The Aging Male

ISSN: 1368-5538 (Print) 1473-0790 (Online) Journal homepage: informahealthcare.com/journals/itam20

Testosterone metabolism, dose–response relationships and receptor polymorphisms: selected pharmacological/toxicological considerations on benefits versus risks of testosterone therapy in men

M. Oettel

To cite this article: M. Oettel (2003) Testosterone metabolism, dose–response relationships and receptor polymorphisms: selected pharmacological/toxicological considerations on benefits versus risks of testosterone therapy in men, The Aging Male, 6:4, 230-256, DOI: 10.1080/13685530312331309772

To link to this article: https://doi.org/10.1080/13685530312331309772

Published online: 06 Jul 2009.

The Aging Male 2003;6:230–256

Review

Testosterone metabolism, dose–response relationships and receptor polymorphisms: selected pharmacological/toxicological considerations on benefits versus risks of testosterone therapy in men

M. Oettel

Jenapharm GmbH & Co. KG, Jena, Germany

Key words: TESTOSTERONE, HYPOGONADISM, TOXICOLOGICAL RISKS

ABSTRACT

In this review selected toxicological problems related to testosterone therapy in hypogonadal men are discussed. Applying 'classical' pharmacological/toxicological findings (e.g. animal studies on short- and long-term toxicity) to clinical situations is not very helpful. Molecular biological knowledge and especially evaluation of epidemiological studies, as well as intervention studies, on testosterone therapy in hypogonadal men are more useful. Potential risks include overdosage for lifestyle reasons, e.g. excessive muscle building and reduction of visceral obesity, when erythrocytosis occurs concomitantly. Modern galenic formulations of testosterone administration (e.g. transdermal gel, suitable testosterone esters for intramuscular application and newer oral preparations) avoid supraphysiological serum concentrations, therefore significantly reducing the toxicological risk. A hypothetical model of the toxicological risks of testosterone therapy is given that is based on the influence of testosterone metabolism (aromatization vs. reduction) of the respective parameter/target chosen. Finally, the great influence of polymorphisms of the androgen receptor on the assessment of toxicological risk and on the individualization of androgen therapy is shown. Already existing national, continental and international guidelines or recommendations for the testosterone therapy should be harmonized.

Presented at the 2003 Annual Meeting of the Applied Endocrinology Section (SAE) of the German Society of Endocrinology (DGE), the German Society of Endocrinology (DGE), the German Society of Applied Endocrinology (DGAE), and at the Diabetes Symposium with the Berlin Diabetes Society, Berlin, 22–25 May 2003.

Correspondence: Dr M. Oettel, Jenapharm GmbH & Co. KG, Otto-Schott-Straße 15, 07745 Jena, Germany

INTRODUCTION

Christoph Wilhelm Hufeland (1762–1836), in his time in Jena, described the attempt to stop or even reverse aging processes simply as 'gerontocomic'. Yet today, so-called 'anti-aging medicines' are booming, with androgen dosage in men holding a particular place[1–15].

In 1941, Adolf Butenandt[16] wrote:

'Die heute synthetisch zubereiteten Hormone sind den natürlichen Wirkstoffen nicht nur ähnlich, sondern mit ihnen . . . identisch; sie stellen demnach keine Kunstprodukte dar im Sinne körperfremder Pharmaka mit hormonartiger Wirkung, sondern natürliche, körpereigene Wirkstoffe. Daher bedeutet die Behandlung eines Kranken mit den heute von der pharmazeutischen Industrie dargebotenen Hormonen eine Therapie auf natürlicher Basis' ['The hormones synthesized today are not only similar to the naturally occurring drug substances, but are identical with . . . them; they are therefore not artificial products in the sense of exogenous pharmaceuticals with hormone-like action, but rather natural, endogenous substances. Thus, the treatment of a patient with the hormones now offered by the pharmaceutical industry means a treatment on a natural basis'].

Is this view still applicable today? Is the administration of testosterone to men a natural form of treatment without serious side-effects?

Classically, androgens are used in the treatment of male hypogonadism; Klinefelter's syndrome; anemia secondary to chronic renal failure; aplastic anemia; protein wasting diseases such as tumors, burns, traumas, AIDS, etc.; treatment of short stature (as in Turner's syndrome or constitutionally delayed growth and puberty); breast cancer (as an anti-estrogen); and hereditary angioedema[8,17].

In the past, androgen use in clinical practice was impeded for a long time, because it was impossible to administer testosterone efficaciously either orally or parenterally owing to its fast degradation, mainly in the liver. It was therefore necessary both to modify the molecule chemically in order to alter its metabolism and to devise new methods of administration in order to obtain and sustain effective testosterone levels in the blood with low or no liver toxicity.

New delivery systems for testosterone, such as transdermal patches, but in particular, very easily applicable gels and suitable testosterone esters for intramuscular and oral application, have been developed to achieve more physiological and sustained hormone levels and to improve tolerability and compliance[18,19].

These new delivery systems may extend the use of androgen therapy. Testosterone in aging males improves body composition, bone and cartilage metabolism, certain domains of brain function and could also decrease cardiovascular risk (at least in biological models). In general, most investigators agree with short-term safety, whereas long-term safety remains a problematic point for scientific discussion[20,21]. Reasonably, we should deal more closely with the basics of androgen toxicology. However, the transfer of 'classical' pharmacological/toxicological findings (e.g. animal studies on short- and long-term toxicity) to the clinical situation is not very helpful and is more or less irrelevant to the judgement of safety in medicinal practice[22]. Molecular biological knowledge, evaluation of epidemiological studies and especially intervention studies on testosterone therapy in hypogonadal men are more useful.

In this review, selected clinical-driven pharmacological and toxicological aspects of testosterone therapy are described and the proven or hypothetical relationships between unwanted side-effects and the metabolic fate of testosterone, dose–response relationships, as well as androgen receptor polymorphisms are discussed.

MODE OF ACTION

Although testosterone mediates a broad range of developmental and homeostatic functions, the main mode of action is via a single androgen receptor (AR). Gobinet and associates[23] and Heinlein and Chang[24] reviewed molecular regulation of androgen-dependent genes by ARs. The receptor is a 120-kDa cytosolic protein encoded on the X chromosome, and to date only one AR cDNA has been identified. As there is only one AR, how does testosterone mediate the well-known diverse pharmacological actions in different tissues? In the past, attempts to isolate a pure anabolic or a pure androgenic receptor have failed[25]. But surprisingly, Hsiao and colleagues[26] found two different kinds of androgen response elements that may respond differentially

to testosterone and 5α-dihydrotestosterone (DHT). Therefore, it cannot be excluded that a selective androgen response element sequence may play a role in differential testosterone vs. DHT for AR transactivation.

One of the best current explanations for the diversity of androgenic actions is that testosterone is also a prohormone, and many of its actions in different tissues are mediated by its metabolites. For example, testosterone is irreversibly converted by two tissue-specific 5α-reductase subtypes to DHT – the main androgen in the prostate – and by aromatase to 17β-estradiol.

In general, DHT has a relatively higher receptor-binding affinity compared to testosterone[27]. Estimates of the relative potency ratio of DHT to testosterone range from 2 : 1 to 10 : 1. Likewise, the dissociation constant for DHT is 0.25–0.50 nmol/l, while the K_d of testosterone is 0.4–1.0 nmol/l, indicating that DHT is a much stronger androgen.

On the other hand, the situation in the muscle in this respect is somewhat different. It is known that skeletal muscle is almost devoid of 5α-reductase activity and therefore testosterone is the major hormone promoting anabolism in skeletal muscle. This, however, does not mean that 5α-reduced androgens or 19-nor-androgens possess weaker pharmacological effects on the muscle. Furthermore, the relative binding affinity of DHT to AR in the muscle is relatively lower than that to AR in the prostate. However, prostate tissue is rich in 5α-reductase activity, and almost all the testosterone that enters the prostate is converted to DHT, which maintains its growth along with that of seminal vesicles and vas deferens, hence exerting its androgenic action[8]. These data show that the conversion of testosterone in various tissues into different metabolites and the activating ability of these metabolites on AR in the tissues are responsible for its diverse pharmacodynamic profile.

Another possible explanation of the tissue-specific actions of testosterone is offered by observations that certain co-regulators of AR, which influence a number of functional properties of the nuclear receptor, including ligand selectivity and DNA binding capacity, can be differently distributed in the body[24].

As to the aromatization of testosterone to 17β-estradiol, we know that there is only one gene known for the expression of aromatase. However, tissue specificity of the aromatization of testosterone is realized by the control of tissue-specific promoters and regulated by different cohorts of transcription factors. In brief, estrogen formation is controlled by glucocorticoid as well as different cAMP-dependent promoters of the sole aromatase gene[28]. The power of local 17β-estradiol biosynthesis has been clinically illustrated by cases of boys and men in whom aromatase expression in adipose tissue, and possibly also in bone, is greatly increased whereas that present in the testes is unaffected. This results in florid gynecomastia and short stature due to premature epiphyseal fusion. This condition is a consequence of chromosomal rearrangements that result in the insertion of a constitutive promoter upstream of the start of translation of the aromatase gene[29].

Finally, there is an increasing amount of information about rapid activation of kinase-signalling cascades and modulation of intracellular calcium levels by androgens. These fast non-genomic effects of testosterone are expected in a number of cellular effects. An example of the membrane-dependent action of testosterone is the ability of testosterone to induce an increase in intracellular calcium in different cell types including T-lymphocytes and macrophages, or stimulating gap junction communication, neuronal plasticity and aortic relaxation. Another example is the increase in intracellular Ca^{2+} owing to DHT in osteoblasts within 50–60 s simultaneously with diacylglycerol formation (5–60 s), phosphorylation of the transcription factor Elk 1 (60 s), and the translocation of PKCα and PKCβ1 within 5 s[30].

The non-genomic action of testosterone can also be mediated by at least two androgen-binding proteins, the classical nuclear AR, which is also detectable in the cell membrane and sex hormone binding globulin (SHBG). In both of these cases, the biological effect of the non-genomic stimulation of second messenger cascades may be the enhancement of AR transcriptional activation like an autocrine loop (for review of non-classical testosterone-actions see references 31 to 33).

Conclusion

The nature of a testosterone-induced signal (genomic vs. non-genomic) may depend on the type of target cell, the receptor location within cells, as well as the ligand itself. However, our

knowledge about the fine-tuned co-ordination between the 'classical' nuclear receptor-mediated mode of action and the membrane-dependent mechanisms is limited at present. The identification of molecules capable of selectively altering genomic vs. non-genomic signalling may be useful in delineating the roles of these pathways in mediating androgen responses, can better explain the toxicological pattern of testosterone therapy and might lead to the development of novel therapeutic compounds that modulate these signals more specifically[34].

MECHANISM OF ANABOLIC ACTION OF TESTOSTERONE ON MUSCLE

Some studies have shown that administration of testosterone to young and elderly hypogonadal men results in an increase in lean body mass and, in some studies, muscle strength[35]. The causal role of declining androgen levels with aging in loss of muscle mass/strength awaits further corroboration. Androgen therapy has a positive effect on lean body mass (muscle volume), but testosterone effects on muscle strength (without concomitant exercise) in hypogonadal men are still controversial at present[36]. The testosterone-induced increase in muscle volume is mainly due to muscle fiber hypertrophy[37].

However, Wang and colleagues[38] reported the effects of 180 days of treatment with 1% testosterone gel preparation (50 and 100 mg/day) in hypogonadal men. Mean muscle strength in the leg press increased by 11–13 kg by 90 days and did not improve further after 180 days of treatment. Supraphysiological doses of testosterone result in an increase in muscle mass and also strength, even in eugonadal men pretreated with gonadotropin releasing hormone (GnRH), especially when combined with strength training[39,40]. Weekly intramuscular injections of 25, 50, 125, 300, or 600 mg testosterone enanthate for 20 weeks in 61 GnRH-suppressed eugonadal men demonstrated clear and linear dose–response relationships from hypogonadal to supraphysiological testosterone levels (without any plateau). Changes in leg press strength, leg power, thigh and quadriceps muscle volumes, hemoglobin and insulin-like growth factor-I (IGF-I) were positively correlated with linearly increasing testosterone concentrations[41]. The responses observed in these men (even though the majority of ARs are likely to have been saturated) suggest that testosterone also mediates anabolic effects indirectly, i.e. not via AR. Therefore, the anabolic actions of testosterone can be divided into direct and indirect mechanisms (for review see references 8 and 42).

Direct mechanism

Administration of testosterone to hypogonadal men results in an increase in both contractile and non-contractile skeletal muscle proteins and also an increased incorporation of leucine into the skeletal muscle[43,44]. Interestingly, oxandrolone administration significantly increased mRNA levels of skeletal muscle AR[45]. Taken together, androgens increase muscle mass and strength by increasing efficient utilization of amino acids and, at least in the case of oxandrolone, by increasing AR expression in skeletal muscle. Aromatization is neccessary for androgen action on bone but not on muscle[46].

Indirect mechanisms

Interaction with IGF-I system

One of the proposed mechanisms by which testosterone increases muscle mass is by alteration of multiple muscle growth factors[47]. Intravenous infusion of IGF-I results in stimulation of skeletal muscle protein synthesis[48]. It has been shown that androgens are necessary for the local production of IGF-I within skeletal muscle regardless of the systemic IGF-I levels and rate of growth hormone (GH) production[35]. When older hypogonadal men were treated with testosterone, there was an increase in IGF-I mRNA levels in skeletal muscle[49,50]. However, treatment of young men with recombinant human IGF-I (60 μg/kg, subcutaneous, twice daily over 10 weeks) mimicked the testosterone effects in enhancing lean body mass[51]. This is also supported by the observation that induction of hypogonadism in normal young men resulted in a reduction in IGF-I mRNA levels in muscle biopsy specimens[52]. Administration of 100 or 200 mg testosterone enanthate intramuscularly weekly over 3 weeks elevated serum IGF-I concentrations only in older, not in younger men. Gentili and co-workers[53]

have proposed that testosterone administration to older men affected the GH/IGF-I axis, resulting in increased burst mass, basal secretion and 24-h rhythmicity of GH secretion, as well as increased IGF-I serum levels. The mechanisms by which testosterone affects muscles also include decreased inhibitory IGF-binding protein 4[54]. These reports show that the interactions between testosterone and the IGF-I system are additional factors for the explanation of anabolic androgen effects on muscle.

Conversely, estrogen and non-aromatizable androgens typically fail to elevate systemic IGF-I concentrations in humans, indicating that the aromatization of testosterone is negligible for direct anabolic effects on muscle, despite the fact that muscle can be an important source of estrogens in men (for review see references 28 and 53).

Repression of myostatin gene

This gene is a member of the transforming growth factor (TGF)-β superfamily, is located on chromosome 2 and is a negative autocrine regulator of muscle growth. The human myostatin gene has already been cloned. Inactivating mutations of the myostatin gene in mice and cattle are associated with double muscling in these animals[55]. The myostatin protein is secreted into the serum and can be measured in the circulation. Myostatin levels were elevated in the serum and skeletal muscle biopsy specimens of patients with AIDS-associated sarcopenia compared with those in AIDS patients without any weight loss and normal controls. In other words, heightened myostatin levels are associated with muscle loss[56]. Furthermore, high levels of circulating myostatin have produced muscle atrophy in the rat[57]. There is some indication that the myostatin protein could also play a role in age-associated sarcopenia[58]. This can be supported by the observation that low gravity-induced muscle wasting accompanies an increase in myostatin mRNA[59].

Marcell and co-workers[60] found no significant relationships between age, lean body mass and transcript levels of GH receptor, IGF-I, AR, or myostatin in 27 healthy men of > 65 years of age. There were also no significant correlations of serum GH, IGF-I, androgens, or testosterone with their corresponding target mRNA levels (GH receptor, intramuscular IGF-I, or AR) in skeletal muscle. However, GH receptor was negatively correlated with myostatin mRNA levels. The lack of apparent relationships between muscle transcripts and their respective ligands in healthy older men suggests that age-related deficits in both GH and testosterone may lead to an increase in myostatin expression and a dissociation of autocrine IGF-I effects on muscle protein synthesis, both of which could contribute to age-related sarcopenia. In this context, it is possible to assume that androgens may exert their anabolic effects, at least partly, by either directly or indirectly suppressing the expression of myostatin. Therefore, the ability to inhibit myostatin expression in humans with age, cancer, HIV, or trauma-related losses of skeletal muscle mass may have profound clinical implications. However, at present the role of myostatin in humans has not been extensively studied, and there is much speculation[8,42].

Antiglucocorticoid action

Men with androgen insensitivity syndrome also show nitrogen retention when given large doses of testosterone despite having non-functional ARs[61]. Similarly, testosterone administration to patients with severe burns (a state of hypercortisolism and hypogonadism) results in a significant decrease in protein breakdown[62]. In this context, there is some indirect evidence that the anabolic effects of testosterone on skeletal muscle may also be mediated through an antiglucocorticoid action[63]. There is a high degree of homology between AR and glucocorticoid receptor, and therefore it is not surprising that evidence exists that the anabolic effect of testosterone on skeletal muscle may also be mediated by the antiglucocorticoid action of this steroid[64]. Testosterone is known to have a high level of affinity to the glucocorticoid receptor and show antagonistic effects against endogenous circulating glucocorticoids[65–67]. These observations are further supported by the fact that antagonism of glucocorticoids prevents muscle atrophy in men who have undergone orchidectomy[68]. Another indirect mode of antiglucocorticoid action of testosterone could be interference with glucocorticoid action at the gene level by interfering with hormone response elements[69,70].

Safety considerations

Despite the apparent positive effects of testosterone in the physiological dose range on muscle strength, alterations in connective tissue structure induced by high-dose anabolic steroids in eugonadal men have been associated with deleterious effects on tendon strength. Evidence suggests that anabolic steroid abuse leads to dysplasia of collagen fibrils, resulting in a decrease in overall tendon tensile strength[71]. The risk of triceps tendon rupture, a relatively uncommon injury, is also increased in association with androgenic steroid administration in an unphysiologically high-dosage regimen[72].

Conclusion

With respect to the anabolic action of testosterone on muscle, as shown by Bhasin and associates[41], there are clear dose–response relationships from hypogonadal to supraphysiological serum testosterone levels (without any plateau). Using extremely high testosterone doses, at which androgen receptors should already have been saturated, a linear dose–response relationship can be seen indicating additional indirect anabolic actions of testosterone on skeletal muscle, such as anti-glucocorticoid action, induction of IGF-I synthesis and repression of the myostatin gene. Exogenous estrogens (or androgens after aromatization) have no anabolic effect on the muscle, at least, in human males.

ERYTHROPOIESIS

The stimulatory effect of testosterone on erythropoiesis in laboratory animals and humans is well documented; there are linear dose–response relationships. DHT was found to be more active than testosterone in stimulating erythropoietin. Since finasteride, a 5α-reductase inhibitor, adversely affected stimulated-erythropoietin production in mice, it is suggested that the testosterone effect on stimulated-erythropoietin secretion is mediated mainly by DHT[73].

In humans, elevated levels of erythropoietin have been found in the urine of healthy hypogonadal and anemic subjects after the administration of testosterone, indicating an increase in erythropoietin synthesis in the kidney. Additionally, testosterone has been found to stimulate the proliferation of erythroid progenitors (in the absence of erythropoietin) and, to some extent, myeloid progenitors. A third mode of action has been shown by the increase of erythrocyte 2,3-diphosphoglycerate owing to testosterone administration. 2,3-diphosphoglycerate decreases hemoglobin-oxygen affinity, facilitating the release of oxygen from hemoglobin to tissue (for review see reference 42). In summary, the administration of testosterone acts in three different ways – by increased erythropoietin activity, by increased pool of erythropoietin-responsive cells and by stimulated 2,3-diphosphoglycerate – which explains the constant dose–response relationship without plateau even in the supraphysiological range.

A significant increase in hemoglobin and hematocrit was also seen with treatment with a DHT gel, indicating that the effect of testosterone on erythropoiesis can be supported by reduction of testosterone to DHT[74].

Whereas a moderate increase in hematocrit in older males is possibly beneficial, some studies have reported an increase in hematocrit over 51% (polycythemia) occurring in up to 25% of older patients[75–77]. This leads to hyperviscosity, requiring temporary withholding of the treatment and even phlebotomy. Available data suggest that the frequency of this side-effect is related to supraphysiological levels and a rise of hematocrit of only 3–5% can be expected with physiological testosterone levels. As transdermal testosterone administration yields testosterone levels within the normal range, this may explain the reported lower frequency of polycythemia with this form of treatment[38,78].

Estradiol has no effects on erythropoietin mRNA in the kidney[79] and high-dose estrogen administration induces anemia in mammals[80].

Conclusion

There are linear dose–response relationships regarding androgenic action on erythropoiesis. Several modes of action are being discussed and DHT is as effective as testosterone. Estrogens are, at least, not stimulatory in this respect.

LIPID METABOLISM

Normally, in middle-aged men endogenous testosterone is positively correlated with high-density lipoprotein (HDL) cholesterol and apolipoprotein B levels, whereas 17β-estradiol is correlated to apolipoprotein E levels[81,82].

However, for a long time, it has been widely held that exogenous testosterone supplementation adversely affects the plasma lipoprotein profile and increases the risk of atherosclerotic heart disease in men. On the other hand, the presently available data do not support this premise. Supraphysiological doses of non-aromatizable androgens frequently employed by eugonadal bodybuilders undoubtedly decrease plasma HDL cholesterol levels and the subclasses HDL2 and HDL3 by inducing hepatic lipase[83]. In contrast to this, Tan and co-workers[81] found that the decrease of HDL induced by exogenous testosterone was mainly in HDL3 cholesterol and apolipoprotein A-I:A-II particles and not in the anti-atherogenic HDL2 and apolipoprotein A-I particles.

Compared with these findings in eugonadal men, physiological testosterone replacement in older hypogonadal men has been associated with a modest decrease, if any, in plasma HDL cholesterol. Cross-sectional studies of middle-aged men have found a direct, rather than an inverse, relationship between serum testosterone levels and plasma HDL cholesterol concentrations, and an inverse correlation between testosterone levels and visceral fat volume. Placebo-controlled studies in older men have demonstrated no significant change in plasma HDL cholesterol levels during long-term administration (for review see reference 84). Whitsel and associates[85] analyzed 19 clinical studies of testosterone replacement (mainly testosterone enanthate) in hypogonadal men. This meta-analysis suggests that administering intramuscular testosterone esters (with supraphysiological testosterone levels in the first days after injection) is associated with a small, dose-dependent reduction of HDL cholesterol concentration. The authors also found treatment-related decreases in total cholesterol and LDL cholesterol.

In GnRH-pretreated eugonadal men, over a wide range of doses, there were weak linear negative correlations between serum testosterone or serum free testosterone concentrations and the decrease of plasma HDL cholesterol levels and plasma apolipoprotein A-I levels[86]. However, androgen-induced declines in circulating HDL cholesterol should not automatically be assumed to be proatherogenic, because these declines may instead reflect accelerated reverse cholesterol transport[87]. The transdermal administration of testosterone (patches or gel) did not markedly affect the lipid or lipoprotein parameters by avoiding the induction of hepatic lipase[38,88].

On the other hand, based on current evidence, the therapeutic use of testosterone in men with cardiovascular problems is presently open to debate and needs further investigation[87]. Observational studies have certainly shown that blood testosterone concentrations are consistently lower among men with cardiovascular disease, suggesting a possible preventive role for testosterone therapy, but critical evaluation by further prospective studies is required[89]. Hak and colleagues[90] found an independent inverse association between serum levels of testosterone and aortic atherosclerosis in 1032 non-smoking men. This means that low testosterone serum levels increase the risk of atherosclerosis in men over 55 years of age. Dockery and associates[91] found that testosterone suppression in men with prostate cancer was associated with arterial stiffness. In the same context, Fukui and co-workers[92] demonstrated that in 253 men with type 2 diabetes, serum free testosterone was inversely associated with carotid atherosclerosis determined by ultrasonographically evaluated intima-media thickness and plaque score. Perusquia[93] postulated that androgens induce vasorelaxation and therefore have a protective effect on the vascular system. This conclusion was not accepted by Zitzmann and co-workers[94,95] who demonstrated that a decrement of endothelium-mediated vasodilatation occurs with increasing testosterone serum levels. In the same context, Sader and colleagues[96] found that subcutaneous implantation of four 200-mg testosterone pellets every 6 months in nine hypogonadal men was associated with decreased endothelium-dependent dilatation of the brachial artery.

Regional fat distribution

Androgen deprivation results in a marked reduction in the mRNA and protein levels in genes

involved in fatty acid (fatty acid synthase and acetyl-CoA carboxylase) and cholesterol synthesis (human menopausal gonadotropin (HMG)-CoA-reductase and farnesyl diphosphate synthase). Readministration of testosterone or DHT immediately following orchidectomy in rats restores the expression of all four genes. In support of the co-ordinated nature of this regulation, androgen-induced upregulation of lipogenic gene expression is accompanied by an increase in the nuclear content of sterol regulatory element binding proteins (SREBPs), a key lipogenic transcription factor[97]. Male androgen receptor-deficient (ARKO) mice show a typical late onset obesity with asymmetrical fat distribution in different organs[98].

Several intervention studies have demonstrated favorable effects of testosterone treatment on fat tissue distribution, insulin sensitivity, blood lipids and blood pressure in men diagnosed with symptoms of the metabolic syndrome[99–101].

Since the publications of Björntorp, we have known that testosterone replacement in hypogonadal men significantly reduces abdominal visceral fat[102]. The reduction of fat mass with testosterone replacement is linearly proportional to the administered dose of testosterone enanthate, which means no S-curve or plateau[84].

Jockenhövel and associates[103] found that the non-aromatizable mesterolone (DHT derivative) had effects on lipids and lipoproteins. Similarly, Kalintchenko[104] found that 25 mg mesterolone taken twice daily significantly reduced visceral adipose tissue, body mass index (BMI), total plasma cholesterol, plasma triglycerides and diastolic pressure in men with BMI > 30, indicating that these androgen effects were, at least partly, dependent on 5α-reduction.

The effects of androgens on lipid profile and body fat distribution cannot be mimicked by estrogens. However, BMI and visceral fat are correlated positively with serum estrogens (for review see references 105 to 109).

Conclusion

Supraphysiological doses of testosterone and DHT can alter the lipid profile unfavorably in a linear manner. However, both androgens are capable of decreasing the amount of visceral adipose tissue, indicating the significance of 5α-reduction. Aromatization of testosterone to estrogen is positively correlated with visceral obesity.

AGGRESSIVE BEHAVIOR

Since the work of Brain and Poole[110] we have known that androgens influence aggressive behavior in mice[111]. This has induced speculation that the same conditions are also found in men. In principle, the subject of aggressiveness and testosterone therapy is a permanent topic in psychoneuroendocrinology. Reports on the connection between testosterone administration and aggressive behavior in several pharmacological models have become more and more differentiated. However, Martinéz-Sanchis and colleagues[112] reported that in gonadally intact male mice the aggression-inducing effect of testosterone was only marginal. The behavioral effects in the total sample were only found in the previously aggressive animals selected on the basis of their latency of attack in the first encounter. McGinnis and co-workers[113] showed that, in gonadally intact rats, androgens alone did not induce indiscriminate and unprovoked aggression characteristics, but testosterone-propionate administration (5 mg/kg subcutaneous over 12 weeks) heightened the animals' sensitivity to external stimuli and lowered the threshold for aggression and dominance in response to provocation.

Animal models for aggression have only limited value for translation to humans. Twenty-five years ago, we found that intranasally administered $ZnSO_4$ made male mice temporarily anosmic. In that status the inter-male aggressiveness was significantly reduced. The same results were seen by clipping the whiskers, the so-called vibrissae[114]. Our assumption, at the time, that the aggressive behavior of rodents was mediated by pheromones, has been confirmed by Martínez-Sanchis and associates[115]. When anosmic opponents were used, animals showed a significantly lower frequency of attack and threat. These are examples of the species specificity of aggressive behavior in rodents. The testosterone effects in mice are related to 5α-reduction to DHT as well as to aromatization to 17β-estradiol[116,117]. In principle, it has been confirmed that the most important CNS effects of testosterone depend either totally or partly on local aromatization to estrogens[14,106,109].

However, high levels of endogenous testosterone in men seem to encourage behavior intended to dominate others. Sometimes dominant behavior is aggressive, its apparent intent being to inflict harm on another person, but often dominance is expressed non-aggressively. Sometimes dominant behavior takes the form of antisocial behavior, including rebellion against authority and law breaking. Measurements of testosterone at a single point in time, presumably indicative of a man's basal testosterone level, predicts many of these dominant or antisocial behaviors. Testosterone not only affects behavior but also responds to it. The act of competing for dominant status affects male testosterone levels in two ways. First, testosterone rises in the face of a challenge, as if it were an anticipatory response to impeding competition. Second, after the competition, testosterone rises in winners and declines in losers[118].

There are few epidemiological studies that suggest a correlation between serum testosterone levels and aggressiveness or antisocial personality[119,120]. However, the majority of epidemiological studies have underlined that there is no significant relationship between serum testosterone and single or aggregate measures of aggression[121–124]. Christiansen[125] believed that hormonal influences on behavior are much less potent in humans than in animals, a view that should be concurred with. Differences in behavior are considered to result mainly from a combination of intrapsychic, social and cultural factors.

In a clinical study comparing intramuscular testosterone undecanoate with intramuscular testosterone enanthate in hypogonadal men, a stimulatory effect of the two androgen preparations on aggressive behavior was not found (Figure 1)[126].

What has been reported on high-dose testosterone administration in eugonadal men? With extremely high doses, of up to 600 mg of testosterone cypionate per week, a higher score of aggressive response has been reported. However, these effects were not uniform for all individuals; most showed little psychological change[127,128]. On the other hand, doses of testosterone cypionate up to five times the physiological replacement dose appear to have a minimal risk of adverse psychological effects in the majority of normal men[129]. A dose of 200 mg testosterone enanthate administered to 30 eugonadal men weekly for

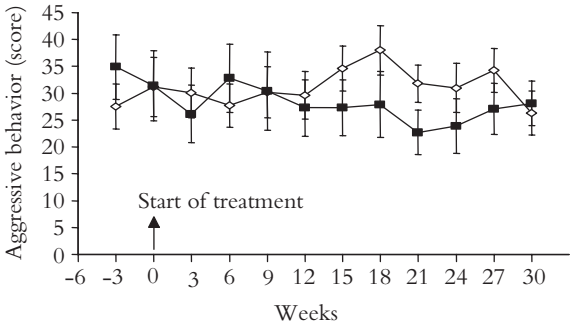

Figure 1 Scores (mean ± SEM) of aggressive behavior in two groups of 20 hypogonadal men before and during testosterone therapy with intramuscular ◇ testosterone undecanoate (1000 mg every 6 weeks for the first 12 weeks and, thereafter, every 9 weeks) or ■ 250 mg testosterone enanthate every 3 weeks. No significant differences at any time (Wilcoxon rank test)[126]

8 weeks produced no significant changes in aggression or mood levels[130]. Tricker and colleagues[131] found no increase in angry behavior, in a double-blind, placebo-controlled study using the extreme supraphysiological dosage regimen of 600 mg testosterone enanthate per week.

Hypogonadal men with certain mood changes were identified as a group also benefiting from testosterone treatment. Significant reductions in tension, anger and fatigue were reported by O'Connor and co-workers[130] in eugonadal as well as in hypogonadal men treated with testosterone enanthate for 8 weeks. Significant reductions in negative mood (tension, anger and fatigue) followed by an increase in vigor were found in response to testosterone therapy in the hypogonadal group. However, no significant changes in aggression or mood levels were found in the eugonadal group indicating that the testosterone effect reaches a plateau in this respect. These findings support other studies that have found testosterone administration to have positive mood effects when testosterone levels are restored to the normal range and suggest that prolonged treatment is likely to maintain these mood benefits, but not to improve them further[132–134]. The marked elevations in self-esteem scores and positive mood states are likely to be concomitant with the restoration of normal sexual function. Therefore, it is possible that improvement in the psychological functioning is related to changes in sexual function.

Conclusion

The influence of exogenous testosterone on aggressive behavior in hypogonadal men is marginal and dose–response relationships are not detectable. However, important CNS effects of testosterone are assumed to be connected with its local aromatization to estrogen.

ANDROGENS AND PROSTATE

Prostate cancer

Androgen receptors (ARs) are widely distributed in the prostate, and therefore testosterone may influence prostate specific antigen (PSA) levels, increase prostate size and obstructive symptoms, and could activate occult prostatic malignancy[135].

The literature on the relationships between the androgenic milieu and prostate cancer is still inconsistent. Since the publication by Huggins and Hodges[136] in 1941, on the favorable effects of castration on the natural history of prostate cancer, many scientists have been investigating the relationships between endogenous androgen activity and development of proliferative prostate pathologies. Androgens have been presumed to activate the proliferation of prostate cells directly via the stimulation of growth factor synthesis[137].

Testosterone therapy in men with erectile dysfunction and hypogonadism is associated with a minor PSA elevation, but there does not appear to be a short-term increase in risk for the development of prostate cancer[138]. Current knowledge of the correlation between exogenous testosterone and the risk of prostate cancer has been summarized in many reviews and is not discussed here in greater detail[84,139–144]. Although there is no evidence that normal levels of testosterone promote the development of cancer of the prostate, it is clear that the administration of testosterone could enhance a pre-existing prostatic malignancy (carcinoma *in situ*). However, current evidence does not support the view that appropriate treatment of older hypogonadal men with androgens has a causal relationship with prostate cancer. Nevertheless, more experience is needed before firm conclusions can be drawn. In this context, we should not ignore the meta-analysis by Shaneyfelt and associates[145], indicating that men with either serum testosterone or IGF-I levels in the upper quartile of the population distribution have an approximately two-fold higher risk of developing prostate cancer.

Although prostate event rates have not been significantly different between placebo- and testosterone-treated men, in most of the recently published studies of testosterone therapy in older men, it is important to recognize that these studies do not have sufficient power to detect significant differences in relative risks. It has been estimated that detection of a 30% difference in prostate cancer incidence rates between the placebo- and testosterone-treated groups would require inclusion of approximately 6000 older men with low testosterone levels for an average of 5 years. To date, no study of this size has been funded[144].

However, publications do exist that prove an inverse correlation between serum testosterone levels and the malignancy of prostate cancer. A higher prevalence of biopsy-detectable prostate cancer was identified in men with low total or free testosterone[146]. Other publications have shown that serum total and free testosterone levels are reduced in patients with prostate cancer[147] and that patients with low free testosterone had more metastatic disease[148,149]. Despite the dependency of the normal prostate and most prostatic cancers upon androgens, and the fact that tumors can be produced in some rodent models by androgen administration, Prehn[150] speculated, and provoked us by maintaining, contrary to prevalent opinion, that declining rather than high levels of androgens probably contribute more to human prostate carcinogenesis and that androgen supplementation would probably lower the incidence of prostate cancer. However, this courageous conclusion remains hypothetical at present.

Benign prostatic hyperplasia

For centuries, it has been known that benign prostatic hyperplasia (BPH) occurs mainly in older men and that it does not develop in men whose testes were removed before puberty. In 1944 Moore[151] reported that a group of 28 eunuchs, eunuchoids and patients with pituitary infantilism showed no evidence of BPH. Another study of 26 eunuchs from China was reported by Jie-ping and Fang-liu in 1987[152]. Seven of these men were castrated before puberty, and 18 were castrated between the ages of 10 and 26. The age of

castration of one man was not known. The follow-up in the men occurred 41–65 years later, and all exhibited non-palpable (81%) or very small prostates (19%). None of the men had obstructive voiding symptoms. However, 22 (85%!) had nocturia two to ten times per night.

It is interesting to note that, more than 100 years after castration was first performed in the Western world for BPH, the exact role of the testes and hormonal androgenic factors remains unknown. In some studies, patients with BPH had higher testosterone levels than healthy men[153,154], and the volume of hyperplastic prostate was positively correlated to the serum free testosterone level[155]. Brochu and Belanger[156] found higher androsterone levels in patients with BPH compared to healthy men. However, according to Hammond and co-workers[157], there were no differences in serum androsterone levels between men with BPH and healthy subjects. Patients with BPH had a reduced urine androsterone/etiocholanolone rate, which probably reflects the diminished activity of 5α-reductase or relatively increased activity of 5β-reductase[158].

Others observed no differences in serum androgen activity between men with and without BPH[159–162], and revealed no relationship between serum free testosterone levels and prostate dimensions. According to Gann and associates[163], endogenous androgen activity in the general male population did not affect the probability of BPH occurrence. In men on hemodialysis, serum levels of total and free testosterone, and estradiol did not correlate with the prostate volume[164].

Is BPH perhaps an androgen-deficiency disease? Some authors found reduced serum testosterone levels in men with BPH as compared to controls[165–167]. A very provocative contribution to the hypothesis that 'BPH is a testosterone-deficiency symptom' came from Peshersky and co-workers[168], who demonstrated inverse relationships between baseline serum testosterone levels and the prostate volume and PSA (see also above). This means that androgen replacement could be beneficial for the treatment of BPH.

Other authors have paid particular attention to the phenomenon of relative hyperestrogenism among men with BPH, i.e. increase in the serum estradiol/testosterone ratio[166,169]. Suzuki and colleagues[162,165] found positive correlations between serum estradiol levels, the serum estradiol/free testosterone ratio, and the prostate size. In hypogonadal male mice, Bianco and associates[170] found that estradiol proliferated stromal fibroblasts, whereas smooth-muscle cells were reduced. There are at least two isoforms of estrogen receptor. Estrogen receptor α is expressed by prostatic stromal cells, while estrogen receptor β is expressed by prostatic epithelial cells[171]. Since estrogen receptors α and β are differentially expressed, the estrogenic responsiveness of the prostate will be dictated by the type of receptor stimulated. Identification of genes that are differentially regulated by two receptors could be essential to defining the role of estrogen in the prostate in more detail. The hormonal changes in the course of the so-called 'andropause' (e.g. increase in the estrogen/androgen ratio within hormone-dependent tissues) seem, for some authors, to be conducive to the development of BPH. The results of Dahle and co-workers[172] are along these lines. Their results in 200 men with newly diagnosed BPH suggest that abdominal obesity (negatively correlated to testosterone and positively correlated to estrogens) and increasing serum insulin are associated with a higher risk of BPH. Shibata and colleagues[173] found an age-dependent decrease in prostatic DHT and constant estradiol concentration leading to a relatively estrogen-dominant local environment compared to that at younger ages. The authors assumed that this relatively estrogen-dominant status induces stromal proliferation by some mechanism and leads to the development of BPH. On the other hand, leptin levels (which are androgen dependent) are unlikely to affect substantially the risk of either prostate cancer or BPH[174]. According to Gann and associates[175], the increased serum estradiol level was presumed to promote the development of BPH. A clinical trial, of 49 men with obstructive BPH treated with relatively weak aromatase inhibitor, initially confirmed the above-mentioned theory[176]. Unfortunately, the larger Schering clinical study with the same compound did not agree with these findings[177].

Despite the fact that male aging is accompanied by an increase in estrogen concentrations in many peripheral tissues, i.e. in the prostate[178], and also by an increase in the serum estrogen/androgen ratio[165], some results do not confirm this increased endogenous serum estrogen activity in men with BPH. In other studies there were no differences

in serum estradiol levels between patients with BPH and healthy men[157,160,161,174].

It has been demonstrated[155] that BPH volume correlates with free testosterone and estrogen levels in the serum when adjusted for age. These findings suggest that androgen levels, acting synergistically with estrogen concentrations, might be important factors in the persistence of BPH. Based on the above studies, it can be assumed that persistent androgenic stimulation, coupled with certain estrogenic synergisms, are involved in the increase in BPH with age. Intuitively, Partin[179] assumed that therapeutic attempts at simultaneously lowering plasma testosterone levels and reducing estrogen levels could prevent progression of BPH. This point of view can also explain, why the administration of aromatase inhibitors is insufficient. There is only a reduction of the serum estrogen levels, however, there is a simultaneous increase in testosterone concentrations[177].

In summary, there is great controversial discussion about the evidence that initiation and development of BPH is definitively and/or dominantly under endocrine control in humans. We do know that BPH requires testicular androgens during prostate development, puberty and aging, and that the stromal cell plays a central role in androgen-dependent paracrine-mediated growth (at least from the point of view of older publications). We also know that canine models suggest estrogen synergism with androgen exposure, particularly DHT, in the development of BPH. However, the translation of these more or less pharmacological-endocrinological findings to the concrete clinical situation in hypogonadal testosterone-treated men remains difficult.

More interesting is the clinical point of view. Based on a meta-analysis of relevant published clinical studies, Bosch and colleagues[180] found no signs indicative of testosterone supplementation in hypogonadal men increasing prostate volume or of PSA levels over the range of same-age eugonadal controls[38,181]. Nor did Bhasin and associates[41] find any dose-effective relationships regarding PSA and prostate volume with testosterone enanthate in an extremely wide dose regimen.

In a recent publication, Gooren[36] stated that relevant epidemiological studies provide no clues that the levels of circulating androgen are correlated with or predict prostate disease. It would seem that non-obstructive BPH is not a contraindication against androgen administration, but obstructive BPH is. In this context, Bhasin and co-workers[144] also argued that testosterone therapy can be administered safely to men with BPH who have mild to moderate symptom scores.

Conclusion

There are no clear relationships between the incidence of prostate cancer and exogenous testosterone. As to prostate volume or PSA and exogenous testosterone, there are no linear dose–response relationships. In hypogonadal men, testosterone replacement restores prostate volume and PSA to levels comparable to those of eugonadal men of the same age. The role of estrogens, and thus aromatization of testosterone to estrogens, has been discussed with regard to being a possible cause or concomitant circumstance of BPH.

REDUCTION VERSUS AROMATIZATION

As discussed above, there are three modes of action of testosterone. It may act directly on the nuclear or membranous ARs, be converted by 5α-reductase enzymes 1 and 2 to DHT before acting on the AR, or be aromatized to estrogens that act on the nuclear or membranous estrogen receptors.

The phenotypic appearance of men with congenital 5α-reductase enzyme 2 deficiency has demonstrated the critical role of DHT on some androgen target tissues such as the external genitalia, prostate and skin. Men with this congenital enzyme defect have very small prostate glands and ambiguous external genitalia, but have increased muscle mass at puberty and are not osteoporotic[182–185].

On the other hand, testosterone is the major source of plasma estradiol in men. Only 20% of this estrogen is secreted by the Leydig cells in the testes[107]. Whereas to date fatty tissue aromatase has been assumed to be the main source of estrogen production in men, Larionov and colleagues[28] have shown that estrogen formation also may be controlled by glucocorticoid as well as by cAMP-dependent promotors of the aromatase gene in the muscle.

Estrogens play a major role in many metabolic processes in men[14,105,106,108,109,186]. Also, mood and cognition[187], cardiovascular diseases[82,188–190], sexual function including libido[191] and bone turnover[192,193] are estrogen-influenced in men.

Figure 2 summarizes the different possible androgenic effects depending on the respective metabolism of testosterone. It becomes apparent that either the natural aromatizable testosterone or aromatizable testosterone derivatives should be preferred to androgen therapy. In contrast to this, the therapeutic potential of DHT or DHT derivatives appears relatively limited[74,194].

Fred Wu (Manchester), in a personal communication, supplied the idea and the work of Shalender Bhasin's group, on GnRH-suppressed eugonadal men with a wide dose range for testosterone enanthate[41], the inspiration for Figure 3. It shows that the metabolism of exogenous testosterone (reduction vs. aromatization) is also of toxicological relevance. All endpoints or targets of testosterone actions showing clear linear dose–response relationships up to extremely high dose ranges depend, at least in part, on reduction of testosterone to DHT, i.e. the 5α-reduction has an accompanying part. In addition, these phenomena cannot be explained by a single mode of testosterone action, but often by testosterone becoming active at different points of action. This group of testosterone actions are skeletal muscle (body lean mass), erythropoiesis and lipid profile including body fat distribution. However, this also means that these targets involve the risk of testosterone abuse or overdosage in self-medication because, for example, muscle growth and reduction of visceral fat can be counted among the desirable 'cosmetic' or life-style effects of testosterone therapy. This point of view should be taken into account with testosterone preparations for self-medication such as transdermal application forms. However, long-term overdosage of testosterone may cause life-threatening increases in hematocrit. Toxicologically, therefore, hematocrit is a critical and reliable parameter for the monitoring of a testosterone therapy and should be checked periodically.

On the other hand, for the CNS effects of testosterone, like improvement of mood or the

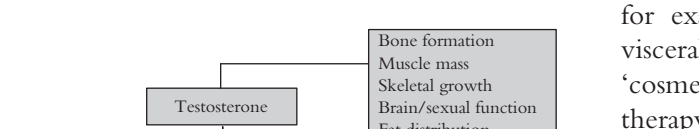

Figure 2 Tissue- and function-specific activities of testosterone and its main metabolites

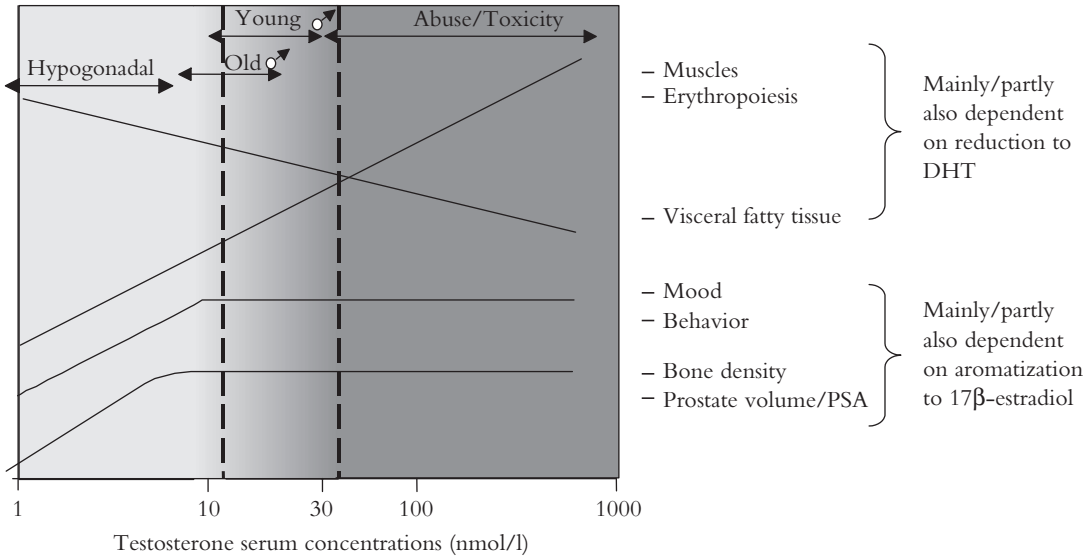

Figure 3 Tissue-specific effects of testosterone in hypogonadal men and the relationships to dosage and metabolism (hypothetical and schematic). DHT, 5α-dihydrotestosterone; PSA, prostate specific antigen

influence on the different domains of behavior, the activities on bone and for the effects of testosterone on the prostate, no clear and linear dose–response relationships have been recognized (plateauing). For most of these targets or parameters with bent dose–response curves, aromatization of testosterone to 17β-estradiol plays a part. Obviously, no risk of abuse or overdosage has been recognized for these targets.

These considerations are hypothetical but should provide some hints to the attending physician to enable prevention or remedy of unwanted side-effects due to testosterone abuse.

Conclusion

Increase in lean body mass and reduction of visceral fat mass are effects of testosterone therapy that are among the preferred endpoints for the hypogonadal patient. As these targets over a wide dose range show linear dose–response curves, testosterone preparations for self-medication involve the risk of abuse due to self-induced overdosage by the patient. The most important toxicological parameter in this context is the rise in hematocrit. However, it is possible to monitor testosterone therapy by checking the hematocrit regularly. On the other hand, the testosterone effects on the CNS, bone and prostate gland are less problematic from a toxicological point of view.

RECEPTOR POLYMORPHISMS

It is well recognized that different people or ethnic groups respond in different ways to the same medication. These differences are often greater among members of a population than they are within the same person at different times (or between monozygotic twins). The existence of large population differences with small intrapatient variability is consistent with inheritance as a determinant of drug response and also for toxicological aspects of testosterone action. It is estimated that genetics can account for 20–95% of variability in drug disposition including pharmacodynamic and toxicological effects[195].

In principle, polymorphisms of genes of androgen-transforming enzymes are also important for the toxicological assessment of testosterone therapy. For example, polymorphisms of the genes for human type 1 and type 2 3β-hydroxysteroid dehydrogenase[196], for type II 5α-reductase[197,198], for CYP3A4 which is responsible for hydroxylation of testosterone[199] and for SHBG[200] have been described. There are significant ethnic variations in total and free serum concentrations of testosterone in healthy men[201]. Finally, a polymorphism of the gene which codes LHβ is also known[202,203].

An androgen insensitivity syndrome can be a result of over 300 mutations in the AR gene known at present[204]. However, what should interest us from a toxicological angle are only the polymorphisms of the AR gene. Three polymorphic markers of AR are known: ARStuI at codon 211 (G1733A), poly-G (GGC)*n* and poly-Q (CAG)*n* at exon 1[205,206]. The latter has met with particular scientific interest.

In the 5′ end of the coding region of the first exon of the AR gene, starting from codon 58, there is a polymorphic $(CAG)_{10–35}$ triplet repeat sequence, which codes for polyglutamine[207,208]. The length of this polyglutamine tract is inversely correlated with the transactivation activity of AR[209,210]. In normal men approximately 14–33 CAG repeats are present[211]. Shorter CAG repeats have been discussed as increasing the risk of prostate cancer and earlier age of onset of prostate cancer[207,208,210–217]. In contrast, Chen and colleagues[218] do not support the hypothesis that a small number of CAG or GGC repeats in the AR increase a man's risk of prostate cancer.

An increase in CAG repeat length is associated with diminished coactivator interaction and transcriptional activity[219]. Long CAG repeats have been reported to be associated with the androgen insensitivity syndrome[220], or with impaired sperm production and undermasculinized genitalia[221]. Expansion of the CAG repeat to ≥ 38 causes spinal and bulbar muscular atrophy (SBMA; Kennedy disease)[222]. This has also been demonstrated clinically in patients with Kennedy's disease (40–62 CAG repeats), where 80% of patients showed clinical signs of partial androgen resistance with gynecomastia being the most prominent[223].

Other evidence for inverse correlations of the number of CAG repeats and androgen-influenced conditions has been provided for BPH[224,225] and sperm production[226,227]. According to Zitmann and associates[228], the number of CAG repeats is negatively correlated with prostate volume as well as prostate growth. The same group found that

vascular reactivity is increased in healthy men with longer triplet residues[229].

As to hormonal contraception, in cases with incomplete gonadotropin suppression the chances of becoming azoospermic were 2.5 times higher in men having more than 22 CAG repeats[230], indicating more distinct reduction of testosterone secretion. The decrease of transactivation activity of the AR with increasing CAP repeats[231] can explain this finding. Furthermore, bone density[232], endothelial function and HDL cholesterol concentrations are correlated with the number of CAG repeats. The CAG repeat polymorphism modulates body fat mass and serum concentrations of leptin and insulin in men. A low number of CAG repeats were independently associated with protective parameters (low body fat mass and plasma insulin), as well as with adverse parameters such as low HDL concentrations[233]. Short CAG repeats in healthy men have been associated with increased bone mineral density[232]. However, Woodhouse and co-workers[234] found that the length of CAG tract was only a weak predictor for the androgenic response in thigh muscle volume and lean body mass.

What can be said about the CNS effects of testosterone and AR polymorphism? Seidman and colleagues[235] found a correlation between CAG repeats and depression. This agrees with our own findings obtained in 213 Finnish men. The repeat number was positively correlated with decreased potency, depression as expressed by the wish to be dead, depressed mood, anxiety, deterioration of general well-being and decreased beard growth[236]. On the other hand, short alleles (≤ 20 CAG repeats) were associated with Alzheimer's disease in men, but not in women[237].

Interestingly, there is no obvious correlation between CAG repeats and serum testosterone[238]. Thus, with androgen production being the same, the clinical androgen effect is substantially influenced by the AR polymorphism. Abnormal expansion of the CAG motif lengths leads to Kennedy's disease, which is accompanied by morphological hypoandrogenic status[26,239]. However, it has been shown in the Massachusetts Male Aging Study that the age-related decline in serum testosterone levels in older men is associated with the number of CAG repeats. Men with shorter CAG repeat length had a greater decline in serum testosterone levels, suggesting that testosterone levels may be modulated by the AR genotype[240]. For clinical practice of testosterone therapy and toxicological considerations, it remains to be elucidated whether these insights are important enough to become part of routine laboratory assessments[241] or for preventive estimating of certain risks of testosterone therapy.

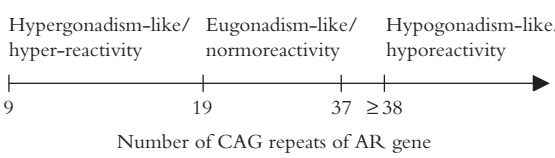

Figure 4 Links between polymorphism of androgen receptor (AR) and transactivation activity (hypothetical and schematic)

Figure 4 shows the postulated links between poly-Q (CAG)*n* polymorphism of AR and the possible clinical as well as toxicological consequences for the testosterone therapy in a schematic way. Of course, this preliminary and incomplete point of view has to be qualified with our increasing knowledge about the relationships between AR polymorphisms and testosterone action.

Conclusion

Polymorphisms of the androgen receptor can significantly influence the quantitative extent of androgenic action. Further investigations are necessary to determine whether the diagnostic determination of polymorphisms of AR are routinely recommendable to individualize the dosages of testosterone, thus minimizing the toxicological risk.

CONCLUDING REMARKS

During the past few years, the relative risk of unwanted side-effects of testosterone therapy in hypogonadal men has clearly been reduced concomitantly with the increase in clinical experience of the signs and symptoms of androgen deficiency and the varying therapeutic options. To date, the key messages of Bhasin and colleagues[242] are still important:

(1) Use natural testosterone;

(2) Aim at a physiological serum testosterone profile;

(3) Allow withdrawal if necessary.

Two points are of particular importance:

(1) Testosterone therapy should be used only in hypogonadal men with clear and stable clinical symptomatology and with repeated and validated estimations of reduced serum testosterone levels.

(2) Use of testosterone preparations which allows a 'physiological' replacement by avoiding supraphysiological serum concentrations. This recommendation is fulfilled by different transdermal preparations such as gels and patches, by buccal preparations and by suitable new intramuscular testosterone esters.

Thus, the discussion about the toxicology of testosterone therapy is changing. The liver toxicity of orally active 17α-methylated or -alkylated androgens and the transient side-effects due to poor pharmacokinetics of the intramuscularly administered old testosterone esters (with short-term phases of supraphysiological testosterone serum concentrations) are no longer to the fore. Interest is now being directed towards the tissue- or target-specific androgen actions and individualizing testosterone therapy.

On the basis of a review of suitable publications, investigation of toxicologically relevant targets or endpoints, e.g. skeletal muscle, erythropoiesis, lipid metabolism including body fat distribution, aggressive behavior and prostate diseases, regarding the correlations between dose–response (linear or non-linear) and metabolism of exogenous testosterone has been attempted. Over a wide dose range there are linear dose–response relationships for the augmentation of lean body mass and erythropoiesis (hematocrit/hemoglobin), as well as for the decline of visceral fat mass. Since the proportions of lean and fat body mass can be given particular attention by the hypogonadal patients (life-style effects), the possibility of overdosage exists with self-medication. Monitoring will succeed with regular checking of the hematocrit.

Other targets of androgenic action such as CNS, bone and prostate show curved, non-linear dose–response relationships with plateauing already occurring at relatively low serum testosterone levels. At these endpoints the risk of overdosage is deemed to be reduced. However, these considerations are still hypothetical and require precise clinical investigation.

Polymorphisms of the AR are also of increasing importance for the assessment of toxicological risk and for the individualization of testosterone therapy. The individual response to a given testosterone dose may for example be influenced substantially by the number of CAG repeats.

What can the future offer from the pharmacological as well as toxicological angle? To date, androgens/anabolics have been screened almost exclusively with the same pharmacological model. The so-called Hershberger assay in immature, castrated male rats was designed as an *in vivo* test system to monitor androgenic and myotropic activity of steroids by their ability to induce growth in the levator ani muscle, seminal vesicles and prostate[243]. Therefore it can be easily imagined that the pharmacological profile of the previously known androgens and anabolics will more or less always be the same. The situation has changed drastically with the introduction of modern molecular biological methods for screening of tissue-specific androgens using certain steroid-transforming enzymes or different co-factors of AR as a target. Selective androgen receptor modulators (SARMs) may act tissue selectively, i.e. they may increase the transactivation of AR in certain target organs, but show reduced or anti-androgenic action in other tissues, and could open a new era in androgen therapy with further reduced toxicological problems[244–248].

Finally, endocrine-toxicological aspects of testosterone therapy should be considered and discussed in context with corresponding guidelines or consensus documents[144,249–258]. To date, the only international society that has issued official recommendations for testosterone treatment is ISSAM[258]. Whilst it seems that these papers are more or less concordant, a harmonization of the national, continental and international documents is desirable.

References

1. Bagatell CJ, Bremner WJ. Androgens in aging men: do men benefit from testosterone replacement? *J Anti-Aging Med* 1998;1:359–64
2. Tenover JL. Testosterone replacement therapy in older adult men. *Int J Androl* 1999;22:300–6
3. Tenover JL. Experience with testosterone replacement in the elderly. *Mayo Clin Proc* 2000;75:77–82
4. Comhaire FH. Andropause: hormone replacement therapy in the ageing male. *Eur Urol* 2000;38:655–62
5. Hayes FJ. Testosterone – fountain of youth or drug of abuse? [Editorial]. *J Clin Endocrinol Metab* 2000;85:3020–3
6. Morales A, Heaton JPW, Caron III CC. Andropause: a misnomer for a true clinical entity. *J Urol* 2000;163:705–12
7. Vermeulen A. Androgen replacement therapy in the aging male – a critical evaluation. *J Clin Endocrinol Metab* 2001;86:2380–90
8. Basaria S, Wahlstrom JT, Dobs AS. Anabolic-androgenic steroid therapy in the treatment of chronic diseases. *J Clin Endocrinol Metab* 2001; 86:5108–17
9. Lejeune H. Age-related androgen deficiency. *Andrologie* 2001;11:241–38
10. Baulieu EE. Androgens and aging men. *Mol Cell Endocrinol* 2002;198:41–9
11. Lunenfeld B. Replacement therapy in the aging male. *J Endocrinol Invest* 2002;25:2–9
12. Wilson JD, Leihy MW, Shaw G, Renfree MB. Androgen physiology: unsolved problems at the millennium. *Mol Cell Endocrinol* 2002;198:1–5
13. De Ligniéres B. L'andropause et sa prise en charge chez l'homme agé. *Dossier Geriatrie* 2002;31: 1750–9
14. Oettel M, Hübler D, Patchev P. Selected aspects of endocrine pharmacology of the aging male. *Exp Gerontol* 2003;38:189–98
15. Vastag B. Many questions, few answered for testosterone replacement therapy. *J Am Med Assoc* 2003;298:971–4
16. Butenandt A. Aufgaben und Ziele der Hormonforschung. *Pharmazeutische Industrie* 1941;8:43–5
17. Gambineri A, Pasquali R. Testosterone therapy in men: clinical and pharmacological perspectives. *J Endocrinol Invest* 2000;23:196–214
18. Bhasin S, Bremner WJ. Emerging issues in androgen replacement therapy. *J Clin Endocrinol Metab* 1997;82:3–8
19. Anawalt BD, Amory JK, Wang C, Swerdloff RS, Dobs AS, Meikle AW, *et al*. A pharmacokinetic study of oral testosterone undecanoate (Org 538). *J Androl* 2002;(Suppl):37
20. Tan RS, Culberson JW. An integrative review on current evidence of testosterone replacement therapy for the andropause. *Maturitas* 2003; 45:15–21
21. Yialamas MA, Hayes FJ. Androgens and the ageing male and female. *Best Pract Res Clin Endocrinol Metab* 2003;17:223–36
22. Oettel M. Testosteron. In Stoffe P-Z, von Bruchhausen F, Dannhardt G, Ebel S, Frahm AW, Hackenthal E, Holzgrabe U, eds. *Hager's Handbuch der Pharmazeutischen Praxis*. Berlin: Springer-Verlag, 1994:820–2
23. Gobinet J, Poujol N, Sultan C. Molecular action of androgens. *Mol Cell Endocrinol* 2002; 198:15–24
24. Heinlein CA, Chang C. Androgen receptor (AR) coregulators: an overview. *Endocr Rev* 2002; 23:175–200
25. Saartok T, Dahlberg E, Gustaffson J-A. Relative binding affinity of anabolic-androgenic steroids: comparison of the binding to the androgen receptors in skeletal muscle and in prostate, as well as to sex hormone-binding globulin. *Endocrinology* 1984;114:2100–6
26. Hsiao PW, Thin TH, Lin DL, Chang C. Differential regulation of testosterone vs. 5α-dihydrotestosterone by selective androgen response elements. *Mol Cell Biochem* 2000; 206:169–75
27. Winters S. Androgens and anti-androgens. In Brody TM, Larner J, Minneman KP, eds. *Human Pharmacology: Molecular to Clinical*, 3rd edn. St. Louis: Mosby, 1998:519–31
28. Larionov AA, Vasyliev DA, Mason JI, Howie AF, Berstein LM, Miller WR. Aromatase in skeletal muscle. *J Steroid Biochem Mol Biol* 2003;84: 485–92
29. Simpson ER, Davis SR. Minireview. Aromatase and the regulation of estrogen biosynthesis – some new perspectives. *Endocrinology* 2001;142: 4589–94
30. Zagar Y, Liebherr M. Signaling cross talk in the rapid action of androgens. Presented at the *6th European Congress of Endocrinology*, Lyon, France, 26–30 April 2003:abstr O–19
31. Heinlein CA, Chang C. The roles of androgen receptors and androgen-binding proteins in

nongenomic androgen actions. *Mol Endocrinol* 2002;16:2181–7

32. Papakonstanti EA, Kampa M, Castanas E, Stounaras C. A rapid, nongenomic, signaling pathway regulates the actin reorganization induced by activation of membrane testosterone receptors. *Mol Endocrinol* 2003;17:870–81
33. Simoncini T, Genazzani AR. Non-genomic actions of sex steroid hormones. *Eur J Endocrinol* 2003;148:281–92
34. Lutz LB, Jamnongjit M, Yang WH, Jahani D, Gill A, Hammes SR. Selective modulation of genomic and nongenomic androgen responses by androgen receptor ligands. *Mol Endocrinol* 2003; 17:1106–16
35. Bhasin S, Storer TW, Berman N, Yarasheski K, Clevenger B, Phillips J, *et al.* Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. *J Clin Endocrinol Metab* 1997;82:407–13
36. Gooren L. Androgen deficiency in the aging male: benefits and risks of androgen supplementation. *J Steroid Biochem Mol Biol* 2003;in press
37. Sinha-Hikim I, Artaza J, Woodhouse L, Gonzalez-Cadavid N, Singh AB, Lee ML, *et al.* Testosterone induced increase in muscle size in healthy young men is associated with muscle fiber hypertrophy. *Am J Physiol Endocrinol Metab* 2002;283:E154–64
38. Wang C, Swerdloff RS, Iranmanesh A, Dobs A, Snyder PJ, Cunningham G, *et al.* Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. *J Clin Endocrinol Metab* 2000;85:2839–53
39. Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, *et al.* The effects of supraphysiological doses of testosterone on muscle size and strength in normal men. *N Engl J Med* 1996;335:1–7
40. Storer TW, Magliano L, Woodhouse L, Lee ML, Dzekov C, Dzekov J, *et al.* Testosterone dose-dependently increases maximal voluntary strength and leg power, but does not affect fatiguability or specific tension. *J Clin Endocrinol Metab* 2003;88:1478–85
41. Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, *et al.* Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab* 2001;281:E1172–81
42. Shahidi NT. A review of the chemistry, biological action, and clinical applications of anabolic-androgenic steroids. *Clin Ther* 2001;23:1355–90
43. Brodsky IG, Balagopal P, Nair KS. Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men – a clinical research center study. *J Clin Endocrinol Metab* 1996;81:3469–75
44. Ferrando AA, Tipton KD, Doyle D, Phillips SM, Cortiella J, Wolfe RR. Testosterone injection stimulates net protein synthesis but not tissue amino acid transport. *Am J Physiol* 1998;275: E865–71
45. Sheffield-Moore M, Urban RJ, Wolf SE, Jiang J, Catlin DH, Herndorn DN, *et al.* Short-term oxandrolone administration stimulates net muscle protein synthesis in young men. *J Clin Endocrinol Metab* 1999;84:2705–11
46. Crawford BAL, Liu PY, Kean MT, Bleasel JF, Handelsman DJ. Randomized placebo-controlled trial of androgen effects on muscle and bone in men requiring long-term systemic glucocorticoid treatment. *J Clin Endocrinol Metab* 2003;88:3167–76
47. Bhasin S, Woodhouse L, Storer TW. Hormones and sport. Proof of the effect of testosterone on skeletal muscle. *J Endocrinol* 2001;170:27–38
48. Fryburg DA. Insulin-like growth factor I exerts growth hormone- and insulin-like actions on human muscle protein metabolism. *Am J Physiol* 1994;267:E331–6
49. Urban RJ, Bodenburg YH, Gilkison C, Foxworth J, Coggan AR, Wolfe RR, *et al.* Testosterone administration to elderly men increases skeletal muscle strength and protein synthesis. *Am J Physiol* 1995;269:E820–6
50. Brill KT, Weltman AL, Gentili A, Patrie JT, Fryburg DA, Hanks JB, *et al.* Single and combined effects of growth hormone and testosterone administration on measures of body composition, physical performance, mood, sexual function, bone turnover, and muscle gene expression in healthy older men. *J Clin Endocrinol Metab* 2002;87:5649–57
51. Hayes VY, Urban RJ, Jiang J, Marcell TJ, Helgeson K, Mauras N. Recombinant human growth hormone and recombinant human insulin-like growth factor I diminish the catabolic effects of hypogonadism in man: metabolic and molecular effects. *J Clin Endocrinol Metab* 2001; 80:2211–19
52. Mauras N, Hayes V, Welch S, Rini A, Helgeson K, Dokler M, *et al.* Testosterone deficiency in young men: marked alterations in whole body protein kinetics, strength, and adiposity. *J Clin Endocrinol Metab* 1998;83:1886–92

53. Gentili A, Mulligan T, Godschalk M, Clore J, Patrie J, Iranmanesh A, *et al*. Unequal impact of short-term testosterone repletion on the somatotropic axis of young and older men. *J Clin Endocrinol Metab* 2002;87:825–34
54. Kamel HK, Maas D, Duthie EH Jr. Role for hormones in the pathogenesis and management of sarcopenia. *Drugs Aging* 2002;19:865–77
55. McPherron AC, Lee SJ. Double muscling in cattle due to mutations in the myostatin gene. *Proc Natl Acad Sci USA* 1997;94:12457–61
56. Gonzales-Cadavid NF, Taylor WE, Yaresheski K, Sinha-Hikim I, Ma K, Ezzat S, *et al*. Organization of the human myostatin gene and expression in healthy men and HIV-infected men with muscle wasting. *Proc Natl Acad Sci USA* 1998; 95:14938–43
57. Mallidis C, Bhasin S, Matsumoto A, Shen R, Gonzales-Cadaid NF. Skeletal muscle myostatin in a rat model of aging-associated sarcopenia. Proceedings of the *81st Annual Meeting of The Endocrine Society*, San Diego, CA, June 1999:abstr OR-9-1
58. Yaresheski KE, Bhasin S, Sinha-Hikim I, Pak-Loduca J, Gonzales-Cadavid NF. Serum myostatin-immunoreactive proteins increased with muscle wasting and advanced age. Proceedings of the *81st Annual Meeting of The Endocrine Society*, San Diego, CA, June 1999:abstr OR-9-2
59. Shivji R, Bhasin S, Byhower F, Tarnuzzer RW, Grant M, Shen R, *et al*. Myostatin and IGF-1 and -2 expression in muscle wasting resulting from exposure to the microgravity environment of a space shuttle flight. Proceedings of the *81st Annual Meeting of The Endocrine Society*, San Diego, CA, 1999, OR-32-2
60. Marcell TJ, Harman SM, Urban RJ, Metz DD, Rodgers BD, Blackman MR. Comparison of GH- IGF-I, and testosterone with mRNA of receptors and myostatin in skeletal muscle in older men. *Am J Physiol Endocrinol Metab* 2001; 281:E1159–74
61. Tincello DG, Saunders PTK, Hodgins MB, Simpson NB, Edwards CRW, Hargreave TB, *et al*. Correlation of clinical, endocrine and molecular abnormalities with *in vivo* responses to high-dose testosterone therapy in patients with partial androgen insensitivity. *Clin Endocrinol* 1997;46:497–506
62. Ferrando AA, Sheffield-Moore M, Wolf SE, Herndon DN, Wolf RR. Testosterone normalization in severe burns ameliorates muscle catabolism. *FASEB J* 2000;14:A797
63. Sheffield-Moore M. Androgens and the control of skeletal muscle protein synthesis. *Ann Med* 2000;32:181–6
64. Raaka BM, Finnerty M, Samuels HH. The glucocorticoid antagonist 17α-methyltestosterone binds to the 10 S glucocorticoid receptor and blocks agonist-mediated dissociation of the 10 S oligomer to the 4 S deoxyribonucleic acid-binding subunit. *Mol Endocrinol* 1989;3:332–41
65. Danhaive PA, Rosseau GG. Binding of glucocorticoid antagonists to androgen and glucocorticoid hormone receptors in rat skeletal muscle. *J Steroid Biochem* 1986;24:481–7
66. Danhaive PA, Rosseau GG. Evidence for sex-dependent anabolic response to androgenic steroids mediated by muscle glucocorticoid receptors in the rat. *J Steroid Biochem* 1988;29: 575–81
67. Hollenberg SM, Weinberger C, Ong ES, Cerelli G, Oro A, Lebo R, *et al*. Primary structure and expression of a functional human glucocorticoid receptor cDNA. *Nature* 1985;318:635–41
68. Wu FC. Endocrine aspects of anabolic steroids. *Clin Chem* 1997;43:1289–92
69. Hickson RC, Czerwinski SM, Falduto MT, Young AP. Glucocorticoid antagonism by exercise and androgenic-anabolic steroids. *Med Sci Sports Exerc* 1990;22:331–40
70. Yen PM, Liu Y, Palvimo JJ, Trifiro M, Whang J, Pinsky L, *et al*. Mutant and wild-type androgen receptors exhibit cross-talk on androgen-, glucocorticoid-, and progesterone-mediated transcription. *Mol Endocrinol* 1997;11:162–71
71. Laseter JT, Russel JA. Anabolic steroid-induced tendon pathology: a review of the literature. *Med Sci Sports Exerc* 1991;23:1–3
72. Stannard JP, Bucknell AL. Rupture of the triceps tendon associated with steroid injections. *Am J Sports Med* 1993;21:482–5
73. Barceló AC, Olivera MI, Bozzini C, Alippi RM, Bozzini CE. Androgens and erythropoiesis. Induction of erythropoietin-hypersecretory state and effect of finasteride on erythropoietin secretion. *Comp Haematol Int* 1999;9:1–6
74. Kunelius P, Lukkarinen O, Hannuksela ML, Itkonen O, Tapanainen JS. The effects of transdermal dihydrotestosterone on the aging male: a prospective, randomized, double blind study. *J Clin Endocrinol Metab* 2002;87:1467–72
75. Hajjar RR, Kaiser FE, Morley JE. Outcomes of long-term testosterone replacement therapy in older hypogonadal males: a retrospective study. *J Clin Endocrinol Metab* 1997;82:3793–6

76. Sih R, Morley JE, Kaiser FE, Perry HM III, Patrick P, Ross C. Testosterone replacement in older hypogonadal men: a 12 months randomized controlled study. *J Clin Endocrinol Metab* 1997; 82:1661–7
77. Jockenhövel F, Vogel E, Reinhardt W, Reinwein D. Effects of various modes of androgen substitution therapy on erythropoiesis. *Eur J Med Res* 1997;2:293–8
78. Dobs AS, Meikle AW, Arver S, Sanders SW, Caramelli KE, Mazer NA. Pharmacokinetics, efficiency, and safety of a permeation enhanced testosterone transdermal system in comparison with bi-weekly injections of testosterone-enanthate for the treatment of hypogonadal men. *J Clin Endocrinol Metab* 1999;84:3469–78
79. Chikuma M, Masuda S, Kobayashi T, Nagao M, Sasaki R. Tissue-specific regulation of erythropoietin production in the murine kidney, brain, and uterus. *Am J Physiol Endrocrinol Metab* 2000; 279:E1242–8
80. Blobel GA, Sieff CA, Orkin SH. Ligand-dependent repression of the erythroid transcription factor GATA-1 by the estrogen receptor. *Mol Cell Biol* 1995;15:3147–53
81. Tan KCB, Shiu SWM, Pang RWC, Kung AWC. Effects of testosterone replacement on HDL subfractions and apolipoprotein A-I containing lipoproteins. *Clin Endocrinol* 1998;48:187–94
82. Van Pottelbergh I, Braeckman L, De Bacquer D, De Backer G, Kaufman JM. Differential contribution of testosterone and estradiol in the determination of cholesterol and lipoprotein profile in healthy middle-aged men. *Atherosclerosis* 2003; 166:95–102
83. Herbst KL, Amory JK, Brunzell JD, Chansky HA, Bremner WJ. Testosterone administration to men increases hepatic lipase activity and decreases HDL and LDL size in 3 wk. *Am J Endocrinol Metab* 2003;284:1112–18
84. Bhasin S, Buckwalter JG. Testosterone supplementation in older men: a rational idea whose time has not yet come. *J Androl* 2001;22:718–31
85. Whitsel EA, Boyko EJ, Matsumoto AM, Anawalt BD, Siscovick DS. Intramuscular testosterone esters and plasma lipids in hypogonadal men: a meta-analysis. *Am J Med* 2001;111:261–9
86. Singh AB, Hsia S, Alaupovic P, Sinha-Hikum I, Woodhouse L, Buchanan TA, *et al*. The effects of varying doses of testosterone on insulin sensitivity, plasma lipids, apolipoproteins, and C-reactive protein in healthy young men. *J Clin Endocrinol Metab* 2002;87:136–43
87. Wu FC, von Eckardstein A. Androgens and coronary artery disease. *Endocr Rev* 2003;24: 183–217
88. Snyder PJ, Peachey H, Berlin JA, Rader D, Usher D, Loh L, *et al*. Effect of transdermal testosterone treatment on serum lipid and apolipoprotein levels in men more than 65 years of age. *Am J Med* 2001;111:255–60
89. Liu PY, Death AK, Handelsman DJ. Androgens and cardiovascular disease. *Endocr Rev* 2003;24: 313–40
90. Hak AE, Wittemann JCM, de Jong FH, Geerlings MI, Hofmann A, Pols HAP. Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study. *J Clin Endocrinol Metab* 2002;87:3632–9
91. Dockery F, Bulpitt CJ, Agawal S, Rajkumar C. Testosterone suppression in men with prostate cancer is associated with increased arterial stiffness. *Aging Male* 2002;5:216–22
92. Fukui M, Kitagawa Y, Nakamura N, Kadono M, Mogami S, Hirata C, *et al*. Association between serum testosterone concentration and carotid atherosclerosis in men with type 2 diabetes. *Diabetes Care* 2003;26:1869–73
93. Perusquia M. Androgen-induced vasorelaxation: a potential vascular protective effect. *Exp Clin Endocrinol Diabetes* 2003;111:55–9
94. Zitzmann M, Nieschlag E. Review about 'Androgen-induced vasorelaxation: a potential vascular protective effect' by M Perusquia. *Exp Clin Endocrinol Diabetes* 2003:111:55–9
95. Zitzmann M, Brune M, Nieschlag E. Vascular reactivity in hypogonadal men is reduced by androgen substitution. *J Clin Endocrinol Metab* 2002;87:5030–7
96. Sader MA, Griffiths KA, Skilton MR, Wishart SM, Handelsman DJ, Celermajer S. Physiological testosterone replacement and arterial endothelial function in men. *Clin Endocrinol* 2003;59:62–7
97. Heemers H, Vanderhoydonc F, Roskams T, Shechter I, Heyns W, Verhoeven G, *et al*. Androgens stimulate coordinated lipogenic gene expression in normal target tissues *in vivo*. *Mol Cell Endocrinol* 2003;205:21–31
98. Sato T, Matsumoto T, Yamada T, Watanabe T, Kawano H, Kato S. Late onset of obesity in male androgen receptor-deficient (AR KO) mice. *Biochem Biophys Res Commun* 2003;300:167–71
99. Kaplan N. The deadly quartet: upper body obesity, glucose intolerance, hypertriglyceridemia and hypertension. *Arch Int Med* 1989; 149:1514–20

100. Rolf C, von Eckardstein S, Koken U, Nieschlag E. Testosterone substitution of hypogonadal men prevents the age-dependent increases in body mass index, body fat and leptin seen in healthy ageing men: results of a cross-sectional study. *Eur J Endocrinol* 2002;146:505–11
101. Boyanov MA, Benova Z, Christov VG. Testosterone supplementation in men with type 2 diabetes, visceral obesity and partial androgen deficiency. *Aging Male* 2003;6:1–7
102. Björntorp P. The regulation of adipose tissue distribution in humans. *Int J Obes* 1996;20:291–302
103. Jockenhövel F, Bullmann C, Schubert M, Vogel E, Reinhardt W, Reinwein D, *et al*. Influence of various modes of androgen substitution on serum lipids and lipoproteins in hypogonadal men. *Metabolism* 1999;48:590–6
104. Kalintchenko S. The role of androgens in the treatment of obesity in men. In *3rd International Conference of the Andropause Society. Androgens and Men's Health*. London, UK: The Royal Society, 2003:40
105. Oettel M. Estrogens and antiestrogens in the male. In Oettel M, Schillinger E, eds. *Handbook of Experimental Pharmacology*. Berlin: Springer-Verlag, 1999;135/II:505–71
106. Oettel M. Is there a role for estrogens in the maintenance of men's health? *Aging Male* 2002; 5:248–57
107. Vermeulen A, Kaufman JM, Goemaere S, van Pottelberg I. Estradiol in elderly men. *Aging Male* 2002;5:98–102
108. De Ronde W, Pols HAP, van Leeuwen JPTM, de Jong FH. The importance of oestrogens in males. *Clin Endocrinol* 2003;58:529–42
109. Gooren LJ, Toorians AW. Significance of oestrogen in male (patho) physiology. *Ann Endocrinol* 2003;64:126–35
110. Brain PF, Poole AE. The role of endocrines in isolation-induced intermale fighting in albino laboratory mice. II. Sex steroid influences in aggressive mice. *Aggress Behav* 1976;2:55–76
111. Kurischko A, Oettel M. Androgen-dependent fighting behaviour in male mice. *Endokrinologie* 1977;70:1–5
112. Martinez-Sanchis A, Salvador A, Moya-Albiol L, González-Bono E, Simón VM. Effects of chronic treatment with testosterone propionate on aggression and hormonal levels in intact male mice. *Psychoneuroendocrinology* 1998;23:275–93
113. McGinnis MY, Lumia AR, Breuer ME, Possidente B. Physical provocation potentiates aggression in male rats receiving anabolic androgenic steroids. *Horm Behav* 2002;41:101–10
114. Oettel M, Kurischko A. Physiologisch-endokrinologische Studien zum Aggressionsverhalten von Mäuseböcken. *Z Versuchtstierk* 1978;20:186–93
115. Martínez-Sanchis S, Arnedo MT, Salvador A. Anti-aggressive effect of high doses of testosterone propionate is modulated by the type of opponent. *Med Sci Res* 1999;27:765–7
116. Oettel M, Kurischko A. Maintenance of aggressive behaviour in castrated mice by sex steroids: modification by neonatal injections of gonadal hormones. In Dörner G, Kawakami M, eds. *Hormones and Brain Development*. Amsterdam: Elsevier/North-Holland Biomedical Press, 1978:49–56
117. Frye CA, Rhodes ME, Walf A, Harney JP. Testosterone enhances aggression of wild-type mice but not those deficient in type I 5α-reductase. *Brain Res* 2002;948:165–70
118. Mazur A, Booth A. Testosterone and dominance in men. *Behav Brain Sci* 1998;21:353–97
119. Aromäki AS, Lindman RE, Eriksson CJP. Testosterone, aggressiveness, and antisocial personality. *Aggress Behav* 1999;25:113–23
120. Räsänen P, Hakko H, Visuri S, Paanila J, Kapanen P, Suomela T, *et al*. Serum testosterone levels, mental disorders and criminal behaviour. *Acta Psychiatr Scand* 1999;99:348–52
121. Campbell A, Muncer S, Odber J. Aggression and testosterone: testing a bio-social model. *Aggress Behav* 1997;23:229–38
122. Archer J, Birring SS, Wu FCW. The association between testosterone and aggression among young men: empirical findings and a meta-analysis. *Aggress Behav* 1998;24:411–20
123. Blanco C, Ibánez A, Blanco-Jerez CR, Baca-Garcia E, Sáiz-Ruiz J. Plasma testosterone and pathological gambling. *Psychiatry Res* 2001;105: 117–21
124. Aromäki AS, Lindman RE, Eriksson CJP. Testosterone, sexuality and antisocial personality in rapists and child molesters: a pilot study. *Psychiatry Res* 2002;110:239–47
125. Christiansen K. Behavioural effects of androgen in men and women. *J Endocrinol* 2001;170:39–48
126. Huebler D, Christoph A, Schubert M, Oettel M, Ernst M, Mellinger U, *et al*. Effect of a new long-acting testosterone undecanoate (TU) formulation vs. testosterone enanthate (TE) for intramuscular androgen replacement therapy on sexual function and mood in hypogonadal men. *Int J Impot Res* 2001;13(Suppl 1):S31
127. Kouri EM, Lukas SE, Pope HG, Oliva PS. Increased aggressive responding in male

volunteers following the administration of gradually increasing doses of testosterone cypionate. *Drug Alcohol Depend* 1995;40:73–9

128. Pope HG, Kouri EM, Hudson JI. Effects of supraphysiological doses of testosterone on mood and aggression in normal men. *Arch Gen Psychiatry* 2000;57:133–40
129. Yates WR, Perry PJ, MacIndoe J, Holman T, Ellingrod V. Psychosexual effects of three doses of testosterone cycling in normal men. *Biol Psychiatry* 1999;45:254–60
130. O'Connor DB, Archer J, Hair WM, Wu FCW. Exogenous testosterone, aggression, and mood in eugonadal and hypogonadal men. *Psychol Behav* 2002;75:557–66
131. Tricker R, Casaburi R, Storer TW, Clevenger B, Berman N, Shirazi A, *et al.* The effects of supraphysiological doses of testosterone on angry behavior in healthy eugonadal men – a clinical research center study. *J Clin Endocrinol Metab* 1996;81:3754–8
132. Wang C, Alexander G, Berman N, Salehian B, Davidson T, McDionald V, *et al.* Testosterone replacement therapy improves mood in hypogonadal men – a clinical research center study. *J Clin Endocrinol Metab* 1996;81:3578–83
133. Alexander GM, Swerdloff RS, Wang C, Davidson T, McDonald V, Steiner B, *et al.* Androgen – behaviour correlations in hypogonadal men and eugonadal men. *Horm Behav* 1998;33:85–94
134. Anderson RA, Martin CW, Kung AWC, Everington D, Pun TC, Tan KCB, *et al.* 7αMethyl-19-nortestosterone maintains sexual behaviour and mood in hypogonadal men. *J Clin Endocrinol Metab* 1999;84:3556–62
135. Svetec DA, Canby ED, Thompson IM, Sabenegh ES Jr. The effect of parenteral testosterone replacement on prostate specific antigen in hypogonadal men with erectile dysfunction. *J Urol* 1997;158:1775–7
136. Huggins C, Hodges C. Studies on prostatic cancer. Effect of castration, of estrogen and androgen injections in serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res* 1941;1:293–7
137. Sciarra F. Sex steroids and epidermal growth factor in benign prostatic hyperplasia (BPH). *Ann NY Acad Sci* 1995;761:66–78
138. Gerstenbluth RE, Maniam PH, Corty EW, Seftel AD. Prostate-specific antigen changes in hypogonadal men treated with testosterone replacement. *Andrology* 2002;23:922–6
139. Dorgan JF, Albanes D, Virtamo J, Heinonen OP, Chandler DW, Galmarini M, *et al.* Relationship of serum androgens and estrogens to prostate cancer risk: results from prospective study in Finland. *Cancer Epidemiol Biomarkers Prev* 1998; 7:1069–74
140. López-Otín C, Diamandis EP. Breast and prostate cancer: an analysis of common epidemiological, genetic, and biochemical features. *Endocr Rev* 1998;19:365–96
141. Heikkilä R, Aho K, Heliövaara M, Hakama M, Marniemi J, Reunanen A, *et al.* Serum testosterone and sex hormone-binding globulin concentrations and the risk of prostate carcinoma. *Cancer* 1999;86:312–15
142. Slater S, Oliver RTD. Testosterone – its role in development of prostate cancer and potential risk from use as hormone replacement therapy. *Drugs Aging* 2000;17:431–9
143. Morales A. Androgen replacement therapy and prostate safety. *Eur Urol* 2002;41:113–20
144. Bhasin S, Singh AB, Phong Mac R, Carter B, Lee MI, Cunningham GR. Managing the risks of prostate disease during testosterone replacement therapy in older men: recommendations for a standardized monitoring plan. *J Androl* 2003; 24:299–311
145. Shaneyfelt T, Husein R, Bubley G, Mantzoros CS. Hormonal predictors of prostate cancer: a meta-analysis. *J Clin Oncol* 2000;18:847–53
146. Morgenthaler A, Bruning CO III, DeWolf WV. Occult prostate cancer in men with low serum testosterone levels. *J Am Med Assoc* 1996;276: 1904–6
147. Zhang PL, Rosen S, Veeramachanemi R, Kao J, DeWolf WC, Bubley G. Association between prostate cancer and serum testosterone levels. *Prostate* 2002;53:179–293
148. Hoffmann MA, DeWolf WC, Morgentaler A. Is low serum free testosterone a marker for high grade prostate cancer? *J Urol* 2000;163: 824–7
149. Massengill JC, Sun L, Moul JW, Wu H, McLeod DG, Amling C, *et al.* Pretreatment total testosterone level predicts pathological stage in patients with localized prostate cancer treated with radical prostatectomy. *J Urol* 2003;169:1670–5
150. Prehn RT. On the prevention and therapy of prostate cancer by androgen administration. *Cancer Res* 1999;59:4161–5164
151. Moore RA. Benign hypertrophy and carcinoma of the prostate: occurrence and experimental production in animals. *Surgery* 1944;16:152–67

152. Jie-ping W, Fang-liu G. The prostate 41–65 years post-castration: an analysis of 26 eunuchs. *Chin Med J* 1987;100:271–2
153. Stege R, Carlstrom K. Testicular and adrenocortical function in healthy men and in men with benign prostatic hyperplasia. *J Steroid Biochem Mol Biol* 1992;42:357–62
154. Zgliczynski S, Baranowska B, Szymanowski J. Zmiany hormonalne w przeroscie i raku gruczolu krokowego. *Endokr Pol* 1979;4:319–30
155. Partin A, Oesterling JE, Epstein JI, Horton R, Walsh PC. Influence of age and endocrine factors on the volume of benign prostatic hyperplasia. *J Urol* 1991;145:405–9
156. Brochu M, Belanger A. Comparative study of plasma steroid and steroid glucuronide levels in normal men and in men with benign prostatic hyperplasia. *Prostate* 1987;11:33–40
157. Hammond GL, Kontturi M, Vihko P, Vihko R. Serum steroids in normal males and patients with prostatic diseases. *Clin Endocrinol* 1978;9: 113–21
158. Matzkin H, Chayen R, Goldfarb H, Gilad S, Braf Z. Laboratory monitoring of androgenic activity in benign prostate hypertrophy treated with a 5α-reductase inhibitor. *Clin Chem* 1992;38: 1304–6
159. Lagiou P, Mantzoros CS, Tzonou A, Signorello LB, Lipworth L, Trichopoulos D. Serum steroids in relation to benign prostatic hyperplasia. *Oncology* 1997;54:497–501
160. Bartsch W, Becker H, Pinkenburg FA, Krieg M. Hormone blood levels and their interrelationships in normal men and men with benign prostatic hyperplasia (BPH). *Acta Endocrinol* 1979;90:727–36
161. Kumar VL, Wadhwa SN, Kumar V, Farooq A. Androgen, estrogen, and progesterone receptor contents and serum hormone profiles in patients with benign hypertrophy and carcinoma of the prostate. *J Surg Oncol* 1990;44:122–8
162. Suzuki K, Ito K, Ichinose Y, Kurokawa K, Suzuki T, Imai K. Endocrine environment of benign prostatic hyperplasia: prostatic size and volume are correlated with serum estrogen concentration. *Scand J Urol Nephrol* 1995;29:65–8
163. Gann PH, Hennekens CH, Longcope C, Verhoek-Oftedahl W, Grodstein F, Stampfer MJ. A prospective study of plasma hormone levels, nonhormonal factors, and development of benign prostatic hyperplasia. *J Steroid Biochem Mol Biol* 1992;42:357–62
164. Hayami S, Sasagawa I, Nakada T. Influence of sex hormones on prostate volume in men on hemodialysis. *J Androl* 2000;21:258–61
165. Suzuki K, Inaba S, Takeuchi H, Takezawa Y, Fukabori Y, Suzuki T. Endocrine environment of benign prostatic hyperplasia – relationships of sex steroid hormone levels with age and the size of the prostate. *Nippon Hinyokika Gakkai Zasshi* 1992;83:664–71
166. Ranniko S, Adlercreutz H. Plasma estradiol, free testosterone, sex hormone binding globulin binding capacity, and prolactin in benign prostatic hyperplasia and prostatic cancer. *Prostate* 1983; 4:223–9
167. Swerdloff RS, Wang C. Androgen deficiency and aging in men. *West J Med* 1993;159:579–85
168. Pechersky AV, Mazurov VI, Semiglazov VF, Karpischenko AI. Androgen administration in middle-aged and ageing men: effects of oral testosterone undecanoate on dihydrotestosterone, oestradiol and prostate volume. *Int J Androl* 2002;25:119–25
169. Soygür T, Küpeli B, Aydos K, Küpeli S, Arikan N, Müftüoglu YZ. Effects of obesity on prostatic hyperplasia: its relation to sex steroid levels. *Int Urol Nephrol* 1996;28:55–9
170. Bianco JJ, Handelsman DJ, Pedersen JS, Risbridger GP. Direct response of the murine prostate gland and seminal vesicles to estradiol. *Endocrinology* 2002;143:4922–33
171. Prins GS, Marmer M, Woodham C, Chang W, Kuiper G, Gustafsson JA, *et al*. Estrogen receptor-β messenger ribonucleic acid ontogeny in the prostate of normal and neonatally estrogenized rats. *Endocrinology* 1998;139: 874–83
172. Dahle SE, Chokkalingam AP, Gao Y-T, Deng J, Stanczyk FZ, Hsing AW. Body size and serum levels of insulin and leptin in relation to the risk of benign prostatic hyperplasia. *J Urol* 2002;168: 599–604
173. Shibata Y, Ito K, Suzuki K, Nakano K, Fukabori Y, Suzuki R, *et al*. Changes in the endocrine environment of the human prostate transition zone with aging: simultaneously quantitative analysis of prostatic sex steroids and comparison with human prostatic histological composition. *Prostate* 2000;42:45–55
174. Lagiou P, Signorello LB, Trichopoulos D, Tzonou A, Trichopoulo A, Mantzoros CS. Leptin in relation to prostate cancer and benign prostatic hyperplasia. *Int J Cancer* 1998;76:25–8

175. Gann PH, Hennekens CH, Longcope C. A prospective study of plasma hormone levels, nonhormonal factors, and development of benign prostatic hyperplasia. *Prostate* 1995;26:40–9
176. Schweikert HU, Tunn UW, Habenicht UF, Arnold J, Senge T, Schulze H. Effects of estrogen deprivation on human benign prostatic hyperplasia. *J Steroid Biochem Mol Biol* 1993;44:573–6
177. Radlmaier A, Eickenberg HU, Fletcher MS. Estrogen reduction by aromatase inhibition for benign prostatic hyperplasia: results of a double-blind, placebo-controlled, randomized clinical trial using two doses of the aromatase-inhibitor atamestane. *Prostate* 1996;29:199–208
178. Krieg M, Nass R, Tunn S. Effect of aging on endogenous level of 5α-dihydrotestosterone, testosterone, estradiol, and estrone in epithelium and stroma of normal and hyperplastic human prostate. *J Clin Endocrinol Metab* 1993;77:375–81
179. Partin AW. Benign prostatic hyperplasia – etiology of benign prostatic hyperplasia. In Lepor H, ed. *Prostatic Diseases*. Philadelphia: WB Saunders, 2000:95–105
180. Bosch JLHR, Hop WCJ, Niemer QHJ, Bangma CH, Kirkels WJ, Schröder FH. Parameters of prostate volume and shape in a community based population of men 55 to 74 years old. *Urology* 1994;152:1501–5
181. Behre HM, Bohmeyer J, Nieschlag E. Prostate volume in testosterone-treated and untreated hypogonadal men in comparison to age-matched normal controls. *Clin Endocrinol* 1994;40:341–9
182. Griffin JE, Wilson JD. Disorders of the testes and the male reproductive tract. In Wilson JD, Foster DW, eds. *Williams' Textbook of Endocrinology*. Philadelphia: WB Saunders, 1992:799–852
183. Mendonca BB, Inacio M, Costa EM, Arnhold IJ, Silva FA, Nicolau W, *et al*. Male pseudohermaphroditism due to steroid 5α-reductase-2 deficiency. Diagnosis, psychological evaluation, and management. *Medicine (Baltimore)* 1996; 75:64–76
184. Imperator-McGinley J. 5α-reductase-2 deficiency. *Curr Ther Endocrinol Metab* 1997;6:384–7
185. Imperato-McGinley J, Zhu YS. Androgens and male physiology; the syndrome of 5α-reductase-2 deficiency. *Mol Cell Endocrinol* 2002; 198:51–9
186. Shape RM. The roles of oestrogen in the male. *Trends Endocrinol Metab* 1998;9:371–7
187. Barrett-Connor E, Goodman Gruen D, Patay B. Endogenous sex hormones and cognitive function in older men. *J Clin Endocrinol Metab* 1999; 84:3681–5
188. Komesaroff PA, Fullerton M, Esler MD, Dart A, Jennings G, Sudhir K. Low-dose estrogen supplementation improves vascular function in hypogonadal men. *Hypertension* 2001;38:1011–16
189. Mukherjee TK, Dinh H, Chaudhuri G, Nathan L. Testosterone attenuates expression of vascular adhesion molecule-1 by conversion to estradiol by aromatase in endothelial cells: implications in atherosclerosis. *Proc Natl Acad Sci USA* 2002; 99:4055–60
190. Giltay EJ, Verhoef P, Gooren LJG, Geleijnse JM, Schouten EG, Stehouwer CDA. Oral and transdermal estrogens both lower plasma total homocysteine in male-to-female transsexuals. *Atherosclerosis* 2003;168:139–46
191. Toda K, Okada T, Takeda K, Akira K, Saibara S, Shiraishi M, *et al*. Oestrogen at the neonatal stage is critical for the reproductive ability of male mice as revealed by supplementation with 17β-oestradiol to aromatase gene (Cyp19) knockout mice. *J Endocrinol* 2001;168:455–63
192. Falahati-Nini A, Riggs BL, Atkinson EJ, O'Fallon WM, Eastell R, Khosla S. Relative contributions of testosterone and estrogen in regulating bone resorption and formation in normal elderly men. *J Clin Invest* 2000;16: 1553–60
193. Orwoll ES. Men, bone and estrogen: unresolved issues. *Osteoporos Int* 2003;14:93–8
194. Ly LP, Jimenez M, Zhuang TN, Celermajer DS, Conway AJ, Handelsman DJ. A double-blind, placebo-controlled, randomized clinical trial of transdermal dihydrotestosterone gel on muscular strength, mobility, and quality of life in older men with partial androgen deficiency. *J Clin Endocrinol Metab* 2001;86:4078–88
195. Evans EW, McLeod HL. Pharmacogenomics – drug disposition, drug targets, and side effects. *N Engl J Med* 2003;348:538–49
196. Thomas JL, Mason JI, Brandt S, Norris W. Differences in substrate and inhibitor kinetics of human type 1 and type 2 3β-hydroxysteroid dehydrogenase are explained by the type 1 mutant, H156Y. *Endocr Res* 2002;28:471–5
197. Shibata A, Garcia MI, Cheng I, Stamey TA, NcNeal JE, Brooks JD, *et al*. Polymorphisms in the androgen receptor type II 5α-reductase genes and prostate cancer prognosis. *Prostate* 2002; 52:269–78
198. Lamharzi N, Johnson MM, Goodman G, Etzioni R, Weiss NS, Dightman DA, *et al*. Polymorphic markers in the 5α-reductase type II gene and the incidence of prostate cancer. *Int J Cancer* 2003; 105:480–3

199. Tayeb MT, Clarc C, Haites NE, Sharp L, Murray GI, McLeod HL. CYP3A4 and VDR gene polymorphisms and the risk of prostate cancer in men with benign prostate hyperplasia. *Br J Cancer* 2003;88:928–32
200. Försti A, Jin Q, Grzybowska W, Söderberg M, Zientek H, Sieminska M, *et al.* Sex hormone-binding globulin polymorphisms in familial and sporadic breast cancer. *Carcinogenesis* 2002;23: 1315–20
201. Heald AH, Ivison F, Anderson GS, Cruickshank K, Laing I, Gibson MJ. Significant ethnic variation in total and free testosterone concentration. *Clin Endocrinol* 2003;58:262–6
202. Elkins DA, Yokomizo A, Thibodeau SN, Schaid DJ, Cunningham JM, Marks A, *et al.* Luteinizing hormone β polymorphism and risk of familial and sporadic prostate cancer. *Prostate* 2003;56: 30–6
203. Powell BL, Piersma D, Kevenaar ME, van Staveren IL, Themmen APN, Iacopetta BJ, *et al.* Luteinizing hormone signaling and breast cancer: polymorphisms and age of onset. *Clin Endocrinol* 2003;88:1653–7
204. Ghali SA, Gottlieb B, Lumbroso R, Beitel LK, Elhaji Y, Wu J, *et al.* The use of androgen receptor amino/carboxyl-terminal interaction assays to investigate androgen receptor gene mutation in subjects with varying degrees of androgen insensitivity. *Clin Endocrinol Metab* 2003;88:2185–93
205. Medeiros R, Vasconcelos A, Costa S, Pinto D, Morais A, Oliveira J, *et al.* Steroid hormone genotypes ARStuI and ET325 are linked to the progression of human prostate cancer. *Cancer Genet Cytogenet* 2003;131:91–6
206. Suter NM, Malone KE, Daling JR, Doody DR, Ostrander EA. Androgen receptor (CAG)n and (GGC)n polymorphisms and breast cancer risk in a population-based case-control study of young women. *Cancer Epidemiol Biomarkers Prev* 2003; 12:127–35
207. Platz EA, Rimm EB, Willett WC, Kantoff PW, Giovannucci E. Racial variation in prostate cancer incidence and in hormonal system markers among male health professionals. *J Natl Cancer Inst* 2000;92:2009–17
208. Hsing AW, Gao YT, Wu G, Wang X, Deng J, Chen YL, *et al.* Polymorphic CAG and GGN repeat lengths in the androgen receptor gene and prostate cancer risk: a population-based case-control study in China. *Cancer Res* 2000;1518: 5111–16
209. Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res* 1994;11:3181–6
210. Beilin J, Ball EM, Favaloro JM, Zajac JD. Effect of the androgen receptor CAG repeat polymorphism on transcriptional activity: specificity in prostate and non-prostate cell lines. *J Mol Endocrinol* 2000;25:85–96
211. Byrne MM, Nieschlag E. Testosterone replacement therapy in male hypogonadism. *J Endocrinol Invest* 2003;26:481–9
212. Gottlieb B, Lehvaslaiho H, Beitel LK, Lumbroso R, Pinsky L, Trifiro M. The androgen receptor gene mutations database. *Nucleic Acids Res* 1998; 26:234–8
213. Nam RK, Elhaji Y, Krahn MD, Hakimi J, Ho M, Chu W, *et al.* Significance of the CAG repeat polymorphism of the androgen receptor gene in prostate cancer progression. *J Urol* 2000;164: 567–72
214. Xue W, Irvine RA, Yu MC, Ross RK, Coetzee GA, Ingles SA. Susceptibility to prostate cancer: interaction between genotypes at the androgen receptor and prostate-specific antigen loci. *Cancer Res* 2000;60:839–41
215. Modugno F, Weissfeld JL, Trump DL, Zmada JM, Shea P, Cauley JA, *et al.* Allelic variants to aromatase and the androgen and estrogen receptors: toward a multigenic model of prostate cancer risk. *Clin Cancer Res* 2001;7:3092–6
216. Terouanne B, Nirdé P, Rabenoelina F, Bourguet W, Sultan W, Auzou G. Mutation of the androgen receptor at animo acid 708 (Gly → Ala) abolishes partial agonist activity of steroidal antiandrogens. *Mol Pharmacol* 2003;63:791–8
217. Hardy DO, Scher HI, Bodenreider T. Androgen receptor CAG repeat length in prostate cancer: correlation with age of onset. *J Clin Endrocrinol Metab* 1996;81:4400–5
218. Chen C, Lamharzi N, Weiss NS, Etzoni R, Dightman DA, Barnett M, *et al.* Androgen receptor polymorphisms and the incidence of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 2002;11:1033–40
219. Hsiao PW, Lin DL, Nakao R, Chang C. The linkage of Kennedy's neuron disease to ARA 24, the first identified androgen receptor polyglutamine region-associated coactivator. *J Biol Chem* 1999;29:20229–34
220. McPhaul MJ. Androgen receptor mutations and androgen insensitivity. *Mol Cell Endocrinol* 2002; 198:61–7
221. Yong EL, Lim LS, Wang Q, Mifsud A, Lim J, Ong YC, *et al.* Androgen receptor

polymorphisms and mutations in male infertility. *J Endocrinol Invest* 2000;23:537–77

222. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 1991;352:77–9
223. Dejager S, Bry-Gauillard H, Bruckert E. A comprehensive endocrine description of Kennedy's disease revealing androgen insensitivity linked to CAG repeat length. *J Clin Endocrinol Metab* 2002;87:3893–901
224. Giovannucci E, Platz EA, Stampfer MJ, Chan A, Krithivas K, Kawachi I, *et al.* The CAG repeat within the androgen receptor gene and benign prostatic hyperplasia. *Urology* 1999;53:121–5
225. Mitsumori K, Terai A, Oka H, Segawa T, Ogura K, Yoshida O, *et al.* Androgen receptor repeat length polymorphism in benign prostatic hyperplasia (BPH): correlation with adenoma growth. *Prostate* 1999;41:253–7
226. Dowsing AT, Yong EL, Clark M, McLachlan RI, de Kretser DM, Trounson AO. Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. *Lancet* 1999; 354:640–3
227. Hiort O, Holterhus PM. Androgen insensitivity and male infertility. *Int J Androl* 2003;26:16–20
228. Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E. Prostate volume and growth in testosterone-substituted hypogonadal men are dependent on the CAG repeat polymorphism of the androgen receptor gene: a longitudinal pharmacogenetic study. *Clin Endocrinol Metab* 2003;88:2049–54
229. Zitzmann M, Brune M, Kornmann B, Gromoll J, von Eckardstein S, von Eckardstein A, *et al.* The CAG repeat polymorphism in the AR gene affects high density lipoprotein cholesterol and arterial vasoreactivity. *J Clin Endocrinol Metab* 2001;86:4867–73
230. Von Eckardstein S, Schmidt A, Kamischke A, Simoni M, Gromoll J, Nieschlag E. CAG repeat length in the androgen receptor gene and gonadotrophin suppression influence the effectiveness of hormonal male contraception. *Clin Endocrinol* 2002;57:647–55
231. Shimohata T, Nakajima T, Yamada M, Uchida C, Onodera O, Naruse S, *et al.* Expanded polyglutamine stretches interact with TAFII130, interfering with CREB-dependent transcription. *Nature Gene* 2000;26:29–36
232. Zitzmann M, Brune M, Kornmann B, Gromoll J, Junker R, Nieschlag E. The CAG repeat polymorphism in the androgen receptor gene affects bone density and bone metabolism in healthy males. *Clin Endocrinol* 2001;55:649–58
233. Zitzmann M, Gromoll J, von Eckardstein A, Nieschlag E. The CAG repeat polymorphism in the androgen receptor modulates body fat mass and serum concentrations of leptin and insulin in men. *Diabetologia* 2003;46:31–9
234. Woodhouse LJ, Reisz-Porszasz S, Javanbakht M, Storer TW, Lee M, Zerounian H, *et al.* Development of models to predict anabolic response to testosterone administration in healthy young men. *Am J Physiol Endocrinol Metab* 2003;284: E1009–W1017
235. Seidmann SN, Araujo AB, Roose SP, McKinlay JB. Testosterone concentration, androgen receptor polymorphism, and depressive symptoms in middle-aged men. *Biol Psychiatry* 2001; 50:371–6
236. Härkönen K, Huhtaniemi I, Mäkinen J, Hübler D, Irjala K, Koskenvuo M, *et al.* The polymorphic androgen receptor gene CAG repeat, pituitary-testicular function and andropausal symptoms in aging men. *Int J Androl* 2003;26:187–94
237. Lehmann DJ, Butler HT, Warden DR, Combrinck M, King E, Nicoll JAR, *et al.* Association of the androgen receptor CAG repeat polymorphism with Alzheimer's disease in men. *Neurosci Lett* 2003;340:87–90
238. Schatzl G, Madersbacher S, Haitel A, Gsur A, Preyer M, Haidinger G, *et al.* Associations of serum testosterone with microvessel density, androgen receptor density and androgen receptor gene polymorphism in prostate cancer. *J Urol* 2003;169:1312–15
239. Mariotti C, Castellotti B, Pareyson D, Testa D, Eoli M, Antozzi C, *et al.* Phenotypic manifestations associated with CAG-repeat expansion in the androgen receptor gene in male patients and heterozygous females; a clinical and molecular study of 30 families. *Neuromusc Disord* 2000;10: 391–7
240. Kritivas K, Yurgalevitch SM, Mohr BA. Evidence that the CAG repeat in the androgen receptor gene is associated with the age-related decline in serum androgen levels in men. *J Endocrinol* 1999;162:137–42
241. Zitzmann M, Nieschlag E. The CAG repeat polymorphism with the androgen receptor gene and maleness. *Int J Androl* 2003;26:76–83
242. Bhasin S, Bagatell CJ, Bremner WJ, Plymate SR, Tenover JL, Korenman SG, *et al.* Therapeutic perspective. Issues in testosterone replacement in older men. *J Clin Endocrinol Metab* 1998;83: 3435–48

243. Hershberger JG, Shipley EG, Meyer TK. Myotrophic activity of 19-nortestosterone and other steroids determined by a modified levator ani method. *Proc Soc Exp Biol* 1953;83:175–80
244. Negro-Vilar A. Selective androgen receptor modulators (SARMs): a novel approach to androgen therapy for the new millennium. *J Clin Endocrinol Metab* 1999;54:3459–62
245. Zhi L, Martinborough E. Selective androgen receptor modulators (SARMs). In Doherty AM, ed. *Annual Reports in Medicinal Chemistry*. San Diego: Academic Press, 2001;36:169–80
246. Elbers JMH, Grootenhuis AJ. New tissue-selective androgens: perspectives in the treatment of androgen deficits. *Ann Endocrinol* 2003;64: 183–8
247. Anderson RA, Wallace A, Sattar N, Kumar N, Sundaram K. Evidence for tissue selectivity of the synthetic androgen 7α-methyl-19-nortestosterone in hypogonadal men. *Clin Endocrinol* 2003;88:2784–93
248. Lutz LB, Jamnongjit M, Yang WH, Jahani D, Gill A, Hammes SR. Selective modulation of genomic and nongenomic androgen receptor ligands. *Mol Endocrinol* 2003;17:1106–16
249. Tremblay RR, Morales AJ. Canadian practice recommendations for screening, monitoring and treating men affected by andropause or partial androgen deficiency. *Aging Male* 1998;1: 213–18
250. Conway AJ, Handelsman DJ, Lording DW, Stuckey B, Zajac JD. Use, misuse and abuse of androgens. The Endocrine Society of Australia consensus guidelines for androgen prescribing. *Med J Aust* 2000;172:220–4
251. Weidner W, Behre H, Beutel M, Brahler E, Hertle L, Krause W, *et al*. The Ageing Male. A Consensus Statement from the Joint Multidisciplinary Working Group of the German Society of Andrology, the German Society of Dermatology, the German Society of Endocrinology and the German Society of Urology. *Urol Int* 2001;66:160–1
252. Andropause. Consensus Panel. *Proceedings of the Andropause Consensus Panel*. The Endocrine Society from 2001; cited by 142
253. Valenti G, Bossoni S, Giustina A, Maugeri D, Motta M, *et al*. Consensus document on substitution therapy with testosterone in hypoandrogenic elderly men. GISEG (Italian Study Group on Geriatric Endocrinology). *Aging Clin Exp Res* 2002;14:439–64
254. Petak SM, Nioukiu HR, Spark RF, Swerdloff RS, Rodriguez-Rigau LJ. American Association for Clinical Endocrinologists AACE guidelines for evaluation and treatment of hypogonadism in adult male patients – 2002 update. *Endocr Pract* 2002;8:439–55
255. Guay AT, Spark RF, Bansal S, Cunningham GR, Goodman NF, Nankin HR, *et al*. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of male sexual dysfunction: a couple's problem – 2003 update. *Endocrine Practice* 2003;9:77–95
256. American College of Physicians/American Society of Internal Medicine. Disease Management Module on Male Hypogonadism from 2002; cited by 142
257. Kim YC. Hormonal replacement treatment & aging – Asian practical recommendations on testosterone supplementation. *Asian J Androl* 2003;in press
258. Morales A, Lunenfeld B. Standards, Guidelines and Recommendations of The International Society for The Study of the Aging Male (ISSAM). Investigation, treatment and monitoring of late-onset hypogonadism in males. Official Recommendations of ISSAM. *Aging Male* 2002; 5:74–86