

TOPICAL REVIEW

Testosterone supplementation therapy as a treatment of hypogonadism

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Abstract: A substantial proportion of older men (ranging from 20% of 60-year-old to 50% of 80-year-old men) have total serum testosterone levels below the normal range for younger men (1). At the age of 75 years, mean plasma testosterone levels are only 65 % of levels in young adults, whereas over 25 % of these men have bioavailable testosterone levels below the lowest normal limit in young adults. The interindividual variations in the plasma levels are, however, very important and a quarter of men over 75 years old have still testosterone levels within the upper quartile of values in young men (2).

Many symptoms associated with aging in men, including muscle atrophy and weakness, osteoporosis, reduced sexual functioning, and increased fat mass, are similar to changes associated with testosterone deficiency in young men. These similarities suggest that testosterone supplementation may prevent or reverse the effects of aging (3), improve the general well being, sexual characteristics and behaviour of elderly men (Fig. 4, Ref. 71). Full Text (Free, PDF) www.bmj.sk.

Key words: andropause, aging of men, hypogonadism, PADAM, ADAM, AMS, testosterone, supplementation, testosterone replacement therapy, testosterone, androgen receptor, AR gene.

Abbreviations: DHEA – dehydroepiandrosterone, AR – androgen receptor, T – testosterone, DHT – dihydrotestosterone, 5 α RD – 5 α -reductases, GTFs – general transcription factors, ARA – androgen receptor associated proteins, P – phosphorylation, TFs – transcription factors, CoR – co-regulators, hsp – heat shock protein, ARE – androgen response element, PADAM – partial androgen deficiency of aging males, AMS – aging male syndrome, ADAM – androgen deficiency in aging man, SHBG – sex hormone binding globulin, TRT – Testosterone Replacement Therapy, LDL – low density lipoproteins, LH – luteinizing hormone, BPH – benign prostate hyperplasia, DBD – DNA binding domain, CHD – coronary heart disease.

Nowadays, a great attention is given on improving the life of both elderly and young men with low levels of testosterone. Testosterone concentrations which are decreased and out of normal physiological range, lead to worsening of nearly all physiological functions, secondary sexual signs and the general well being. These symptoms are generally named by the term PADAM – partial androgen deficiency of aging males or AMS – aging male syndrome or androgen deficiency in aging man – ADAM. The factors, which lead to the andropause, are apparently multifactorial. The first evidence of association of testosterone decreasing

levels with aging in men, was assessed by Hollander in 1958 (4). This syndrome is the consequence of hormonal and non-hormonal changes, which are connected with number of symptoms and low testosterone level in men over 50 years of age (5). Decrease of free testosterone levels is induced by the age-associated increase in binding capacity of the sex hormone binding globulin (SHBG), which results in lower levels of bioavailable free or albumin-bound testosterone in tissues.

The levels of free testosterone in plasma can be influenced by various genetic factors. Action of testosterone is provided via androgen receptor (AR) on nuclear membrane of human cells. After binding the ligand (testosterone or dihydrotestosterone) to AR receptor, the expressions of target genes are extensively affected. AR gene is highly polymorphic, mainly in numbers of CAG repeats in exon 1. Determination of AR polymorphism is very important during the testosterone supplementation in order to expect positive and/or possible side effects of treatment.

Testosterone Replacement Therapy (TRT) or testosterone supplementation provides a possibility to achieve normal physiological levels of testosterone. It can help to prevent or reverse the symptoms typical for aging males, which include endocrine, metabolic, somatic and psychological effects.

Functions of testosterone

Testosterone as the main steroid hormone in males is produced in smooth endoplasmatic reticulum of Leydig's cells in testes. Other possible places of testosterone formation are central nervous system, adipocytes and even some microorganisms

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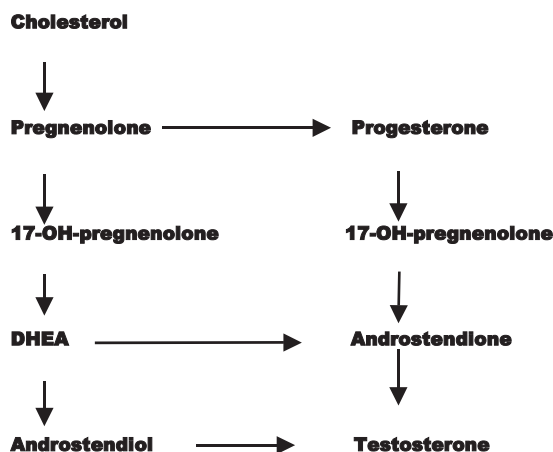


Fig. 1. The biosynthesis pathway of testosterone. DHEA – dehydroepiandrosterone (70).

in gastrointestinal tract can produce this hormone (6). Primary precursor for testosterone synthesis is cholesterol, which is transferred by low-density lipoproteins (LDL) in blood. The biosynthesis pathway (Fig. 1) of testosterone starts with placement of cholesterol to inner mitochondrial membrane where desmolase, the enzyme coded by gene *CYP11A1* catalyzes the removal of side chain, and pregnenolone is created. Then pregnenolone is changed in microzomes to androstendione in two different pathways. The first includes conversion of pregnenolone to progesteron and the second possibility is to convert it to dehydroepiandrosterone, while both processes are catalyzed by 17α -hydroxylase, coded by gene *CYP17*. 17β -hydroxysteroiddehydrogenase causes the transformation of androstendione to main androgen – testosterone. In adipocytes, bones, muscles, liver, Sertoli's cells, Leydig's cells and in some structures of brain, aromatase coded by *CYP19* gene converses testosterone to estradiol. Single nucleotid polymorphism *C¹⁵⁵⁸-T* is localized in 3'-untranslated region of *CYP19* aromatase gene (exon 10). This polymorphism is associated with increased aromatase activity, especially in brain, which leads to improvement of spatial abilities. In prostate, testes, kidneys, skin, brain, liver and in some another tissues testosterone is reduced to dihydrotestosterone

(DHT) by 5α -reductase, which is coded by *SRD5A1* and *SRD5A2* genes. Substitution in codon 89 (*V89L*) of *SRD5A2* gene leads to reduction of 5α -reductase activity *in vivo* and also *in vitro*. Alanin to treonin transpozition in codon 49. increases enzyme's *in vitro* activity 5-folds, so this polymorphims could be responsible for raised levels of dihydrotestosterone and therefore also the higher risk of developing prostate cancer.

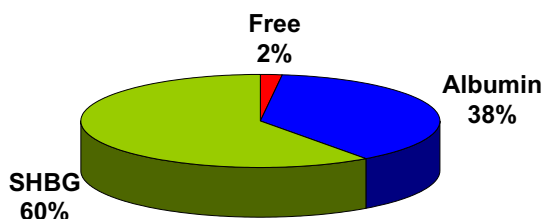
In young men about 1 % to 2 % of circulating testosterone is free, 38 % is bound to albumin and approximately 60 % of circulating testosterone is bound to sex hormone binding globulin (SHBG) (Fig. 2). Only the free fraction and albumin-bound testosterone fraction are able to enter the cells in physiologic conditions and activate the testosterone receptor via changing its conformation. The levels of free testosterone in plasma might be different in healthy elderly men but also in young men with lower levels of non-bound testosterone. This can be influenced by various genetic factors, which seem to play an important role and according to Meikle (33) they account for 30 % of the variability of free testosterone levels.

During the aging there is an increase in SHBG levels and this change is associated with an alternation in the total binding affinity of SHBG (7, 8, 9). This leads to higher concentrations of SHBG bound testosterone resulting in lower levels of free tissue-bioavailable testosterone in older persons. In other words we can say that testosterone is inappropriately split in elderly men who suffer from hypogonadism. The differences between binding capacities of SHBG could be caused by various forms of SHBG, which predominate in older persons, increased glycation of SHBG, or a tissue factor (10).

There are various mechanisms of testosterone activity, which include direct action using nuclear receptor (11), different membrane receptors associated with G-proteins (12) and also via using simple plasmatic channels (13). Activity of testosterone can be provided by conversion to active metabolits such as DHT (14) or estradiole (15).

Testosterone influences also brain and mental functions of human, changes of muscle and fat tissue, it determines development of neuronal synapses, which are crucial for behaviour and thinking (16). Testosterone stimulates growth of muscles, bones, hair and prostate; it can cause men's baldness and is responsible for dominant and aggressive behaviour of males. Testosterone

Levels of testosterone in young men.



Levels of testosterone in old men.

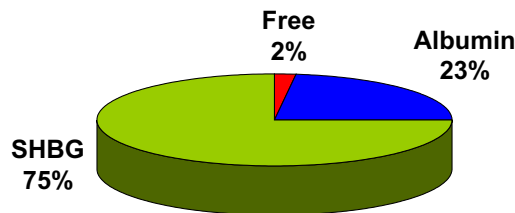


Fig. 2. Levels of testosterone in young and elderly men.

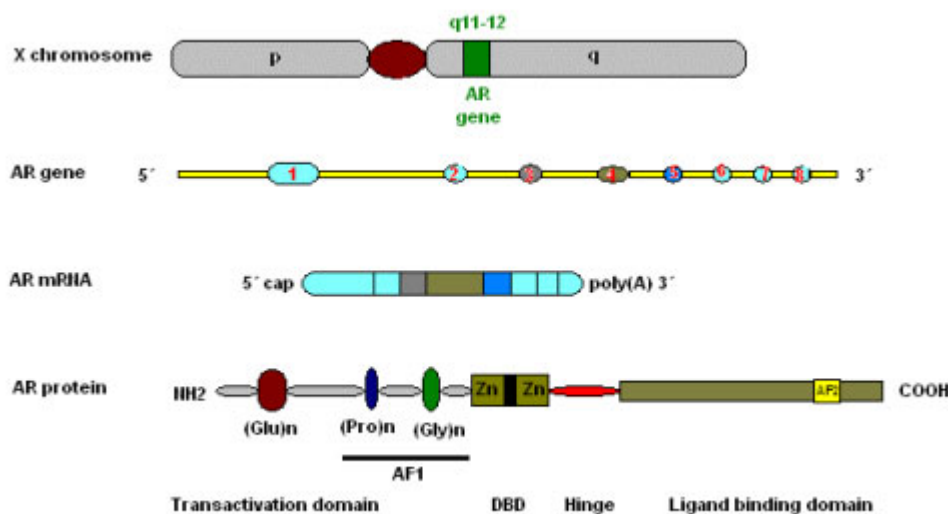


Fig. 3. X chromosome, AR gene, AR mRNA, AR protein.

also influences the cognitive abilities of men in positive way, which is determined not only by hormone presence, but also by activity of its receptors in tissues (17). One of the most important functions of testosterone is its ability to inhibit luteinizing hormone (LH) response to gonadotropin-releasing hormone by aromatization of testosterone.

Testosterone is a predictor of muscle mass, but the best predictor of muscle mass is free androgen index, which also predicts muscle strength on relatively good level (18, 19). Testosterone also reduces myocardial infarction (20), decreases cholesterol and low-density lipoprotein cholesterol levels, as well as high-density lipoprotein cholesterol level (21).

Genetics of testosterone receptor

Androgens functioning via androgen receptor (AR) play an essential role in the prostate development, growth and pathogenesis of benign prostate hyperplasia (BPH) and prostate cancer (22). Testosterone affects via androgen receptor (AR) (Fig. 3), whose gene sequence is localized on the long arm of X chromosome in the region q11–12. The AR gene itself is a single-copy gene that covers approximately 90 kilobases of genomic DNA and its polypeptide product includes around 910–919 amino acids, with a molecular mass of approximately 110 kDa. The encoding region of the AR gene is comprised of 8 exons separated by 7 introns.

AR receptor as a ligand-dependent nuclear transcription factor belongs to the superfamily of nuclear receptors, which includes receptors for steroid hormones (estrogen, progesterone), thyroid hormones, all-trans and 9-cis retinoic acid, 1,25 dihydroxy-vitamine D, ecdysone and peroxisome proliferator-activated receptors (23). The common polypeptide structure of nuclear hormone receptors consists of 4 different functional domains: an amino-terminal A/B domain, a DNA binding domain (DBD,

domain C), a hinge region (domain D), and a ligand-binding domain. By exon 1 is encoded a large amino-terminal domain that comprises nearly half of the AR and involves transactivation domain. This domain plays an integral role in AR functions via intramolecular and/or intermolecular interaction with other co-regulators (24). This transactivation domain on amino-terminal side of AR protein also includes three highly polymorphic direct repeats of amino acid residues: glutamine, proline, and glycine.

Kennedy's disease is related to expansion of the size of the glutamine homo polymeric segment (25) (CAG)_nCAA – repeat, which is presented in the NH₂-terminal domain. The normal range of this region is between 9–38 glutamine residues and pathological number is more than 40 residues. On the other hand, the shortening of the lengths of glutamine (CAG repeats) or glycine (GGN repeats) may correlate with higher risk of development of prostate cancer in earlier age, a higher tumor grade and aggressiveness (26, 27). There are many possible mechanisms of how the varying length of repeats influences the activity of receptor: alternation in its binding affinity to its ligand, interaction with co-regulators, responsiveness to phosphorylation, or changes in its AR interdomain interactions (28). Size of the CAG repeat might also influences the cognitive abilities of individuals. Repeat expansion leads to worsening of spatial abilities, mental operations, visual processing, executive behaviour and attention.

The most conserved region of AR receptor is DNA binding domain (29), which is encoded by exon 2 and 3. It contains two “zinc finger” motifs that are signs of all nuclear steroid receptors and are responsible for specific binding interaction to the androgen response element (ARE) of target genes. The ligand-binding domain is located in the C-end of the AR, encoded by 3'-end of exon 4 and exons 5, 6, 7, 8. It is responsible for specific high-affinity ligand binding through hydrophobic interaction as well as some hydrogen binding (30). The carboxyl-end also contains subdomains involved in dimerization and transcriptional

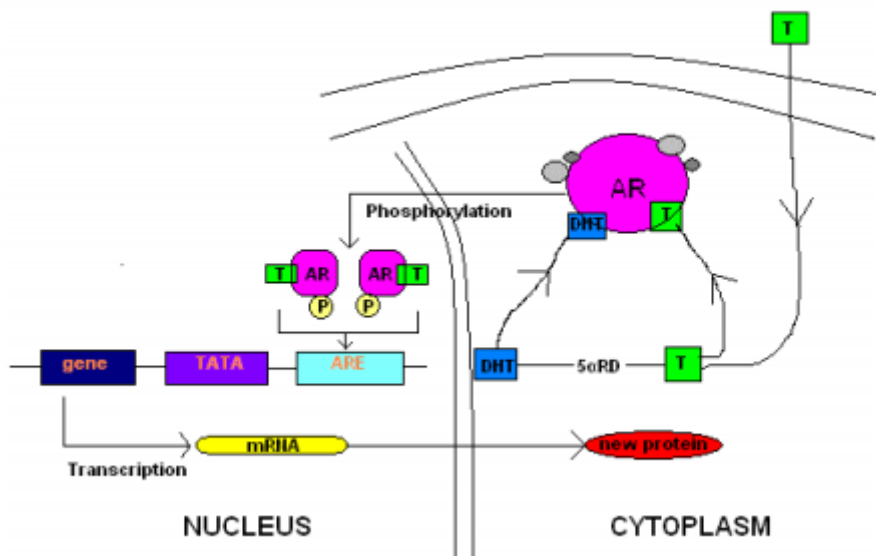


Fig. 4. Molecular mechanism of action of testosterone. AR – androgen receptor, T – testosterone, DHT – dihydrotestosterone, 5 α RD – 5 α -reductase, ARE – androgen response element.

activation, e.g. AF-2 domain (22). The fourth region of AR receptor is hinge region, which is located between the DNA-binding domain and the steroid-binding domain and contains the nuclear translocation signal.

The binding of ligand to the androgen receptor results in conformation change of AR that promotes the dissociation of heat shock proteins and facilitates receptor dimerization, transportation into nucleus, phosphorylation, and DNA binding (31). Translocation of the receptor complex to nucleus via intrinsic nuclear localization signal is the next step. The complex, which interacts with androgen response element (ARE) on the target gene, is created by receptor dimerization with the second molecule. All this events, especially creation of homodimer, lead to the induction of the general transcription complex, co-regulators (CoR) and transcription factors (TF) which can interact with AF-2 domain and mediate or enhance androgen-target gene expression and affection of AR (24). A recent study indicates that the AR-AF2 domain may also stabilize the overall structure of the receptor, allowing the amino-end to interact with appropriate coactivators (32). The transcription of target genes can be inhibited or induced at particular sites on the chromatin. The consequence of AR gene expression is protein synthesis, which finally results in androgen response, including changes of biological and cellular structures and functions. Testosterone can bind to the AR receptor directly or after its conversion to 5 α -dihydrotestosterone by 5 α -reductases. In several cell lines there have been identified two androgen receptor mRNA species (8.5 and 11 kb), which appear as a result of differential splicing in the 3'-untranslated region. The function of AR can be activated or modified by non-ligand factors such as growth factors, EGF and IGF-1 (22) (Fig. 4).

Ageing of men and hypogonadism

Ageing is accompanied by a gradual decrease of nearly all physiological functions, what includes mainly decrease of muscle mass, bone mineral density, strength and energy, what leads to overall weakness and increased risk of falls and fractures. The regression of secondary sex characteristics and decrease of the general well being also occur. The changes also include decreased virility, sexual pilosity, libido, potency and sexual activity, as well as increased fatigue, and a decrease of cognitive functions and intellectual ability. Some of the physiological characterizations and functions are increased, this group of changes involves increased frequency of impotence, forgetfulness, nervousness, memory problems, insomnia, sleep disturbances, numbness, tingling, hot flushes and irritability, increased sweating, development of abdominal obesity and overall fat mass, insulin resistance, osteoporosis and atherosclerosis (2, 3, 10, 34–40).

The discussed decrease of free testosterone levels is the consequence of the age-associated increase in binding capacity of the sex hormone binding globulin (SHBG). Despite this fact, there exists an important variability between individual levels, about 20 % of men over 75 years old have plasma testosterone levels still within the upper quartile of values of young men, whereas over 25 % of men in this young age group have clearly subnormal (11 nmol/l total testosterone and 220 pmol/l free testosterone), i.e. hypogonadal plasma levels (41). The rate of decline of testosterone is between 1 % and 2 % per year (10). When bioavailable testosterone (free and albumin-bound testosterone) is used as a measure of gonadal functions in men between the age of 40–50 years and 70 years old men, 3 % to 5 % and 30 % to 70 % (respectively) are found hypogonadal (7, 42).

In 1958 Hollander and Hollander (4) provided the first evidence of decreasing levels of testosterone, which was associated with aging in men. This hypoandrogenism is generally moderate and it used to be called by term PADAM – partial androgen deficiency of aging males or AMS – aging male syndrome. Other names used for the andropause include androgen deficiency in aging man – ADAM, male climacteric, male menopause or even viropause. This syndrome is the consequence of hormonal and nonhormonal changes, which are connected with the senescence of men. Andropause has been defined as a cluster of symptoms in men over 50 years of age who have low testosterone levels (5).

The factors, which lead to the andropause, are apparently multifactorial. For older men with low levels of testosterone it is typical that concentrations of follicle-stimulating hormone are more elevated outside the normal range (secondary hypogonadism) than LH (7). In testes of elderly men the amount of Leydig's cells are decreased as well as whole process of spermatogenesis, which leads to decline in fertility rates (43). Older men have less testosterone secreted per burst and a decline in secretory bursts in response to LH suggesting a partial desensitization of Leydig's cells to LH with aging (44). It means that a decline in testosterone is the consequence of the testicular failure. Another factor, which accompanies the male climacteric, is a decreased testosterone response to human chorionic gonadotropin (43). Leifke et al (45) evaluated that pituitary responsiveness to gonadotropin-releasing factor, which is restrained by testosterone, is declined with age. There also appears a reduction in CAG repeats in androgen receptor, which leads to its greater activity (46). In older men there is also a relative increase in aromatization of testosterone to estradiol and in 5α -reduction to dihydrotestosterone (DHT). In comparison with testosterone, DHT levels do not usually fall down (47). The levels of inhibin B also decrease with age and vice versa the concentrations of activin slightly increase at middle age (48).

Moderately decreased levels of total testosterone (2.5–3.0 ng/ml) can be detected in healthy older men. This condition is accompanied by symptoms such as erectile dysfunctions, loss of libido and muscle weakness. In weak older men in rehabilitation units or living in nursing homes, low testosterone levels are more prevalent than in healthy men of the same age (49, 50). Testosterone levels are often low in number of chronic medical illnesses (e.g. renal failure, malignancy – e.g. prostate cancer) and with the use of certain medications (e.g. glucocorticoids, opiates) in older men (3). Epidemiologic studies have shown a strong correlation between low bioavailable testosterone levels and cognitive decline with aging (51). Low testosterone levels are also related to atherosclerotic coronary artery disease and the degree of atherosclerosis (52). But testosterone influence on the development of the coronary heart disease (CHD) in men are controversial, because the incidence of CHD is higher in male than in women in the same age and there is a possibility that testosterone may predispose the development of disease.

It is well proved that fertility of men is maintained until very old age and does not decline with aging, compared to the women. Some studies demonstrate paternity achieved by men over 90

years old (53). This appears to be largely the consequence of the age associated decrease in sexual activity rather than of the decrease in sperm quality (54). Androgen deficiency is rarely the major cause of impotence in elderly men, although hormonal alterations may play an important role in some cases of impotence, but generally the reasons for impotence are mainly non-hormonal such as atherosclerosis or polyneuropathy (55). Development of early impotence can be frequently caused by diabetes mellitus and some medications e.g. antihypertensives. Both diabetes mellitus and hyperprolactinemia are often accompanied by hypotestosteronemia (2).

Testosterone replacement therapy

Nowadays, a great attention is given on the improvement of physiological and psychological health of population of elderly men. Testosterone Replacement Therapy (TRT) or testosterone supplementation provides a possibility to achieve normal physiological levels of testosterone in young as well as aging male with low levels of testosterone. It can help to precede or reverse symptoms typical for aging males, which includes endocrine, metabolic, somatic and psychological effects.

There are many studies that suggest improvement in libido and quality of erections in older men, what was reached by testosterone replacement (56–59). Testosterone therapy consistently caused the activation of sexual behaviour and libido rather than an improvement in erectile function (3). DHT has been shown to improve the ability to maintain erections (60). On the other hand, impotence is seldom significantly improved (2). Testosterone supplementation increases hematocrit levels (61), prostate-specific antigen increases mildly or not et al (62), size of prostate is also a little bit influenced (19) and it either improves or has no effect on urinary symptoms (46). Testosterone supplementation influences the increase in muscle mass and strength, decrease of fat mass but the effect on strength in elderly men is not clear (21). Uncertain is also the mechanism how testosterone leads to muscle mass increase. It might be direct effects of testosterone on muscle mass protein synthesis or indirect influence on progenitor muscle cells (10). Sexual functioning, mood, upper and lower body strength, functional performance and some of cognitive domains (e.g. spatial, working and verbal memory) were improved or unchanged in different ways by testosterone replacement. Positive effects were reported in men with coronary heart disease, where supplementation improved exercise-induced coronary ischemia, while angina pectoris was also improved or unchanged (3). Furthermore, testosterone was found to induce relaxation of coronary arteries and to increase coronary blood flow in animal models (63). Adequate substitution will be manifested in patients by improvement of general well being, alertness, vigor, bone mineral density as well as increase in hematocrite and metabolic profile improvement (64). Testosterone therapy also reduced or did not change the levels of total and LDL cholesterol, while HDL cholesterol levels remained unchanged (64).

In young men, testosterone supplementation increases poor body mass and strength not only in men with hypogonadism, but

also in men with normal testosterone levels receiving more than physiological doses of testosterone (65). In this group of young patients, testosterone replacement is used because of maintenance of normal libido, sexual functioning, ability to concentrate and it influences the spatial ability, mood and well being in positive way. The goal of therapy in older men is not only to improve age-related changes in body composition, but also to affect meaningful changes in strength, prevent fall and fracture risk and improve functional status (3).

Also testicular cancer belongs to etiologic factors leading to male hypogonadism. Decreased testosterone level may lead to the symptoms of androgen deficiency with subsequent negative impact on to the patient's quality of life. Ondrusova et al (71) recommend hormonal profile examination and testosterone supplementation as an important part of patient's follow-up in bilateral testicular cancer, moreover in unilateral disease. The important part of standard examination algorithm should be also osteological examination to prevent osteopenia or even osteoporosis development.

Optimal supplementation dose for treatment is based on two indicators: the plasma testosterone levels and on the other hand on the clinical effects. The final decision concerning application of testosterone treatment must be considered in comparison with expected benefits and possible serious side effects. It is recommended to examine hematocrit, especially in patients with chronic respiratory disease, as well as lipid profile. Before the start of any type of supplementation therapy, patients should be carefully screened for the presence of contra-indications. The most important are clinical prostate carcinoma, apparently very highly androgen sensitive tumor, rarely presented mammary carcinoma as well as prolactinoma, whose growth might be stimulated by increasing levels of estradiol, the product of testosterone conversion. Relative contra-indications are polycythemia and also erythrocytosis, developed frequently during intramuscular testosterone therapy (62), but probably less frequently during transdermal (66) or oral testosterone-undecanoate therapy (67). A clear atherosclerotic lipid profile also belongs to relative contra-indications (2). Some patients are sensitive to levels of testosterone because it can induce water and salt retention, mainly in those who suffer from cardiac decompensation or hypertension, and in this case it may be required to reduce the dose of applied testosterone.

Side effects of testosterone replacement therapy include gynaecomastia – as a result of increased concentration of estradiol, whose levels are markedly higher after intramuscular application of testosterone therapy. Water retention and hypertension occur rarely and sleep apnoea was worsened by this supplementation (68, 69). During the treatment eventual side effects should be carefully monitored by repeated rectal examinations and determination of PSA (prostate specific antigen) every six months, as well as hematocrit and lipid profile (2). Tolerant of testosterone supplementation in majority of patient was acceptable, although in many cases abnormal elevations in the hematocrit occurred, especially in patients treated by high dose of parenteral testosterone. PSA levels often in-

creased slightly but significantly, mostly within the normal range (<4 ng/mL) (3).

There are several ways of testosterone application in supplementation therapy: intramuscular way, transscrotal, transdermal patch, transdermal gel, subcutaneous implants, buccally and orally. This kind of therapy is not recommended to patients with normal or slightly below normal levels of testosterone and with no clinical symptoms of hypogonadism, although in men with markedly decreased levels of testosterone, but with no symptoms, it may be used.

Summary and perspective

In almost all men, aging is associated with a decline of testosterone levels, which is accompanied by the whole group of symptoms typical for testosterone deficiency. It includes not only sexual dysfunction (decrease in virility, sexual pilosity, libido, potency and sexual activity), but also the decrease in bone mineral density, muscle mass, strength, energy, cognitive functions and intellectual ability, and in many cases low testosterone levels play role as a metabolic risk factor for cardiovascular disease and diabetes. Some of the related symptoms are worsened: nervousness, sweating, frequency of impotence, forgetfulness, memory problems, insomnia, sleep disturbances, numbness, tingling, hot flushes and irritability, development of fat mass, insulin resistance, osteoporosis and atherosclerosis (2, 3, 10, 34–40).

Testosterone supplementation therapy is used in both groups of patients, in elderly men as well as in young men. To achieve significant results of treatment there must be an attention to the detection of the plasma testosterone levels and on the other hand to the clinical benefits and possible serious side effects. A crucial contra-indication is clinical prostate carcinoma, rarely presented mammary carcinoma as well as prolactinoma, polycythemia and also erythrocytosis. The group of side effects includes development of gynaecomastia, rare water retention and hypertension, as well as worsened sleep apnoea (68, 69). The testosterone application in prostate cancer patients stimulates formation of badly aggressive and fast-developing tumors.

After testosterone supplementation, improvements in almost all factors and characteristics, including libido, sexual behaviour, quality of erections, muscle mass, strength, mood, cognitive functions, increases in hematocrit levels, decreasing in fat mass, improvement of general well being, alertness, vigor, bone mineral density as well as improvement of metabolic profile appear (56–61, 64). Benefits were reported in men with coronary heart disease, where supplementation improved exercise-induced coronary ischemia, while angina pectoris was also improved or unchanged (3). Testosterone therapy also reduced or did not change levels of total and LDL cholesterol, and HDL cholesterol levels remained unchanged (64).

Up to present only a few hundred elderly subjects have been treated with testosterone, so the safety and efficacy of testosterone replacement therapy in long-term period is still uncertain. The long-term study on representative group of patients in the age over 50 is necessary to evaluate the real benefits as well as the frequency of side effects of testosterone replacement therapy.

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