 men over 13 years the continuation rate was 93% overall. One or more adverse effects were observed after 11% of the implantations consisting of extrusions (8.5%), bleeding (2.3%) and infections (0.6%).[112]

3.4 Androgen Replacement with 5α-Reduced Testosterone: 5α-Dihydrotestosterone

The effects of testosterone are mediated directly as testosterone or after conversion to either DHT or E_2 locally in target tissues. The reduction of testosterone to DHT is an amplification mechanism of the androgenising effects of testosterone. DHT binds to the same receptor as testosterone but its receptor binding is stronger, resulting in a considerably higher biopotency than testosterone itself. DHT, as opposed to testosterone, cannot be aromatised to E_2 and therefore acts as a pure androgen. In certain clinical conditions a pure androgen might have advantages over aromatisable testosterone, such as cases of a microphallus, hypogonadal men with a proneness to gynaecomastia or constitutionally delayed puberty in boys. Estrogens are pivotal in closure of the epiphyses in puberty, and a non-aromatisable androgen might allow some extra gain in height by slowing the closure of the pubertal epiphyses.

As argued in section 1.2, in view of the effects of estrogens on bones, brain and the cardiovascular system in men, aromatisable androgens may have potential benefits. In contrast, estrogen effects on the prostate might be deleterious and in this regard DHT might be the preferred androgen for the androgen-deficient aging male. Wang and Swerdloff[114,115] have hypothesised that the decrease in E_2 levels following administration of the non-aromatisable DHT gel may be favourable at the level of the prostate, where estrogens supposedly stimulate the proliferation of the stroma.

In France and Belgium, DHT gel is available as a form of androgen replacement. This is a 2% DHT hydroalcoholic gel (Andractim™, Besins-Iscovesco, Paris, France) administered between 5g and 10g of gel per day. It is applied to a large area of skin of arms, shoulders, chest and abdomen. The gel is rapidly absorbed through the skin into the dermis, which forms a reservoir where absorption into capillaries occurs. A steady-state plateau level of serum DHT is attained after 2–3 days of application.[116-118] Studies of DHT administration to hypogonadal men

show that DHT maintains sex characteristics, increases muscle mass and improves sexual functions without significant increases in prostate size.[116,118] Since DHT is such a potent prostate-stimulating androgen it is paradoxical to recommend DHT for androgen replacement of aging men. However, the available evidence suggests that administration of DHT is relatively prostate sparing. In one study of elderly men, DHT administration resulted in a 15% decrease in prostate size.[116] This effect was ascribed to the lack of aromatisation of DHT to E_2, therewith reducing the hypothesised synergism between androgens and estrogens on the prostate. An alternative explanation is that DHT is less well transported from the circulation to the prostate. In a more recent study of 3-month DHT administration to aging men,[119] no effect on circulating E_2 levels was noted. Prostate disease markers such as serum PSA, the prostate symptom score and central and peripheral volume of the prostate measured with sonography, showed no changes following DHT replacement. In another recent study,[120] DHT was administered for 6 months. In this study a reduction of plasma E_2 was noted, but again effects on the prostate were not observed. Neither were detrimental effects on lipid profiles noted. The two recent studies were of short duration and did not address the question whether the administration of non-aromatisable DHT had deleterious effects on bone, brain functions and the cardiovascular system. On the basis of these findings DHT cannot be dismissed as a potentially useful androgen for the aging male, but a number of issues that have been raised will have to be addressed in future studies.

4. Adequacy of Androgen Replacement: Biochemical and Clinical Endpoints

A general principle in hormone replacement therapy (HRT) is that plasma levels to be achieved over the 24 hours of the day must come close to normal reference values, and should ideally follow the normal diurnal pattern. Therefore, an impression of adequate levels might be gained by determining plasma testosterone before administration of the next dose of the androgen preparation, but this measurement does not reveal deviations from reference values between two administrations. Fluctuations of plasma testosterone levels are strong with injectable testosterone esters but much less so with transdermal testosterone preparations.

It is common clinical practice to judge the adequacy of androgen replacement by the effects on general well-being, mood, sexual interest and sexual activity. An improvement of these variables might well be due to the effects of testosterone replacement but this parameter is rather subjective. Apart from the effects of androgen replacement on general well-being, mood, sexual interest and sexual activity, haemoglobin and haematocrit levels might provide another index in the sense that anaemia, for which no other explanation can be found, might point to undersubstitution. Conversely, polycythaemia might be an index of oversubstitution. BMD, though determined by multiple factors, can be regarded as another indicator of adequacy of sex steroid replacement, but changes in BMD are slow and a higher frequency of measurement of BMD than every 2 years usually does not provide a sufficiently quantitative difference to be informative. In conclusion, these parameters do not provide quick reference regarding whether androgen is adequate.

Plasma levels of LH have not been widely used to guide the dosage of androgens. This principle is less useful when testosterone injections are used with its strongly fluctuating plasma testosterone levels. Snyder and Lawrence,[89] adhering to this principle, established that administration of testosterone ester 200mg every 2 weeks or 300mg every 3 weeks, with fluctuating testosterone levels, were able to maintain LH levels in a physiological range. Reporting on the merits of testosterone implants, Handelsman et al.[103] have included plasma LH to assess the physiological range of plasma testosterone generated by testosterone pellets implanted subcutaneously. There might indeed be reasons to explore more carefully whether monitoring plasma LH might provide guidance as to dosage adequacy. In a study of transsexuals receiving long-term cross-sex hormones, plasma LH was inversely correlated with BMD in these patients. In view of this correlation, plasma LH might be an indicator of adequacy of hormone dosages, at least with regard to their bone-sparing action.[121] In the aging male the situation is complex. Though several studies have found that LH levels are elevated in response to the decline of

testosterone levels with aging, the elevation is less than in younger men with similarly decreased testosterone levels. Therefore, it would seem that there is a shift in the setpoint of the negative feedback action of testosterone on the hypothalamic-pituitary unit resulting in an enhanced negative feedback action of androgens which leads to a relatively lower LH output.[122]

A recent study found that serum LH levels increase with age in independently living elderly men and correlate inversely with a variety of indicators of frailty. The observed relation between LH and frailty, independent of testosterone, suggested that LH reflects serum androgen activity in a different way than testosterone, possibly reflecting more closely the combined feedback effect of estrogen and androgen.[123] Therefore, there may be grounds to give greater attention to plasma LH, at least with those androgen treatment modalities that generate more or less stable levels of testosterone in the range of reference values. Obviously, for newer types of androgens (e.g. nonsteroidal androgens) it remains to be seen whether plasma LH remains an indicator of the adequacy of androgen replacement.

4.1 Recent Insights into Androgen Action

Blood testosterone levels provide insight into the strength of the hormonal signal, but expression of androgen action in target organs depends on properties of the androgen receptor. While androgen receptors are very widespread in the body, a biologically significant action depends on properties of the androgen receptor, its tissue distribution and regulation of co-regulators of the androgen receptor, enhancing or restraining gene expression. Further androgen metabolism by aromatase, 5α-reductase and the cytochrome P450 isoenzyme CYP3A4,[124] and androgen clearance play a role in androgen action. Moreover, a number of androgen actions are nongenomic. The clinical relevance of co-regulators of the androgen receptor is becoming evident in prostate disease. Studies into the transition of hormone-sensitive to hormone-insensitive prostate cancer have elucidated the significance of co-regulators in androgen receptor signalling.[124] Functional polymorphism of the androgen receptor modulates the strength of the genomic signal transduced from the interaction with an androgen as a bound ligand. The best studied functional polymorphism is the exon 1 triplet repeat CAG (i.e. polyglutamine).[125]

Several studies document that the repeat length is inversely correlated with androgen sensitivity. Pathological extension, with over 40 repeats, is the molecular basis of Kennedy's disease which is characterised by spinal muscular bulbar atrophy and deficient androgen action.[126]

An inverse relationship has been found between the repeat length and androgen sensitivity of target organs such as the prostate,[127] bone[128] and cardiovascular risk markers,[129] but not all studies are able to establish this relationship.[130-132] It remains to be seen how soon these insights will result in personalised androgen therapy.[133]

5. Towards Novel Androgen Preparations

In cases of hormone deficiencies, traditional endocrinology aims to replace the missing hormone with a substitute. To afford the full biological action of the hormone, this substitute ideally mimics the natural hormone in molecular structure as closely as possible. Increasing insight on how hormones exert their biological effects has paved the way for rethinking of this traditional aim.

There may be clinical situations where the full spectrum of biological action is redundant or may be even harmful by carrying a (long-term) health risk. Future research must document whether conditions, such as male aging, muscle wasting as in AIDS, osteoporosis and sexual dysfunction (all usually not associated with deeply hypogonadal testosterone levels) can be improved with a form of androgen treatment. Therefore, there may be a need for androgenic compounds intentionally designed not to render the full spectrum of androgenic actions that the normal testosterone molecule has. In fact, this idea is not completely novel. Examples are the so-called anabolic steroids designed to exert the anabolic effects of androgens but without the typical androgenic effects so that they also could be used in women or in children. Critical examination shows that this goal was not (fully) achieved, hence the justified suggestion to use the new term androgenic-anabolic steroids.[134]

HRT in postmenopausal women has set the stage for new developments in the use of gonadal hormones. While HRT with traditional estrogens successfully reduce postmenopausal complaints and are beneficial with regard to preservation of bone mass, estrogens also increase the risk of endometrial and breast cancer,[135] which limits the acceptability of HRT. The observation that the nonsteroidal estrogen antagonist tamoxifen preserved bone mass in postmenopausal women with breast cancer renewed interest in the concept of biological action of both estrogens and estrogen antagonists and their potential clinical use. It appeared that this class of compounds binds to estrogen receptors and has tissue-specific effects that allow them to function as estrogen agonists in some tissues and estrogen antagonists in other tissues, hence the term 'selective estrogen receptor modulator' (SERM). Insight into the molecular basis of estrogenic actions of antiestrogens is growing,[136,137] but is still incomplete.[138] The therapeutic potential of SERMs in women has set the stage for the search for compounds that bind to the androgen receptor and exert tissue-specific agonist/antagonist actions, for which the term 'selective androgen receptor modulators' (SARMs) has been proposed.[139-141]

One area in which SARMs may find an application is the relatively mild androgen deficiency occurring in a subpopulation of aging men. The almost immediate reflex is to dismiss the idea of administering androgens to aging men in view of the preconceived harmful effects on the prostate and the cardiovascular system. Several preconceptions of the dangers of androgen administration to aging men may be exaggerated as the first results of androgen administration to aging men indicate. However, it is certainly worthwhile investigating whether the full spectrum of effects of normal testosterone is needed in old age or whether certain effects are potentially harmful. Modifications of androgenic compounds specifically designed to achieve the specified goals may be a progress, particularly when (some) adverse effects of androgens can be minimised. Postmenopausal and other women whose ovarian endocrine function is insufficient may also benefit from selective actions of androgens, such as effects on libido and mood, and anabolic actions on bone and muscle; however, virilising effects are obviously undesired. Recent progress in the area of gene regulation by steroid receptors and by selective receptor modulators provides an opportunity to examine whether SARMs could address some of the problems associated with current androgen therapy. Since the composition of the transcriptional initiation complex recruited by liganded androgen receptor determines the specificity of gene regulation, synthetic ligands aimed at initiating transcription of tissue and promoter-specific genes offers hope for developing better and more specific ART. Establishment of assays that predict synthetic ligand activity is critical for SARM development. Advancement in high throughput compound screening and gene fingerprinting technologies, such as microarrays and proteomics, will facilitate and accelerate identification of effective SARMs.[140-143]

There are several candidate compounds for SARMs studied in the laboratory.[140-142,144] They are nonsteroidal compounds selective for the androgen receptor. Because of their nonsteroidal nature, they do not undergo conversions to estrogen or are not potentiated as in the case of conversion of testosterone to DHT. In animal experiments, these compounds have shown anabolic action on bone and muscle and LH suppression.[140-143] As indicated in section 1.3, androgens also have non-androgen receptor-mediated effects or nongenomic effects. The nature of a steroid-induced signal (genomic vs nongenomic) may depend on the type of target cell, the receptor location within cells, as well as the ligand itself. Classes of new androgens have not only to be tested for their receptor modulation but also for their nongenomic actions (or lack of) on membranes. The identification of molecules capable of selectively altering genomic versus nongenomic signalling may be useful in delineating the roles of these pathways in mediating androgen responses, and may lead to the development of novel compounds that specifically modulate these signals *in vivo*.[145]

6. A Selective Androgen: 7α-Methyl-19-Nortestosterone

The compound MENT has a much higher biopotency per molecule than testosterone and does not undergo 5α-reduction, which may or may not be an advantage for its effects on the prostate. However, it

retains its capacity to be aromatised to E_2.[146] It cannot be regarded as a SARM since there is no tissue selectivity based on receptor mechanisms, but it is potentially prostate sparing. MENT has a biopotency ten times greater than testosterone.[33] In view of its high biopotency a dosage of 500 μg/day (10% of the amount of normal daily testosterone production) would suffice for ART. In primates the effects of MENT on gonadotropin suppression and on anabolism could be achieved with 10% of the required dose of testosterone.[33,146] The effects of MENT on the prostate are two to three times as potent as those of testosterone, but studies with MENT given in a dose sufficient to sustain gonadotropin suppression and anabolism (10% of the amount of testosterone) showed a 50% loss in prostate volume. The effects of MENT on lipid profiles are approximately ten times as potent as of testosterone.[146] MENT undergoes aromatisation to an estrogen, 7α-methyl-estradiol,[147] which may be relevant for its effects on bone, brain and the cardiovascular system. MENT is capable of restoring and maintaining sexual behaviour in the human male with plasma levels in the order of 15–20% of normal testosterone levels.[148] Pharmacokinetic studies show that the metabolic clearance rate (MCR) of MENT, in both men and monkeys, is much faster than the values reported for testosterone. The faster MCR can be attributed in part to the finding that, in contrast with testosterone, MENT showed no binding to SHBG.[149] The pharmacokinetics of MENT administered as MENT acetate subdermal implants have been studied. Serum MENT levels remained at a steady-state level during the 4 weeks of implant use and were clearly dose dependent. The release rate of MENT from one, two or four implants of MENT 112 ± 4mg were 0.3, 0.8 and 1.3 mg/day, respectively.[150]

7. Target Organs of Androgens

7.1 Muscles

There is little 5α-reductase activity in muscle and, indeed, patients with a 5α-reductase deficiency develop normal male muscle mass in puberty. Significant aromatase levels are present in muscle but non-aromatisable anabolic steroids are effective in animal models. Men with a disruption in the biological action of E_2 had normal male muscle contours. Therefore, it would seem that testosterone itself is capable of its anabolic effects on muscle.

7.2 Bones

Sex steroid deficiencies in both men and women are associated with loss of BMD and increases in bone fractures. The evidence that estrogens protect women from osteoporosis is very convincing. In men, androgen replacement increases BMD.[151,152] Cases of men with an impairment of the biological effects of estrogens presenting with delayed epiphyseal closure and osteopenia have stirred up attention for the role of estrogens in acquiring and maintaining BMD in men. In other cases of men with aromatase deficiency it could be shown that estrogen administration had a significant beneficial effect on skeletal growth and bone maturation (for review, see Rochira et al.[153]). Androgen receptors are present at low densities in osteoblast, which express 5α-reductase activity. (Non)aromatisable androgens probably induce proliferation and differentiation of osteoblasts. Androgen action may be direct or through interaction with the growth hormone-IGF-1 axis. Androgen excess in women is associated with an increased BMD (for a review of the relation of androgens and bone, see Vanderschueren et al.[154]). Although estradiol is required for the attainment of maximal peak bone mass in both sexes, the additional action of testosterone on stimulating periosteal apposition accounts for the larger size and thicker cortices of the adult male skeleton. Therefore, there is convincing evidence that androgens exert effects on (peak) bone mass in men in their own right. Indeed, recent findings support a role of estrogens in the bone loss of aging men[22,24] and some studies in aging men show that estrogen levels correlate better with BMD than androgen levels (for review, see Khosla et al.[155]).

Therefore, with the present state of knowledge it would seem desirable that the types of androgens used in replacement are aromatisable for formation and maintenance of bone mass androgens, since both androgens and estrogens are required for attainment and preservation of male bone mass. Aromatisation of testosterone may, however, be disadvantageous for age-related prostate disease.

7.3 Skin

The action of testosterone on the skin is induction of male-type hair growth and stimulation of sebum production in the pilosebaceous unit that is comprised of the hair follicle and sebaceous gland. Pubertal increase in testosterone and administration of testosterone is often associated with acne. Further male pattern baldness may occur over time.[156] DHT mediates the actions of testosterone on the skin. It was initially believed that the predominant form of 5α-reductase was type 1, but recent studies show that type 2 5α-reductase, is present in the inner layer of the outer root sheath of hair and in the more proximal regions of the hair follicle.[157] This observation explains why finasteride, a selective inhibitor of type 2 5α-reductase, can reduce hair loss in men with male pattern baldness.[158] In sebaceous glands the dominant 5α-reductase is type 1 but finasteride was also able to reduce sebum production.[159] However, type 1 5α-reductase inhibitor is a potential therapeutic target in the prevention/treatment of acne, while a type 1 and 2 5α-reductase inhibitor, such as dutasteride, will prevent or reduce male pattern baldness.[160] Alternatively, androgens which cannot be 5α-reduced may achieve the same results.

7.4 Androgens and Sexual Functioning

Reliable studies on the relationships between androgens and psychological functions have been performed quite recently. There is now solid evidence that androgens stimulate sexual appetite. However, with regard to erectile function the situation is somewhat less clear. It has become clear that in males aged between 20 and 50 years approximately 60–80% of the normal physiological levels of testosterones suffice to maintain sexual functions and that increasing testosterone levels above that threshold adds little to sexual functioning.[34,36] Whether this holds true for aging men remains to be established. Both Schiavi[161] and Bancroft[162] have suggested that circulating androgen levels in elderly men might be insufficient to sustain nocturnal penile tumescence and adequate sexual function.

It is evident that a multitude of factors impact on sexual functioning at all ages, but certainly in old age. Testosterone is only one of these factors. Aging is generally associated with a decline in sexual desire, arousal and activity. The studies on the relationship between testosterone levels and sexual functioning in old age are certainly not unanimous. Men who desired sexual intercourse with a greater frequency than once a week had higher testosterone levels than men with lower frequency.[161] Also, men with the primary diagnosis of hypoactive sexual desire had significantly lower testosterone levels than controls.[163] It has also been reported that men with a greater sexual activity had higher bioavailable testosterone levels than men with a lower frequency.[164] In an epidemiological study of 439 men over 51 years old, low levels of bioavailable testosterone were associated with low sexual activity.[165] Therefore, from these studies it would seem that the age-related decrease of androgens may be a factor in the decline in male sexuality. In contrast, other studies[36,166] failed to observe any correlation between plasma testosterone levels, as long as they were within the normal range, and sexual activity. Interestingly, an inverse relationship was found between sexual inactivity and bioactive LH and testosterone, reversible upon resumption of sexual activity.[167] If these findings can be replicated this factor must be taken into consideration when interpreting plasma testosterone in elderly (and maybe in young) men.

The relationship between erectile dysfunction and androgens is more difficult to establish. Erectile dysfunction increases dramatically with age. Physiologically, testosterone acts primarily on the brain, increasing sexual appetite, but animal experimentation shows that androgens stimulate nitric oxide synthesis in the corpora cavernosa.[168,169] Erectile dysfunction, particularly when associated with a normal libido, is only rarely explained by androgen deficiency.[34,161,170,171] However, recent studies indicate that in men whose erectile dysfunction is not successfully treated with phosphodiesterase inhibitors, androgen administration may be helpful.[172]

7.5 Androgens and Cognitive Performance

There is some evidence to suggest that testosterone may influence performance on cognitive functions,[173,174] which is supported by the finding that testosterone administration to older men enhances performance on measures of spatial cognition. The correlation between testosterone levels and cogni-

tive performance, such as spatial abilities or mathematical reasoning,[175,176] has been confirmed in Western and non-Western cohorts of healthy males.[176]

Testosterone has also been associated with general mood elevating effects. Some studies have found associations between lowered testosterone levels and depressive symptoms.[177,178] Depression is not rare in aging men and impairs their quality of life,[177] therefore the effects that declining levels of androgens may have on mood and on specific aspects of cognitive functioning in aging are well worth researching.

While earlier studies have questioned the relevance of estrogens in human male sexuality,[170,179] a recent study found that estrogen replacement in an aromatase-deficient man increased libidinous aspects of sexuality.[180]

There is also a range of nonsexual effects of androgens on the brain and for (some of) these effects aromatisation of androgen to estrogens might be relevant. Indeed, effects of estrogen on the brain are increasingly being recognised,[27] though studies have mainly been carried out using animal models. Estrogens have been observed to influence many processes in many regions of the brain throughout the entire lifespan. These effects include those on cognitive function, coordination of movement, pain and affective state, involving both the estrogen receptor-α and -β genes. Only some of the estrogen actions on the brain are intracellular receptor mediated, while others take place on the cell membrane, mediated via second messenger mechanisms, neuronal excitability and ion channels.[181]

With regard to Alzheimer's disease, men are relatively protected in comparison with women. One intriguing possibility is the putative neuroprotective effect of estrogens in preventing or retarding Alzheimer's disease.[27] Estrogens increases choline acetyltransferase, the enzyme needed to synthesise acetylcholine.[182] The assumption that postmenopausal women enjoy protection from Alzheimer's disease when they receive estrogen replacement therapy has been challenged by the recently discontinued Women's Health Initiative (WHI) study.[183] The WHI Memory Study showed that the combination of conjugated equine estrogens and progestogens increased the risk of probable dementia in postmenopausal women aged 65 years or over.[183] This could be due to the antagonistic effects of medroxyprogesterone acetate on the positive effects of estrogens on cognitive functions,[184] and not to estrogenic effects *per se*. There is evidence that androgens confer protection from Alzheimer's disease in their own right.[185] Therefore, there may be an advantage in supplementing androgens in aging men, whose testosterone levels have fallen below a certain limit, thereby in fact substituting both androgens and estrogens. However, not all studies in aging men are in agreement. A recent study[186] found a link between cognition and estrogens in women, but not in men, whereas Yaffe et al.[187] found a correlation between cognitive functioning and bioavailable testosterone, but not E_2.

Estrogen contributes to explicit (or declarative) memory function through its action on hippocampal neurons. The implication of this estrogen effect is improved (conscious) recall of facts, events and autobiographical memories.[27,188] Explicit memory is considered the cognitive function that is most vulnerable to loss of estrogen. Women receiving estrogen replacement and men whose estrogen levels are above those of postmenopausal women score better on explicit memory tasks.[189]

In summary, aromatisation of testosterone is probably not required for the effects of androgens on sexual functioning but the evidence for other effects on the brain is much stronger; it seems recommendable that androgen-deficient men, including the androgen-deficient aging male, receives an aromatisable androgen preparation.

It is of note that almost all the studies discussed in this section are observational and in need of replication. Placebo-controlled studies proving the benefits of (aromatisable) androgens on cognitive functioning and prevention or slowing of dementia are lacking. Therefore, it is too early to recommend androgens to improve the age-related decline of cognition in men.

7.6 Estrogenic Actions of Androgens

From the discussion in sections 1.2 and 7.5 it appears that for a number of androgen actions the aromatisation of testosterone is required. Consequently, the question arises whether there is an

'estrogen deficiency syndrome' in men? In other words, are there critical plasma levels of E_2 below which there are signs and symptoms of estrogen deficiency? The symptoms of men who either had an estrogen receptor defect or an aromatase deficiency on the basis of a genetic defect clearly show signs of osteoporosis and of premature atherosclerosis with a negative biochemical cardiovascular risk profile. In the case of men with aromatase deficiency these symptoms improved upon estrogen administration of approximately 0.25μg twice weekly of estradiol.[190] Furthermore, epidemiological studies have indicated that aging men whose plasma E_2 levels fall below 40 pmol/L have an increased risk of loss of bone mass.[23] As long as circulating androgen levels are normal, androgens provide men with sufficient estrogens. There is a direct relationship between levels of plasma (free) testosterone and plasma (free) E_2.[18] All pharmaceutical androgen preparations that deliver normal testosterone also produce a 50–100% increase in plasma E_2 levels.

8. Modifications to Limit Potentially Negative Effects of Androgens

While there may be well founded indications for the use of androgens, (relative) contraindications may exist. The following considerations may be relevant in trying to reduce the potential negative effects of androgen use.

8.1 Cardiovascular Aspects

The higher frequency of cardiovascular disease in men than in premenopausal women has usually been attributed to the atherogenic effects of androgens. It has become clear that both endogenous and exogenous testosterone account for the lower HDL-cholesterol levels in men compared with women. However, this view is too narrow; both androgens and estrogens exert a wide range of favourable and unfavourable effects on laboratory variables related to cardiovascular disease, such as plasma endothelin, clotting factors, insulin sensitivity, homocysteine and lipolysis (for review, see Wu and Von Eckardstein[191]). There is no longer any reason to believe that estrogen administration is cardioprotective. The available evidence does not suggest that testosterone exposure shortens the life span in either gender. Effects of androgen on biochemical variables related to cardiovascular disease do not explain the gender difference in age-specific cardiovascular death rates. It cannot be excluded that androgens are a factor in an earlier start of the atherosclerotic process in men compared with women with a subsequent similar progression in men and women.[192]

In cross-sectional studies of men, relatively low levels of testosterone appear to be associated with coronary artery disease and myocardial infarction. A recent longitudinal study confirmed this observation. In a follow-up study of 66 men, aged 41–61 years over 13 years, the decline in endogenous testosterone was associated with an increase in plasma triglycerides and a decrease in HDL-cholesterol in multivariate analysis controlling for obesity and other lifestyle covariates.[193] There is some evidence that administration of androgens to middle-aged obese men improves their cardiovascular risk profile.[194,195]

Whether (local) aromatisation of androgens mitigates the negative effects that androgens have on some (but certainly not on all!) biochemical cardiovascular risk factors remains to be established. Studies suggest that the aromatisation of testosterone prevents largely the negative effects of androgens on HDL-cholesterol.[196] Estrogens also decrease lipoprotein (a) and prevent lipid peroxidation. E_2 further mediates vasodilatation, inhibits vascular smooth muscle cell proliferation and modulates vascular inflammatory response.[191] Furthermore, in a man with a disruptive mutation in the estrogen receptor gene, flow-mediated endothelium-dependent peripheral vasodilatation and insulin sensitivity was impaired.[25] Therefore, with the present limited knowledge it would seem that aromatisable androgens are preferable in regard to cardiovascular risk factors.

8.2 Prostate

Available evidence indicates that prostate carcinomas only occur rarely in men who are deprived of androgen action from an early age. It is further well established that androgen ablation is palliative in men with prostate carcinomas. As a result, it meets with disbelief that there is no serious evidence that androgens initiate prostate carcinoma.[197,198] The

same applies to the induction of benign prostate hyperplasia (BPH); pharmacological inhibition of androgen action (with LHRH agonists and antagonists, anti-androgens and 5α-reductase inhibitors) also improves clinical signs of BPH. The observation that prostate volumes increase with age regardless of androgen deficiency or replacement indicates that other factors than ambient testosterone levels are determinants of prostate growth in middle age.[198] Treatment of hypogonadal men with conventional androgen preparations induces prostate volumes and PSA levels similar to age-matched controls or smaller.[198-200] Twin studies have shown that elevated testosterone and DHT levels do not predispose to prostate enlargement or symptoms of BPH.[201] A designer androgen that cannot be converted to DHT would, at face value, be a step forward with regard to safety, but whether that is really true remains to be established. It is highly remarkable that androgen-related prostate diseases such as BPH and prostate carcinoma develop in a period of life of men when serum testosterone levels are declining in most men, therefore androgens alone are unlikely to serve as the only causative factor. However, it is of note that finasteride, a 5α-reductase inhibitor reducing DHT levels, is capable of reducing prostate volume in patients with BPH,[202] evidencing a role of DHT in BPH. Similarly, LHRH agonists and flutamide are capable of reducing prostate volume in men with BPH through reducing, respectively, androgen production and androgen biological action. Plasma DHT levels in elderly men are either unchanged or slightly decreased,[203] but a slightly elevated DHT level has been found in men with BPH.[204] Studies of aging men receiving DHT did not indicate that there was a significant increase in prostate size or symptoms of lower urinary tract in spite of elevated plasma DHT levels.[116,118-120] Very recently, the results of the prostate cancer prevention trial with finasteride were published. There was a 24.8% decrease in prostate cancer prevalence in the finasteride-treated group versus placebo,[205] but there were more high-grade cancers in the finasteride-treated group. This possibly fits with the observation that men with low testosterone levels tend to have more aggressive tumours and worse outcomes. The occurrence of cancer was much higher in the placebo group (24.4%) than anticipated (6%), probably as a result of close surveillance of the men in the study. Five men in both groups died of prostate cancer in the study period. The editorial comment suggests that the strong decrease in intraprostatic DHT levels may have created an environment in which high-grade cancers, less dependent on androgens for their growth, had a competitive advantage.[206] The disease burden of low-grade prostate carcinomas which were more prevalent in the placebo group, is unknown and, in fact, more patients might die with, rather than from, a prostate carcinoma. The high rate of detection of cancer in both groups raises concern about the clinical significance of the cancers that were detected and of the clinical relevance of the reduction in the finasteride group. The clinical course of the cancers detected is difficult to predict and it remains unknown how the diagnosed prostate cancer would have affected longevity and/or quality of life. Administration of finasteride caused considerable adverse effects, such as loss of libido, erectile dysfunction and gynaecomastia,[205] but had beneficial effects on lower urinary tract symptoms. The author concluded that the benefits of chemoprevention of prostate cancer with finasteride have not been convincingly demonstrated as long as we do not know the risks posed by prostate cancer once it has been diagnosed.[206] Even with the most benevolent interpretation of the effects of finasteride as chemoprevention, results compare unfavourably with the effects of tamoxifen in the chemoprevention of breast cancer resulting in a 49% reduction of the risk.[207] In conclusion, as outlined, the trial leaves too many questions unanswered to recommend finasteride for chemoprevention of prostate cancer.

8.2.1 Estrogens and Prostate Disease

Not only androgens but also estrogens play an important role in prostate development.[208] Recent knowledge indicates that prostate development depends on the synergistic effect between androgens and estrogens.[209,210] Furthermore, this synergistic effect characterises the different stages of prostate development in human life. There are indications that estrogens may be implicated in the pathophysiology of BPH and prostate cancer. It is believed that estrogens stimulate stromal proliferation and condition the response of epithelium to androgens through IGF-1.[211]

There is evidence that the development of BPH is correlated to serum E_2 levels.[212] Since BPH occurs typically in the aging male, the resultant increase in the E_2 : testosterone ratio has been implicated in its pathogenesis, mainly on the basis of observation in dogs.[208] Histologically, BPH is more of a stromal disease than an epithelial disease. For example, one study found that levels of E_2 and E_1 increased in the stroma, but not in the epithelium, as a function of age.[213] In the normal situation, as opposed to BPH, the levels of E_2 and E_1 are higher in the epithelium of the prostate than in the stroma. The stromal DHT level shows no correlation with age.[213] One hypothesised mechanism for the effects of estrogens on the prostate is that estrogens can induce transcriptional activity of the androgen receptor.[214] From this, it would seem that the inhibition of the biological effects of E_2 might have a beneficial effect on stromal hyperplasia, but studies using the aromatase inhibitor atamestane show that the reduction of estrogen level has no consistently beneficial effect on clinically established BPH.[215,216] The relationship between estrogens and prostate cancer has received less attention, but several recently published case-control studies show a possible role for estrogen receptor gene polymorphisms in the genesis of prostate carcinoma.[217-219] The notion that androgens and estrogens are both necessary in causing prostate malignancy is emphasised by the findings that neither hormone alone is capable of inducing malignant disease of the prostate in the mouse model.[220] The study by Suzuki et al.[219] also showed an association between prostatic cancer and a polymorphism in the cathechol-*O*-methyltransferase (*COMT*) gene. The conversion of guanine to adenosine at codon 158 of the *COMT* gene lead to a four times lower enzyme activity, resulting in a high estrogen environment.[219] Arguing against an important role of estrogens in prostate cancer is a recent study showing that E_2 levels were lower, while cortisol levels were higher, in men with prostate cancer compared with age-matched men with lower urinary tract symptoms.[221] Studies in the (estrogen deficient) aromatase knockout (ArKO) mouse showed that the exposure to high levels of androgens combined with low levels of estrogens did result in BPH, but no malignant changes were observed at all, indicating that estrogens are necessary in prostate cancer development.[222] In addition, treatment of mature male hypogonadal mice with estrogens alone induced direct proliferative changes in the prostate stroma and epithelium, although no evidence of malignancy was found.[220] While the studies in laboratory animals and *in vitro* show a convincing role for estrogens in prostate pathology of old age, reduction of estrogen action has as not yet resulted in successful therapeutic interventions in established prostate disease. Another consideration is that if the increasing E_2 : androgen ratio, commonly encountered in aging men, is significant for the development of prostate disease, administration of androgens to aging men might restore the E_2 : androgen ratio to more youthful values, even though part of the administered androgens will be aromatised to estrogens; however, androgen administration might still tip the balance to a stronger androgenic component. If estrogens are indeed significant in the development of prostate disease in old age, the use of non-aromatisable androgens may be advantageous, but their use must be weighed against the potential disadvantage non-aromatisable androgens may have for their effects on bone, brain and the cardiovascular system.

9. Requirements for the Various Androgen Treatment Indications

9.1 Induction of Virilisation

For induction of virilisation one would like to administer an androgenic compound that preserves most androgen properties. Normal pubertal development of the prostate and induction of a normal male hair pattern require sufficient levels of DHT, and aromatisation of androgens is significant for epiphyseal closure and bone mass. It would be advantageous if the induction of acne could be avoided. Actions of androgens on the skin are postnatally mediated by type 1 5α-reductase; selective inhibition of type 1 5α-reductase would probably prevent acne but would also interfere with the development of sexual hair, though of late it is becoming clear that type 2 5α-reductase is also involved in sexual hair growth.

9.2 Maintenance of Virilisation

Once complete virilisation has been induced, or when hypogonadism occurs past pubertal development, the requirements for androgen replacement may be different. Our large experience with removal of sexual hair in orchiectomised, estrogen-treated (Caucasian) male-to-female transsexuals shows convincingly that only minimal androgen stimulation is required for maintaining sexual hair growth.[223] The relatively small effects of finasteride on prostate function and on sexual interest/functions of aging men provide an indication that, in adulthood, low levels of DHT suffice to maintain sexual functioning.[15] Therefore, it would seem that a high degree of conversion of androgens to DHT is not a requirement. In view of the long-term use of androgens in substitution treatment aromatisation may be an advantage, thus limiting cardiovascular adverse effects and possibly preserving bone mass more efficaciously.

9.3 Aging Males

Androgen deficiency occurs in a subset of aging men.[224] Reliable criteria for androgen deficiency have not been defined. It is uncertain whether reference values of testosterone should be age stratified. This being so, it has been proposed to adopt the same criteria for testosterone deficiency in aging as for the normal population.[224] Androgen deficiency in aging men is usually not severe, so only partial supplementation of androgens is needed. One aspect is the increased negative feedback sensitivity to androgens with aging, so the androgen molecule should preferably be a little anti-gonadotropic so as to preserve the residual testosterone production of the aging male. Potent anabolic effects on muscle and bone are desirable. The actions on bone aromatisation may be relevant which may also limit cardiovascular adverse effects, though this may be less favourable for effects on the prostate. A limited conversion to DHT might be an advantage for the androgen effects on the prostate, although this remains to be established.

9.4 Androgen Administration to Women

It appears that a total absence of (adrenal) androgens has negative effects on a woman's well-being and sexuality, and the benefits of replacement of androgens have recently been corroborated.[225] The effects of dehydroepiandrosterone replacement on indices of sexual functioning in women and men with complete adrenal insufficiency who are virtually devoid of adrenal androgens were very convincing.[226-228] The positive effects of androgen administration to women after ovariectomy for their sexual functioning have been known for the last two decades, although it is difficult to differentiate between effects of physiological replacement and pharmacological effects.[229,230] The beneficial anabolic effects that androgen levels have for female bones are also becoming clear.[154,155] The problems resulting from androgen administration to women are the undesired effects on the female voice and sexual hair growth and clitoromegaly. Effects of high-dose androgens on the breasts and uterus have been studied in female-to-male transsexuals and were not alarming.[231] Studies in women with polycystic ovary syndrome show that they have a higher risk of cardiovascular disease and type 2 diabetes mellitus than normo-androgenic women. A recent publication reviewing the available evidence on the association between polycystic ovary syndrome and cardiovascular disease arrives at the conclusion that there is little evidence of an association of hyperandrogenism *per se* and cardiovascular events.[232] Designer androgens could be specifically tailored for women and should be devoid of undesired biological actions of androgens.

10. Conclusion

Most medical conditions requiring ART are irreversible. As a consequence, ART often extends over many decades. Therefore, patient compliance is of the utmost importance. Noncompliance not only impairs sexual functioning but is also associated with reductions in muscle and bone mass and with negative effects on mood and vitality. In other words, chronic testosterone deficiency negatively affects quality of life.

There is consensus among experts as to the requirements of ART.[1] Replacement with unmodified testosterone is preferred, with a treatment modality and in a dose which maintains plasma testosterone in the physiological range over 24 hours of the day. For some of its functions testosterone is a

pro-hormone to be further metabolised to E_2 and DHT. Ideally, plasma levels of E_2 and DHT, generated by administration of testosterone, should also lie in the physiological range. Adverse effects of ART should be minimal.

The different modalities of ART must be judged with these requirements in mind. The merits and limitations of the preparations available for ART have been extensively outlined in this article. Overall, the traditional injectable testosterone esters (testosterone enantate, testosterone cipionate, etc.) do not meet the aforementioned requirements, but they are inexpensive compared with newer forms of treatment. Oral testosterone undecanoate in a dose of two 40mg capsules twice daily generate plasma testosterone levels which in 80% of patients lie within the normal range 95% of the time. They may be suitable for the aging male with plasma testosterone levels indicating hypogonadism by conventional standards. Plasma DHT tends to be above normal with oral testosterone undecanoate. Transdermal ART with both the scrotal (although generating elevated plasma DHT levels) and nonscrotal testosterone patch and the testosterone gel meet these requirements. This is also the case with testosterone implants and injectable testosterone undecanoate.

As indicated earlier, patient compliance is of great importance. Therefore, the various ART modalities must be discussed with the individual patient to explore personal preferences and idiosyncrasies which, in the end, will impact on patient compliance.

Another consideration is the safety of ART. In the elderly man, short-acting testosterone preparations are preferable. In case a prostate malignancy is diagnosed, plasma testosterone levels should be reduced within days.

In the years to come, with a better understanding of the biological actions of testosterone in the various target organs and of the safety aspects, particularly with regard to the prostate, the goals of ART may have to be reformulated. Androgenic compounds with different degrees of tissue selectivity may find their place in ART.

Acknowledgements

No funding was received to assist in the preparation of this manuscript. Louis Gooren has been a speaker at scientific meetings sponsored by Organon International, Oss, The Netherlands, manufacturer of oral testosterone undecanoate.

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