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Androgens and the ageing male and female

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Androgens play a number of important physiological roles in the human. In the male, testosterone is required for virilization, normal sexual function, and both stimulation and maintenance of bone and muscle mass. Epidemiological studies have shown a progressive decline in testosterone levels with ageing in men. The clinical significance of this decline is still unclear, and there is controversy as to whether a specific syndrome of androgen deficiency or 'andropause' exists. The benefits of testosterone supplementation in this age group have yet to be equivocally established, and long-term safety data on testosterone administration in this setting are lacking. In the female, a decline in testosterone levels with ageing has been less clearly established due, at least in part, to the absence of sensitive assays. Available data suggest that the major role of testosterone replacement after menopause may be in those women who have had an oophorectomy.

Key words: testosterone; ageing; osteoporosis; sarcopenia; libido; post-menopausal.

Testosterone was first synthesized in 1931.[1] However, much time lapsed before the development of testosterone preparations that were easy and safe to use owing to what was perceived as a rather small market. In recent times, there has been a renewed interest in testosterone replacement because of the expanding population over 65 years of age[2] and the possibility that a relative androgen deficiency exists in the older male and post-menopausal female. It is still controversial as to whether such syndromes do indeed exist. Much interest has been focused on the impact of androgen deficiency on sexual function, bone health, muscle mass and cognition. To date, most of this research has been done in the male, although the role of androgens in the post-menopausal female is now attracting increasing attention.

ANDROGENS AND THE AGEING MALE

Changes in testosterone levels with ageing

Numerous cross-sectional studies have evaluated testosterone levels in the ageing male with conflicting results. Some of these studies have shown a decline in

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testosterone levels with ageing[3–7], whereas others have shown similar testosterone levels in older and younger men.[8,9] The different conclusions of these studies may be explained by small sample size, differences in the overall health of the study subjects, and the timing of sample collection. Data from a meta-analysis have shown that, for any given age, men with chronic disease have testosterone levels that are up to 15% lower than those in healthy controls.[10] Given the diurnal pattern to its secretion, testosterone levels are highest in the early morning and decline in the late afternoon. Therefore, differences in testosterone levels between older and younger men are most likely to be seen in studies where sampling was done in the early morning.[5]

Due to the conflicting cross-sectional data, longitudinal studies were undertaken to answer more accurately the question of whether testosterone levels decline with ageing in men. The two largest longitudinal studies are the Massachusetts Male Ageing Study (MMAS)[11] and the Baltimore Longitudinal Study of Ageing (BLSA).[12] The MMAS studied 1156 men aged 40–70 years and found that the decline in total testosterone was 1.6%/year. The decline in bioavailable testosterone was greater at 2–3%/year due to an age-related increase in levels of sex-hormone-binding globulin (SHBG). The BLSA studied 890 healthy American men with an average age of 53.8 years. Using a definition of hypogonadism as a total testosterone level below the 2.5th centile for healthy men (<11.27 nmol/l) or a free testosterone index (total testosterone/SHBG) <0.153 nmol/l, they reported a significant increase in the incidence of hypogonadism with age (Figure 1). Using total testosterone criteria, the percentage of men with hypogonadism in their 50s, 60s, 70s and 80s was 12, 19, 28 and 49%, respectively. Using the free testosterone index criteria, an even larger number of men were deemed hypogonadal: 9, 34, 68 and 91% of men in their 50s, 60s, 70s and 80s, respectively. These longitudinal studies thus confirm that there is a decline in testosterone as men age and that this decline is gradual. However, unlike women, who inevitably go through menopause accompanied by a dramatic reduction in oestrogen levels, a significant number of men do not become hypogonadal with age.

Aetiology of androgen deficiency in the ageing male

Secretion of testosterone is precisely regulated by complex positive and negative feedback interactions within the hypothalamic–pituitary–gonadal (HPG) axis. In the healthy adult male, the hypothalamus releases gonadotropin-releasing hormone (GnRH) in a pulsatile fashion which, in turn, stimulates the pulsatile release of luteinizing hormone (LH) and, to a lesser extent, follicle-stimulating hormone (FSH) from the pituitary gland.[13] Pulsatile LH stimulates Leydig cells to produce testosterone. Testosterone, in turn, exerts negative feedback on GnRH and gonadotropin secretion.[14,15]

The decline in testosterone levels with ageing in men can be explained by a defect in both the hypothalamic–pituitary axis as well as decreased testicular Leydig cell function. Comparisons of frequent sampling data for LH and testosterone secretion in younger and older men reveal that older men have increased LH pulse frequency and decreased LH pulse amplitude.[16] In addition, testosterone negative feedback appears to be enhanced in older men.[17] Due to the decline in testosterone levels in older men, one would anticipate that the area under the curve (AUC) of LH would be increased. In fact, the reverse is actually true, and the AUC of LH is decreased in older compared with younger men.[18] To
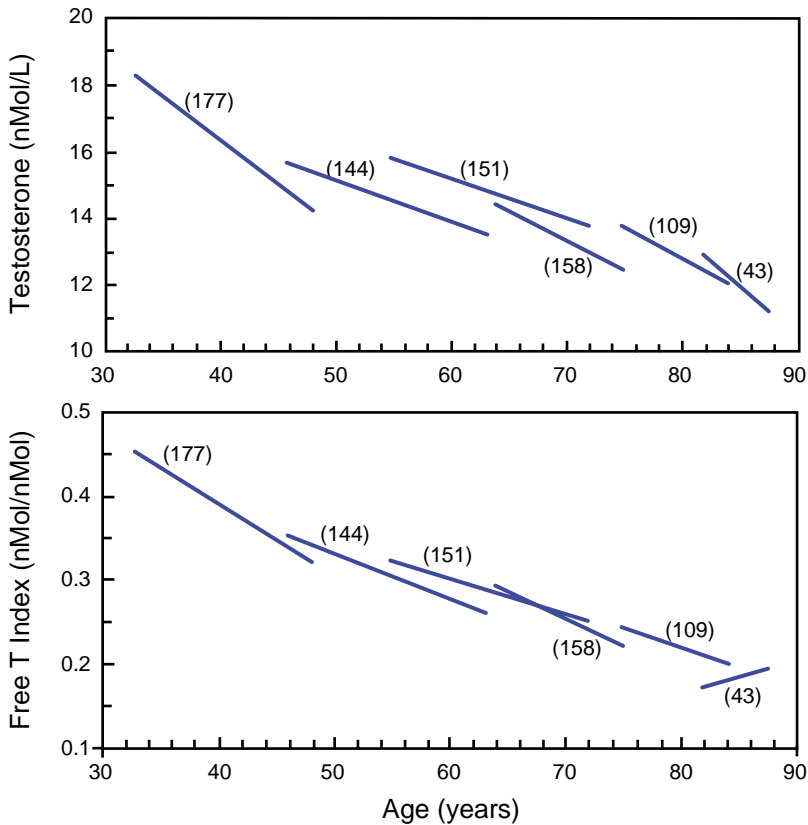
Figure 1. Longitudinal effects of ageing on date-adjusted serum testosterone levels and free testosterone index. Each longitudinal segment has a slope equal to the mean of individual longitudinal slopes in each decade and is centred on the median age in each cohort. The numbers in parentheses represent the number of men in each cohort. Significant downward slope for each decade is observed, except in the ninth decade. No significant change in the slopes is seen over the entire age range. Reproduced from Harman SM, Metter EJ, Tobin JD, Pearson J and Blackman MR (2001, Longitudinal effects of aging on serum total and free testosterone levels in healthy men, *Journal of Endocrinology and Metabolism* 86: 724–731) with permission of The Endocrine Society.

determine whether the defect in the HPG axis is in the hypothalamus or pituitary gland, studies have been conducted to examine the LH response to exogenous GnRH administration. The demonstration of a normal pituitary LH response to GnRH administration in elderly men[19] points to a defect in the hypothalamus, with decreased GnRH secretion resulting in decreased LH secretion.

A defect at the level of the testes also appears to be present in older men as evidenced by a decrease in both Leydig cell mass[20] and function.[21,22] Administration of the anti-oestrogen, clomiphene, results in similar increases in mean LH levels and LH pulse frequency in younger and older men. However, the testosterone levels remain significantly lower in the older than in the younger population.[6] In addition, careful frequent sampling for LH and testosterone has revealed decreased testosterone production for each pulse of LH release in older men.[16]

Diagnosis of androgen deficiency in the ageing male

The diagnosis of androgen deficiency can be a challenging one to make in the ageing male. Clinically, many of the symptoms of decreased testosterone levels, such as fatigue, decreased strength, and loss of libido and potency are non-specific, especially in the older population. The results of biochemical evaluation are not always helpful or consistent in this setting.

Ninety-eight percent of testosterone is protein-bound, 60% to albumin and 40% to SHBG. Testosterone can easily disassociate from albumin but is tightly bound to SHBG. Measurement of total serum testosterone, which includes both the protein and non-protein bound fractions, has long been considered the gold standard for the diagnosis of androgen deficiency. In fact, the Endocrine Society's most recent consensus statement defined hypogonadism in older men as a total testosterone level < 6.93 nmol/l.[23] The reality, however, is that many men have testosterone levels that are just below normal or in the low-normal range (normal 10.40 − 34.67 nmol/l). The clinical importance of such measurements is unknown, especially in the light of previous studies from our group which have shown that as many as 15% of healthy young men have testosterone levels that fall transiently below the normal range during 24 h of frequent blood sampling.[24] In addition, testosterone is highest in the early morning and declines as the day progresses. Therefore, the diagnosis of androgen deficiency should be based on multiple early morning measurements. The timing of the blood draw may not be as important in older men who do not appear to have the same circadian rhythmicity as younger men.[5]

Many researchers have questioned the utility of total testosterone in diagnosing androgen deficiency in the ageing male given the proportionately greater decline in the free than in the total testosterone fraction with ageing. However, measurement of free testosterone is plagued by methodological difficulties. The commercially available kits for measurement of free testosterone are extremely inaccurate, often underestimating levels by as much as 100%.[25] The only truly accurate method for measuring free testosterone is equilibrium dialysis. This assay, however, is difficult to perform, expensive and not available in all laboratories. Another method of assessing a patient's androgen status is to measure bioavailable testosterone, defined as testosterone which is bound to albumin but not SHBG. Measurement of bioavailable testosterone may be particularly useful in situations where SHBG levels are known to change, i.e. with ageing, where they increase, and obesity, where they decrease. However, total testosterone is still the best validated method for measuring testosterone.

While much attention tends to be focused on the measurement of serum hormone levels in diagnosing androgen deficiency, it is important not to overlook the role of the androgen receptor (AR). Testosterone interacts with the AR in target organ tissues. In patients with prostate cancer, a decreased number of N-terminal polyglutamine repeats in the AR has been reported to be associated with increased receptor activity manifested by earlier onset and more aggressive disease.[26] In addition, testosterone is converted to other hormones by various enzymes: to oestrogen by aromatase, and to dihydrotestosterone (DHT) by 5α-reductase. DHT binds more avidly to the AR than testosterone and is thought to mediate most of testosterone's effect in the prostate. In addition, data on men with congenital oestrogen deficiency indicate that oestrogen plays a critical role in stimulating and maintaining bone mass in men.[27,28] Thus, the true androgen status of an individual most likely reflects the combination of serum testosterone levels, the degree of aromatization to oestradiol, 5α-reduction to DHT, and androgen receptor activity.

Osteoporosis and testosterone levels in ageing men

Osteoporosis is a disease of elderly men as well as women.[29] Young men with overt hypogonadism have increased bone loss, which improves with testosterone replacement as shown by increased bone mineral density and improvement in bone resorption markers.[30] Owing to this observation, there has been much interest in the role of testosterone in bone health in older men. A number of observational studies have attempted to correlate bone mineral density with testosterone levels, with conflicting results.[31] To date, the best study on the effect of testosterone therapy on bone density in middle-aged men is a randomized, double-blind, placebo-controlled trial of 108 men over 65 years of age. To be eligible for the study, subjects had to have a baseline testosterone level < 16.47 nmol/l and were then randomized to either a testosterone patch or placebo for 36 months.[32] Overall, there were no differences in bone mineral density at the hip or spine between the testosterone- and placebo-treated subjects. Linear regression analysis, however, showed that lumbar spine bone mineral density did increase in those men whose baseline testosterone levels were < 6.93 nmol/l. These data thus support the hypothesis that there is a critical threshold level of testosterone below which bone health is compromised.

Body composition, muscle strength and testosterone levels in men

Young men with overt hypogonadism have been observed to have decreased lean body mass and muscle strength, which improve with testosterone replacement. These observations led to many studies regarding the effect of testosterone on body composition and muscle strength in eugonadal subjects. In a randomized, double-blind, placebo-controlled study of testosterone administration to young healthy men, those subjects who received testosterone demonstrated an increase in muscle strength and lean body mass, especially when combined with exercise.[33]

These data on the beneficial effects of testosterone on body composition in young men led investigators to attempt to correlate testosterone levels with lean body mass and muscle strength in elderly men. One group studied 108 men over the age of 65 years with testosterone levels < 16.47 nmol/l.[34] The men were randomized to either testosterone or placebo patch in a double-blind fashion for 36 months. Although a significant increase in lean body mass was observed in the subjects who received testosterone, this did not translate to increased muscle strength as tested by a dynamometer. It remains to be seen whether testosterone replacement will result in increased muscle strength in older frail men and whether increased muscle strength in this setting will translate to a decreased number of falls.

Coronary artery disease (CAD) and testosterone levels in men

No randomized, double-blind, placebo-controlled trials have attempted to evaluate the effect of testosterone on CAD. One interventional study demonstrated increased coronary vasodilatation with intracoronary testosterone administration in men with CAD.[35] Given the absence of data on the effect of testosterone on coronary events, attention has been focused on the effect of testosterone administration on CAD risk factors such as lipid levels and insulin resistance, using both as surrogates of cardiovascular disease. Cross-sectional studies have shown a positive correlation between testosterone and high-density lipoprotein

(HDL) cholesterol.[36] In contrast, non-aromatizable androgens, such as methyltestosterone, increase low-density lipoprotein (LDL) and decrease HDL.[37] Most studies of testosterone administration in middle-aged men show a decrease in total cholesterol with no significant change in HDL levels, most likely mediated by the beneficial effect on body composition.[38-40]

The relationship between testosterone and insulin resistance, an important cardiovascular risk factor, has not been fully elucidated. Cross-sectional studies have consistently demonstrated an inverse relationship between testosterone and insulin levels.[41,42] However, in obese middle-aged men with low-normal testosterone levels, testosterone administration has been shown to increase insulin sensitivity.[43,44] In contrast, young healthy men with normal testosterone levels have no change in insulin sensitivity after being treated with supraphysiological doses of testosterone.[45] Unlike testosterone, however, anabolic steroids have been described to increase insulin resistance in men.[46] Thus, existing data regarding the relationship of insulin resistance and testosterone levels are confusing, and more careful physiological research needs to be done to define more accurately the exact nature of this relationship.

Sexual function and testosterone levels in men

Decreased libido and potency are the most common complaints among men with hypogonadism. It is well established that, in men with overt hypogonadism, physiological testosterone replacement restores normal libido and potency.[47] However, most studies have shown no correlation between libido or potency and testosterone levels within the normal range.[48,49] Similarly, increasing testosterone levels within the normal range does not improve libido and potency further. Although the incidence of erectile dysfunction increases significantly with age, and testosterone levels decline with age, it has been shown that erectile dysfunction and hypogonadism are independently distributed in this population.[50,51] These observations suggest that there is a critical value of testosterone in the low-normal range below which libido and potency are compromised.

Testosterone preparations

The first testosterone preparations were oral androgens that were chemically modified by 17α-alkylation, such as methyltestosterone. These synthetic androgens are hepatotoxic and are not recommended for androgen replacement in men. The preparations available today are easy to use and lack the hepatotoxicity of the 17α-alkylated testosterone preparations (Table 1). These preparations include intramuscular injections of testosterone esters, which are inexpensive but have the disadvantage of causing wide fluctuations in testosterone levels.[52] Transdermal preparations provide more steady levels of testosterone and include testosterone patches and a testosterone gel. In Europe and Australia, short-acting oral androgens, such as testosterone undecanoate, are available—which are absorbed by the lymphatics and are not hepatotoxic[50]—in addition to testosterone pellets which are inserted under the skin with a trochanter.[53] Future testosterone preparations include longer-acting intramuscular testosterone esters that will maintain testosterone levels within the normal range for 3 months.[54] Analogous to selective oestrogen receptor modulators (SERMs), much interest has arisen in developing selective androgen receptor modulators (SARMs). The ideal SARM would have positive effects on libido and potency, bone health and muscle strength, with no proliferative effects on the prostate

Table 1. Advantages and disadvantages of various testosterone preparations.

Preparation	Advantages	Disadvantages
IM (Testosterone enanthate and cypionate)	Low cost, flexibility in dosing	Fluctuations in serum levels, need for intramuscular injection
Oral (Testosterone undecanoate)	Ease of administration	Short half-life
Scrotal patch	Physiological testosterone levels	Cost, site of application, high DHT levels
Non-genital patch	Physiological testosterone levels	Cost, skin irritation, poor skin adherence
Testosterone gel	Physiological testosterone levels, not visible	Cost, odour, possible absorption by female partner
Testosterone pellets	Physiological testosterone levels, dosing every 6 months	Need for trochanter implantation, extrusion with intense physical activity

gland. The synthetic androgen 7α-methyl-19 nortestosterone (MENT) is an example of a SARM which is not converted to DHT and does not appear to stimulate prostate growth. MENT has been tested in primates with good results.[55] In addition, administration of MENT to a group of hypogonadal men for 6 weeks has been shown to produce and maintain normal sexual function.[56]

Adverse effects of testosterone replacement in men

Side-effects of testosterone include stimulation of erythropoiesis, sleep apnoea and possibly increased rates of benign prostatic hypertrophy (BPH) and prostate cancer. An increase in haematocrit levels has been well described with androgen therapy and may result in hyperviscosity.[57] A few cases of sleep apnoea have been described in men receiving testosterone.[58,59] Androgen therapy increases prostatic volume and PSA levels to levels similar to age-matched eugonadal controls.[39,44,60] No significant increase in the incidence of BPH or prostate cancer has yet been described. However, no large randomized, double-blind, placebo-controlled trials have been undertaken to assess more accurately the true incidence of BPH and prostate cancer with testosterone replacement.

Recommendations regarding testosterone therapy in men

The Advisory Panel of the National Institute of Ageing[61] and Second Annual Andropause Consensus Meeting of the Endocrine Society[23] have made suggestions regarding testosterone replacement in the elderly. The Advisory Panel of the National Institute of Ageing recently drew attention to the limitations of our existing knowledge concerning androgen deficiency in the ageing male. One of the first steps the Panel recommends is to define the stability of testosterone measurements over time in this age group to have a better appreciation of the clinical significance of a single low level of testosterone. The panel also highlighted the importance of determining the dose–response curves for testosterone at different target organs and of clarifying the relative importance of oestradiol and DHT in mediating testosterone's effects. Finally, they emphasized the importance of conducting large, randomized placebo-controlled trials

in an ethnically diverse population of both healthy and frail elderly men to determine more accurately the risks and benefits of testosterone replacement.

The Endocrine Society's Andropause Consensus Meeting recently made some preliminary recommendations to provide guidance to clinicians pending the availability of results from large clinical trials. The consensus reached was that men with symptoms of androgen deficiency and early morning testosterone levels <6.93 nmol/l on two separate occasions should be offered testosterone replacement as a possible treatment option. Due to the uncertainty regarding the effect of testosterone on the prostate, the panel suggested a baseline measurement of prostate specific antigen (PSA) and a digital rectal examination (DRE). PSA levels should be checked at 3, 6 and 12 months, and DRE should be checked at 3 and 12 months. Referral to an urologist should occur with the following: (i) an increase in PSA ≥ 1.5 µg/l per year, (ii) an increase of PSA ≥ 0.75 µg/l over 2 years, or (iii) PSA level > 4.0 µg/l.

ANDROGENS AND THE POST-MENOPAUSAL FEMALE

Despite optimal oestrogen replacement, a significant number of post-menopausal women continue to remain symptomatic, with symptoms of decreased energy and decreased libido. These observations led to the hypothesis that androgens might play an important role in the female as well as in the male. Unlike the male, in whom a syndrome of androgen deficiency has been well described, there is no widely accepted definition of androgen deficiency in the female. Due to the lack of a specific definition of androgen deficiency in women, and the low testosterone levels present in women[62] which are often difficult to measure with current assays, the existence of such a deficiency remains uncertain.

The two major circulating androgens in the female are androstenedione and testosterone. Fifty percent of androstenedione is produced by the ovary, and 50% is produced by the adrenal gland.[63] Circulating levels of androstenedione in pre-menopausal women are similar to those found in males. Testosterone production in women originates from three sources: 25% of testosterone is produced by the ovaries, while the remainder is derived in approximately equal proportions from the conversion of androstenedione and from the adrenals.[64,65] Total testosterone levels in pre-menopausal women are about one-tenth of those found in men.[62]

As women go through menopause, androstenedione production from the ovaries decreases by 50%.[66] Similarly, oophorectomy has been reported to result in up to a 50% decline in serum testosterone levels.[63] However, whether a similar decline in testosterone levels occurs as women go through natural menopause is controversial. There is some evidence to support a slight decline in testosterone levels at the time of menopause.[67] In addition, a cross-sectional study of follicular phase testosterone levels in women aged 31–51 years revealed a decline in testosterone levels with ageing even prior to menopause.[68] Other studies, however, have shown no decline of testosterone levels in post-menopausal compared with pre-menopausal women.[69,70] Therefore, at present, it is unclear whether a syndrome of androgen deficiency exists as women age, a question that is unlikely to be answered until sensitive assays for the measurement of testosterone levels in women become available.

In addition to the epidemiological data on testosterone levels in ageing women, other studies have attempted to evaluate the effect of androgens on target end organs in post-menopausal women. Some studies have shown an improvement in bone mineral density with testosterone and oestrogen combinations compared with oestrogen

treatment alone in post-menopausal and in oophorectomized women.[71−79] Limitations of some of these studies include the use of supraphysiological doses of testosterone and the inability to conclude whether the positive effect on bone is due to testosterone itself or aromatization to oestradiol oestrogen with resultant higher oestrogen levels. Even in men, the correlation between bone mineral density and oestradiol appears to be stronger than that with testosterone.[27]

Research regarding the effect of sex steroids on body composition in post-menopausal women has revealed that oestradiol alone can decrease central body fat[80], while treatment with anabolic steroids increases lean muscle mass and visceral fat, although total body fat is decreased.[81] As in men, the effects of testosterone on lipids depend on the type and dose of androgen given. If a normal testosterone level is achieved with transdermal testosterone or subcutaneous implants, no significant effect on lipids is observed.[80,82,83]

Last, testosterone replacement may have a positive effect on libido and sexual function in post-menopausal women, particularly those who have had an oophorectomy.[83−85] Many of the studies that did show improvement in libido and quality of life measures, however, achieved testosterone levels in the high-normal to supraphysiological range.

Current testosterone preparations available to women include methyltestosterone, which may be given orally in a combination pill with conjugated equine oestrogen. Oral testosterone undecanoate is available in Europe, and subcutaneous testosterone implants are used in Australia and the UK. Transdermal preparations are being developed to deliver physiological doses of testosterone with minimal side-effects. Potential side-effects of testosterone replacement in women include hirsutism, acne and virilization, which is of greatest concern given that it is not always reversible.[79,81−83,86]

SUMMARY

The role of androgen replacement in older men is still uncertain. Many of the symptoms that are indicative of hypogonadism in young men are non-specific in the older population. In older men, existing data support a threshold testosterone level below which bone health and sexual function may be compromised. More research needs to be done to determine the dose−response curves for testosterone at different tissues and whether the positive end-organ effects with testosterone replacement are due to testosterone itself, oestrogen or DHT. Due to the lessons learned from hormone replacement therapy in post-menopausal women[87,88] and the possible adverse effects of testosterone therapy on the prostate gland, large randomized, double-blind, placebo-controlled trials will need to be undertaken to explore the effects of testosterone therapy in older men.

In post-menopausal women, longitudinal studies need to be done to determine whether a true syndrome of androgen deficiency exists. If androgen deficiency does exist in this population, then careful studies with physiological testosterone replacement will be needed to determine the effects of testosterone, *not* oestrogen, on target end organs.

In summary, much research needs to be done to evaluate the role of androgens in older men and women, with possible implications for bone health, muscle strength, cardiovascular disease and libido.

Practice points

- the role of androgen replacement in elderly men is uncertain
- symptoms of hypogonadism are similar to those of normal ageing and include loss of libido and potency, decreased muscle mass, increased fat mass and osteoporosis
- the combination of these symptoms with a serum testosterone level <6.93 nmol/l may be an indication for testosterone treatment in men
- a diagnosis of hypogonadism must never be based on a single testosterone measurement
- testosterone levels should be drawn in the early morning, given the diurnal rhythm in its secretion
- the existence of androgen deficiency in post-menopausal women is uncertain and the benefit of androgen replacement is controversial

Research agenda

- in older men, dose−response studies need to be done in order to evaluate the minimal testosterone level needed to improve target organ function while minimizing risks
- in older men, more research needs to focus on whether the androgen effects on target organs are due to testosterone, oestrogen or DHT
- in older men, large randomized, placebo-controlled trials are needed to address the effects of androgens on bone health, muscle mass, insulin resistance, lipids, and libido and potency
- in post-menopausal women, longitudinal studies with sensitive testosterone assays are needed to evaluate whether a syndrome of androgen deficiency exists

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