ORIGINAL RESEARCH—ENDOCRINOLOGY

The Paradox Dividing Testosterone Deficiency Symptoms and Androgen Assays: A Closer Look at the Cellular and Molecular Mechanisms of Androgen Action

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ABSTRACT

Introduction. Central to the diagnosis and treatment of testosterone deficiency syndrome in the adult male is the remarkable paradox that there is a very poor correlation between the characteristic symptoms and levels of serum androgens.

Aim. Because androgen deficiency can be associated with severe symptomatology, as well as diverse conditions such as coronary heart disease, diabetes, and metabolic syndrome, the aim was to present an evidence-based working hypothesis to resolve this confusing clinical paradox.

Methods. A review of the possible mechanisms in testosterone deficiency syndrome was carried out, and a hypothesis to explain this paradox and associated problems in the diagnosis and clinical management of androgen deficiency was established on the basis of a review of the literature.

Main Outcome Measures. The mechanisms by which androgen deficiency could arise were studied at five different levels:

1. Impaired androgen synthesis or regulation.
2. Increased androgen binding.
3. Reduced tissue responsiveness.
4. Decreased androgen receptor activity.
5. Impaired transcription and translation.

Results. As with insulin in maturity onset diabetes mellitus, there can be both insufficient production and variable degrees of resistance to the action of androgens operating at several levels in the body simultaneously, with these factors becoming progressively worse with aging, adverse lifestyle, other disease processes, and a wide range of medications.

Conclusions. Using this model, androgen deficiency can be redefined as an absolute or relative deficiency of androgens or their metabolites according to the needs of that individual at that time in his life. There are important ways in which the considerations raised by this hypothesis affect the etiology, terminology, diagnosis, and treatment of androgen-deficient states. **Carruthers M. The paradox dividing testosterone deficiency symptoms and androgen assays: A closer look at the cellular and molecular mechanisms of androgen action. J Sex Med 2008;5:998–1012.**

Key Words. Aging Male Symptoms Questionnaire; Testosterone Deficiency Syndrome; Androgen Assays; Androgen Resistance; Androgen Synthesis; Androgen Action

Introduction

In an elegant article entitled "Mechanism of diabetes mellitus" published in The Lancet in 1939, Sir Harold Himsworth drew the distinction between "insulin-sensitive" and "insulin-insensitive" diabetes, shedding new light on the nature, diagnosis, and treatment of the condition [1].

This article explores the possibility that similar principles might explain the different causes and endocrine background of what has become known as the "testosterone deficiency syndrome" (TDS). It also reviews the evidence that androgen resistance may be an important factor in the onset of this condition and may cause problems with its diagnosis and treatment.

Central Paradox in the Diagnosis of TDS

The typical symptoms of TDS have been recognized and consistent since they were first described nearly 70 years ago by Dr. August Werner (Table 1) [2–7].

The paradox is that these characteristic symptoms of testosterone deficiency are very poorly correlated with total testosterone (TT) or other androgen levels in the blood. A recent report on the best validated of all the symptom scales, the Aging Male Symptoms (AMS) scale, states that "the total AMS score was not significantly associated with TT" [8]. Similarly, using three questionnaires, including the AMS and Androgen Deficiency in the Adult Male—St Louis (ADAM) scales, no relationship was found between symptomatology and any of a battery of eight endocrine assays, including TT and free testosterone (FT), other than possibly age-related declines in dehydroepiandrosterone (DHEA) and insulin-like growth factor 1 (IGF-1) [9].

Further, investigation of a group of 81 Belgian men aged 53–66 (mean 59) concluded "there was no correlation between AMS (total and subscales) and testosterone levels [10], while the same group in a study of 161 more elderly men aged 74–89 (mean 78) also showed no correlation between symptom scores and TT, FT, or bioavailable testosterone (BT)" [11].

However, possibly because the ADAM symptom scores might be more age-related than the AMS, in a study of men aged 23–80 years, unlike TT, BT and FT were found to correlate significantly with a number of the individual questions, both on that and the AMS scale [12]. Also in an evaluation of assays measuring androgens over a similar wide age range, TT showed no correlation with age, and if taken alone would have resulted in misclassification of deficiency in 42% when compared with BT [13].

One of the studies most clearly highlighting the paradox dividing androgen deficiency symptom scales and laboratory measures is that of Miwa et al. in 2006, who found no correlation between the total and psychological, somatic or sexual domain scores of the AMS and serum levels of TT, FT, estradiol (E2), luteinizing hormone (LH), follicle stimulating hormone (FSH), dehydroepiandrosterone-sulfate (DHEA-S), or growth hormone (GH) [14].

Similarly, while individual symptoms, such as reduced libido [15] or erectile dysfunction [16], have some association with androgen levels in

Table 1 Frequency of symptoms of testosterone deficiency syndrome described by various authors [2–7]

Author	Werner	Heller	Reiter	Carruthers	Carruthers	Tremblay	Heinemann
Year the study began	1938	1944	1963	UKAS 1989	Web 1996	1998	1999
Number in study	273	23	100	1,500	1,533	300	116
Reference number	[2]	[3]	[4]	[5]	[5]	[6]	[7]
Symptoms							
Erectile dysfunction	95	++	++	84	83	++	88
Libido/sex drive/desire	90	++	++	82	87	++	84
Fatigue/energy reduced	76	++	+	76	94	+	80
Depression	89	+	++	60	88	+	75
Anxiety/nervousness	100	++	++	++	85	+	69
Memory/concentration	87	+	+	37	90	+	
Irritability/anger	59	+	+	54	85	+	72
Aches/pains joints	75	+		55	83		77
Sweating especially at night	35	+		49	63	+	66
Vasomotor/flushes	46	+		27		+	
Aging/older than years				40	55		59
Dry skin/thinning	30	+		39	63		

+ = mentioned; ++ = frequent; AKAS = UK Androgen Study.

epidemiological studies, they do not appear to be related to them in individual cases [17], and the different symptoms and metabolic effects appear at different levels, i.e., there seem to be various organ thresholds of sensitivity, and hence, possible pathology. Without any clear-cut threshold for overall symptoms of testosterone deficiency, there was a pattern of increasing prevalence of symptoms and metabolic risk factors with decreasing androgen levels [18].

In this study, androgen-induced prevalence of loss of libido or energy increased significantly below testosterone concentrations of 15 nmol/L (430 ng/dL), whereas depression and diabetes mellitus type 2 in non-obese men were more common with testosterone concentrations below 10 nmol/L (300 ng/dL). Erectile dysfunction was identified as a composite pathology of metabolic risk factors, smoking, and depressivity, whereas only testosterone concentrations below 8 nmol/L (230 ng/dL) contributed to that symptom.

The authors concluded that in this cohort from an andrology clinic, which might not be representative of the general population, symptoms accumulated gradually with decreasing testosterone levels, and that various strata of TT concentrations exist, which are associated with specific symptoms.

Sexual responses to treatment, especially erectile function, have been found to vary according to initial TT and FT levels across a wide range of values including men with low-normal levels [19].

There is also a considerable variation between individuals in levels of testosterone at which symptoms appear. Kelleher et al. [20] investigated 52 androgen-deficient men who underwent 260 implantations over a 5-year period. At the time of return of androgen deficiency symptoms, the blood TT and FT concentrations were highly reproducible within individuals, but each person had a consistent testosterone threshold for androgen deficiency symptoms that differed markedly between individuals.

Further divergence of symptoms and androgen levels is seen in population screening studies and in the selection of androgen-deficient patients for trials of testosterone treatment. Symptom scales such as the AMS suggest an incidence of 40–50% in men over the age of 50, but only between 1 and 7% of men with raised symptom scores prove to have testosterone levels sufficient to be declared "hypogonadal" and therefore suitable for treatment according to various international guidelines.

Despite this contradictory evidence, the lower limit of TT, which is regarded as diagnostic of androgen deficiency, varies between 8 nmol/L (230 ng/dL) in Australia [21] and 12 nmol/L (350 ng/dL) in Europe and the United States [22,23], with some trials accepting patients with levels up to 15 nmol/L (430 ng/dL) in an effort to recruit sufficient symptomatic subjects.

This review emphasizes the problems of diagnoses based on TT alone, especially considering the questionable validity of androgen assays overall when all the variables in sampling, analysis, and interpretation are taken into account [24].

This dichotomy between clinical and laboratory findings urgently needs to be explained to reduce the confusion over what one does for men who have symptoms of androgen deficiency unsubstantiated by laboratory tests. For this, we need to take a detailed look at the multiple levels at which testosterone production and action could be impaired (Figure 1).

Level 1—Impaired Androgen Synthesis and Regulation

Aging

The aging process affects androgen production and regulation at every level of the hypothalamogonadal axis (Figure 2) [26]. The reduction in TT, and more especially in both BT and CFT with age is well recognized [27–29] and may be accelerating because of health and environmental effects [30]. As well as lower mean levels, there is a reduction in the circadian rhythm in older men [31,32].

Partly, this is because of a decrease in the efficacy of LH pulses in stimulating androgen production [33]. The question arises as to what proportion of these changes is due to testicular degeneration, and how much to impaired regulation?

Testes

Impaired Development

Men with nondescent, or late descent, of one or both testes often show signs and symptoms of testosterone deficiency throughout their lives, and when testosterone treatment is stopped, they develop typical symptoms. Even when there has been anatomic correction of the defect by orchidopexy, testicular function may well still be impaired, both in terms of sperm and testosterone production.

Sometimes, there is no overt history of testicular problems, but when the patient presents in

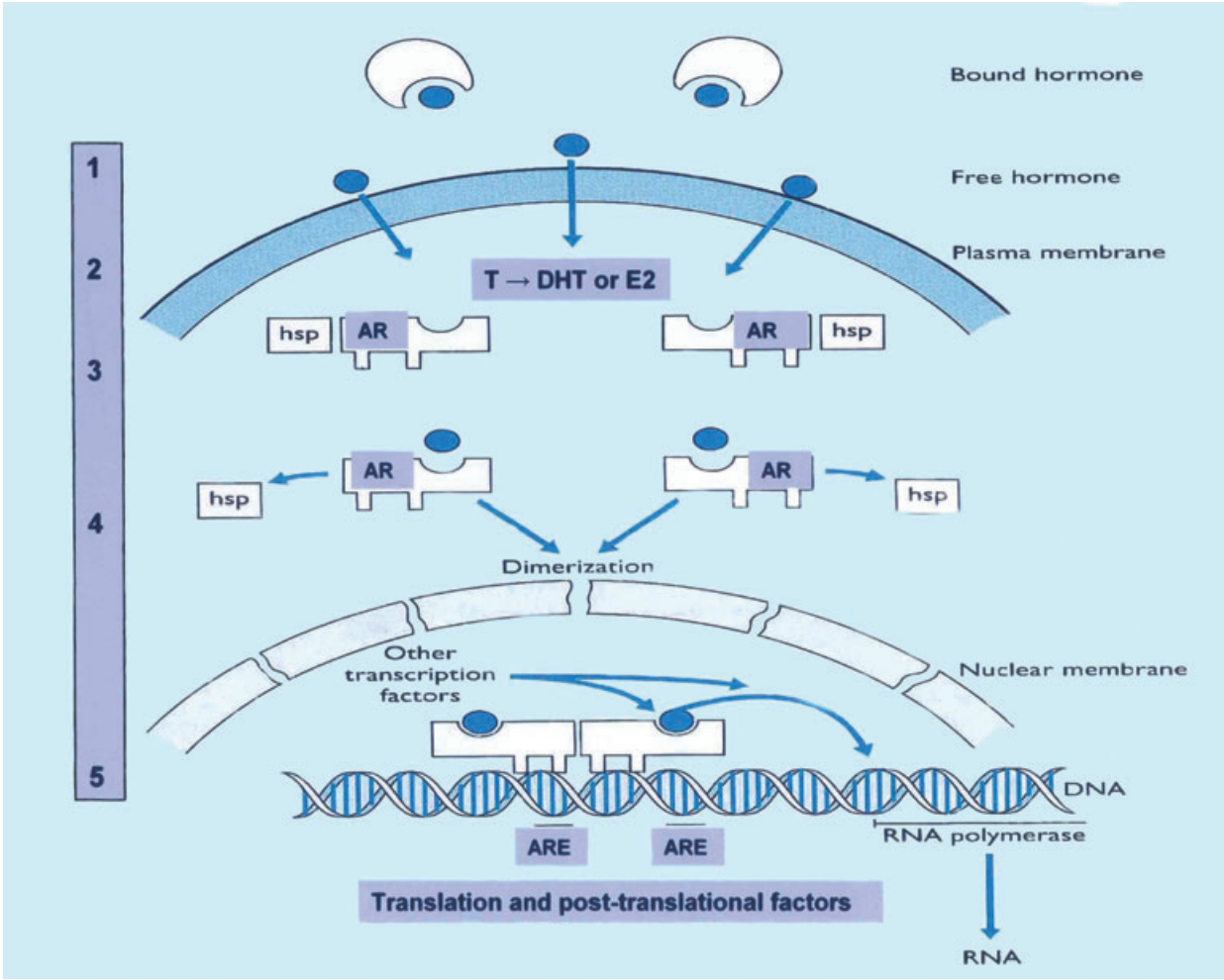

Figure 1 Cellular and molecular mechanisms of androgen action: a cascade of multiple levels (modified from Nussey and Whitehead [25]).

middle age or later, there may be a lifelong history of low sex drive and activity, unexplained infertility, and poor secondary sexual characteristics. Physical examination may show small, easily retractile testes in a poorly developed scrotum, with a small penis.

Aging

A wide range of degenerative changes have been reported in the aging testis. These include a decrease in the number of Leydig cells, increased fibrosis, decreased perfusion, and hypoxia-dependent changes in steroidogenesis, resulting in reduced precursor DHEA synthesis [34,35]. As will be discussed later, testicular failure may also develop after a period of compensatory Leydig cell overactivity and hypertrophy.

Infections

Mumps is the classic example of an infection causing an endocrine disorder. Orchitis occurs in 25–35% of postpubertal cases, and like many testicular disorders, may affect its endocrine function as well as sperm production. This potential for testicular damage to be caused by a wide variety of viruses may be linked to damage to the immunological defense system of the testes, which is only established at puberty.

Other viruses, including those causing glandular fever (infectious mononucleosis), may also be associated with clinical or subclinical orchitis and damage. This has also been reported with herpes, coxsackie, arbo, *Dengue*, and *Marburg* viruses. The testes can also be affected by nephritis, prostatitis, vesiculitis, and epididymitis, especially with gonorrhea, chlamydia, and other causes of nonspecific urethritis.

Temperature

Varicocele and hydroceles impair the temperature regulation function of the scrotum, which nor-

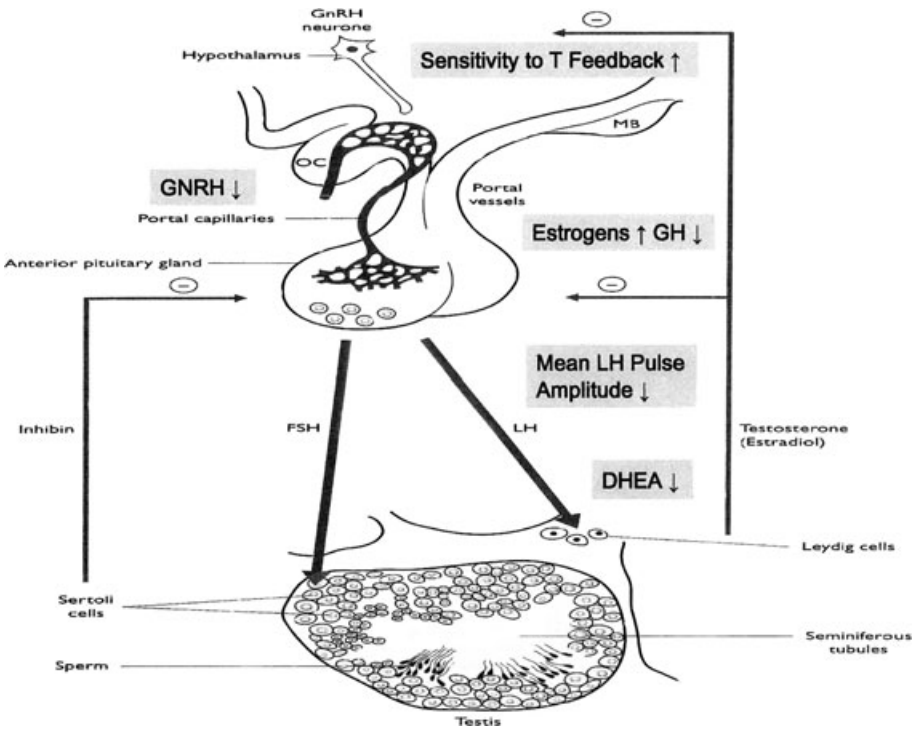

Figure 2 Changes in the regulation and synthesis of testosterone with age (modified from Nussey and Whitehead [25]).

mally keeps the testes 3–4°C cooler than core body temperature, and about 1.5–2.5°C below the temperature of the scrotal skin [36].

Many andrologists interested both in infertility and androgen deficiency encourage scrotal cooling measures such as the wearing of loose-fitting boxer shorts, and avoidance of tight jeans [37] and prolonged periods of driving [38].

Trauma

Testicular trauma as a cause of androgen deficiency is not always obvious from the history. It can include hernial repair at any age, but particularly in infancy when it may be an aspect of partial nondescent of the testes and impaired development of the inguinal canal. Direct blows to the testes, sufficient to cause bruising, may cause unilateral testicular atrophy, as can torsion, even when surgically corrected at an early stage. This may be due to either a breach in the immunological defenses of the testis or a prolonged sympathetic spasm that can affect both sides.

Similar mechanisms could account for testicular atrophy or hypofunction, which may follow any operation on the testis, particularly when it involves trauma to the capsule, as in removal of a varicocele, or damage to the vas, particularly with vasectomy [5]. Operations on the prostate, including transurethral resection, may also damage the vas or their outflow, as shown by retrograde ejaculation of semen into the bladder, and may possibly cause an autoimmune orchitis.

Removal of one testis for testicular cancer, especially where there has been chemotherapy or radiation, or following herniorrhaphy, may not be immediately followed by infertility or symptoms of testosterone deficiency [39]. However, these may appear later in life at a relatively early age because of a lower reserve of testicular function.

Heredity and Familial Influences

Studies of monozygotic and dizygotic twins [40] have shown that familial factors accounted for twice as much of the concordance in TT and FT, and dihydrotestosterone (DHT) as genetic factors, and virtually all sex hormone binding globulin (SHBG) and aromatase activity. In all these factors, nurture appeared more important than nature. Only in estradiol and luteinizing hormone levels did heredity have a slightly greater influence. It is suggested that similar diet and physical activity levels in families may explain most of these factors in determining androgen levels, and hence, liability to androgen deficiency.

Changes in the Regulation of Testosterone Synthesis

Advancing years take their toll on the brain as the biggest sex organ in the body in many ways. Psychologically, sexual stimuli tend to be less frequent and less intense. Feedback of sensory impulses from the wrinkled skin and flaccid penis creating arousal is similarly reduced. The reduced penile sensitivity has been shown to be due to lower testosterone levels and a reduction of the number of androgen receptors (ARs) in the penis [26].

Physically, apart from neuronal dropout in the cortex and various brain nuclei mediating sexual activity, there can be an insidious cognitive impairment leading in extreme case to dementia. Lowered testosterone levels have been found in Alzheimer's disease [41], stroke [42], and Parkinson's disease [43].

Stress

Both excessive and unpleasant physical and mental stress can suppress the hypothalamo-gonadal axis and can reduce either the production or activity of androgens [44]. For example, extreme endurance training in military cadets, involving psychic stress and deprivation of food and sleep, resulted in a marked drop in testosterone levels [45].

Less acute psychological stress, such as redundancy, divorce, financial problems, and loss of close friends or relatives, has been shown to lower androgen levels [44]. Retirement, boredom, bereavement, isolation, and illness also contribute to stress in the elderly.

Physical illnesses ranging from life-threatening trauma to a variety of chronic diseases have been found to reduce testosterone levels, although it is always difficult to establish which came first [46].

Alcohol

Although excess alcohol intake is well recognized as a cause of infertility, its short-term and long-term effects on testosterone production are often overlooked.

Long term in men, it has been found that moderate levels of stable alcohol intake (nonbinge drinking) had no adverse effects on gonadal function, as estimated by testosterone levels and the FT index [47].

In contrast, excess alcohol intake, short or long term, has a variety of adverse effects on androgen status in men. Acutely, high doses cause a decrease in androgen levels by a variety of mechanisms. Partly, these are related to a direct inhibition of testosterone production by acetaldehyde derived from the metabolism of alcohol [48]. Also, alcohol suppresses luteinizing hormone-releasing hormone (LHRH) release by stimulating beta-endorphinergic neurons that inhibit the production of norepinephrine, which drives the nitric oxide-mediated release of LHRH [49]. However, the majority of the endocrine effects of alcohol are probably indirect, resulting from either the stress of intoxication, with stimulation of cortisol, catecholamines, and prolactin, or changes in the level of intermediary metabolites, e.g., free fatty acids (FFA), resulting from alteration in intracellular redox state or tissue damage [50].

Diet, Xenoestrogens, and Antiandrogens

Strict low-cholesterol diets have been shown to lower TT and FT levels by 14% [51]. Vegetarian diets, especially if low in protein, can increase SHBG, further reducing FT. However, men who put on a low-fat, high-fiber vegetarian diet have an 18% reduction in both TT and FT, which is reversed when they go back on a normal diet. This parallel reduction in both androgen measures would seem to indicate that in this situation, the decrease is primarily in testosterone [51]. Conversely, high-protein, low-carbohydrate diets, such as the fashionable weight reduction Atkin's diet, may partly exert their slimming action by raising TT and lowering SHBG.

Drugs/Medications

As well as psychotropic drugs that interfere with gonadotrophin-releasing hormone (GnRH) and LH production, there are many drugs that can directly reduce the production of androgens at the testicular level or can alter their metabolism.

Severe hyperprolactinemia, with consequent reductions in TT, sexual desire, and erectile function, was found to be related to the use of antidepressants, antipsychotic drugs, and benzamides [52].

Drugs such as aminoglutethamide and ketoconazole can inhibit steroidogenic enzymes, causing rapid and dramatic reductions in testosterone levels [53].

Long-term use of phosphodiesterase type 5 (PDE5) inhibitors, such as tadalafil, has been found to increase the TT : E2 ratio, mainly by reducing E levels, considered because of "androgen–estrogen cross-talk and possible inhibition of aromatase activity" [54].

Oral hypoglycemic agents, especially the most frequently used glitazones, rosiglitazone

(Avandia, GlaxoSmithKline, Research Triangle Park, NH, USA), and pioglitazone (Actos, Takeda, Osaka, Japan), by their action as peroxisome peroxidase gamma angonists both reduce testosterone synthesis and raise SHBG, which together greatly decrease FT [55,56]. While these actions can be beneficial in treating polycystic ovarian syndrome, in diabetics with already reduced testosterone levels, it may explain many of the adverse side effects of these drugs, especially on the heart [57], and in causing anemia and osteoporosis.

Level 2—Androgen Binding to Plasma Proteins

Of the TT circulating in the blood, 40–50% is weakly bound to albumin, and 50–60% is strongly bound to SHBG. Only 1–3% of the hormone is free (FT), and together with the albumin-bound fraction is referred to as the BT.

The albumin-bound fraction of testosterone and its metabolites estradiol (E2) and DHT is thought to be biologically available to all tissues and organs. However, the availability of this fraction of the hormones varies widely among different organs according to the capillary transit time in relation to the dissociation constants of the binding proteins and the rate of diffusion through the capillary wall [58,59].

SHBG not only regulates the absolute but also the relative amounts of sex steroids available to tissues because it is an "estradiol amplifier" [60], having a fivefold greater affinity for testosterone. This explains why with age, as SHBG levels rise in men, FT levels fall, and as evidence of greater estrogenic action, both benign enlargement of the prostate and gynecomastia become more common conditions. Also, estrogenic feedback on the pituitary gonadal axis inhibits testosterone production by the testes (Figure 2).

Regulation of SHBG Protein Expression

Given the pivotal role of SHBG in regulating the activity of the sex steroids, by sequestering them in the bound state, it is important to recognize the factors that modulate SHBG levels (Table 2).

Cellular Actions of SHBG

In addition to its function as a steroid-binding protein and estrogen amplifier, SHBG also functions as part of a novel steroid-signaling system that is independent of the classical intracellular steroid receptors. Recent research has shown that SHBG is a modular protein, which comprises an N-terminal steroid-binding and dimerization domain, and a C-terminal domain containing a highly conserved consensus sequence for glycosylation that may be required for other biological activities such as cell-surface recognition [61].

Unlike the intracellular steroid receptors that are hormone-activated transcription factors, SHBG mediates androgen and estrogen signaling at the cell membrane via a cyclic adenosine monophosphate (cAMP)-dependent pathway [62] (Figure 3). That this is a separate pathway of steroid action is shown by the fact that inhibitors of the transcriptional activation of the AR and estrogen receptor do not affect the cAMP response [63].

In the prostate, it has been suggested that the estradiol-activated SHBG/sex hormone binding globulin-receptor (SHBG-R) complex cross-talks with the AR and is able to activate the AR even in the absence of DHT [64]. These factors may be of importance in relation to the actions of androgens and estrogens in the causation and treatment of both benign and malignant prostatic disorders.

Level 3—Reduced Tissue Responsiveness

Structural Changes

Aging produces changes in many tissues, which reduce their responses to androgenic stimulation. Most of the research in this area has been focused on the structural changes in the penis with age and androgen deprivation, for obvious clinical and commercial reasons.

Testosterone stands at the crossroads in the evolution of stromal precursor cells, directing their differentiation toward muscle tissue, whether smooth or striated, rather than the default state of adipose tissue (Figure 4). Therefore, androgens exert a direct effect on penile tissue to maintain

Table 2 Summary of factors influencing sex hormone binding globulin synthesis and sex steroid binding

Increased by
• Age
• Estrogens and xenoestrogens
• Glucocorticoids
• Thyroxine
• Free fatty acids (FFAs), especially saturated FFA
• Drugs, e.g., anticonvulsants
Decreased by
• Androgens
• Insulin and obesity
• Growth hormone
• Diet—high protein and low carbohydrate
• Drugs, e.g., Danazol

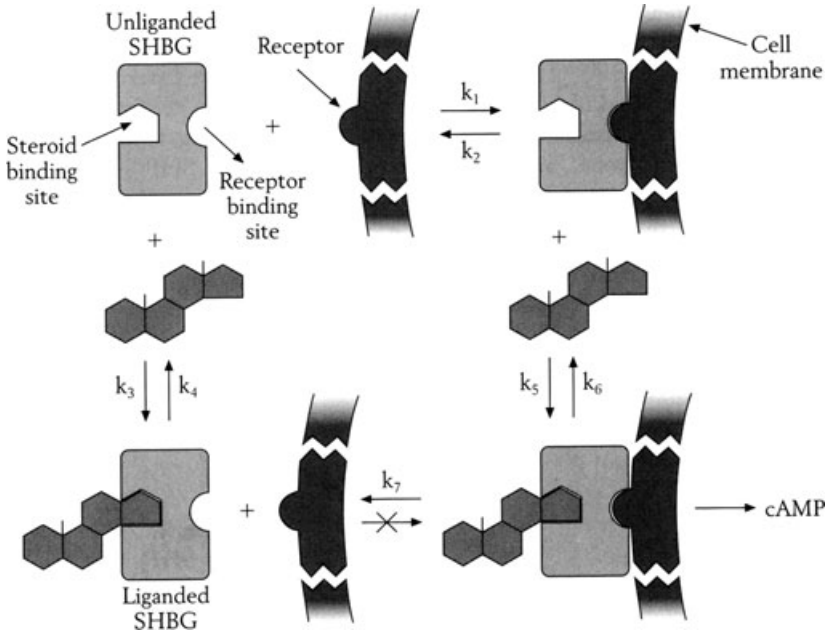

Figure 3 The steroid sex hormone binding globulin (SHBG)–SHBGR signaling system [62].

erectile function, and deficiency produces metabolic and structural imbalances in the corpus cavernosum, resulting in venous leakage and erectile dysfunction [65].

Fortunately, as with the reduction in muscle mass and accumulation of visceral fat seen in diabetes and metabolic syndrome, these changes are reversible by androgen treatment, with a consequent improvement in erectile function [66–69].

Decreased blood flow to many tissues with age also reduces the supply of testosterone to the cells. Endothelial damage as part of the accelerated atherosclerosis seen in androgen-deficient states has been shown to be associated with a decrease in arterial inflow to the penis [70] and is reversed by testosterone treatment [71].

The number of ARs in various tissues has been shown to decrease with age, and these can also undergo downregulation [72]. There are neurovascular changes, particularly in diabetics, which further reduce tissue responsiveness.

Tissue-Specific Prereceptor Actions

When testosterone enters the cell, variable amounts are converted to the metabolically more active form, DHT, by 5α-reductase enzymes, and

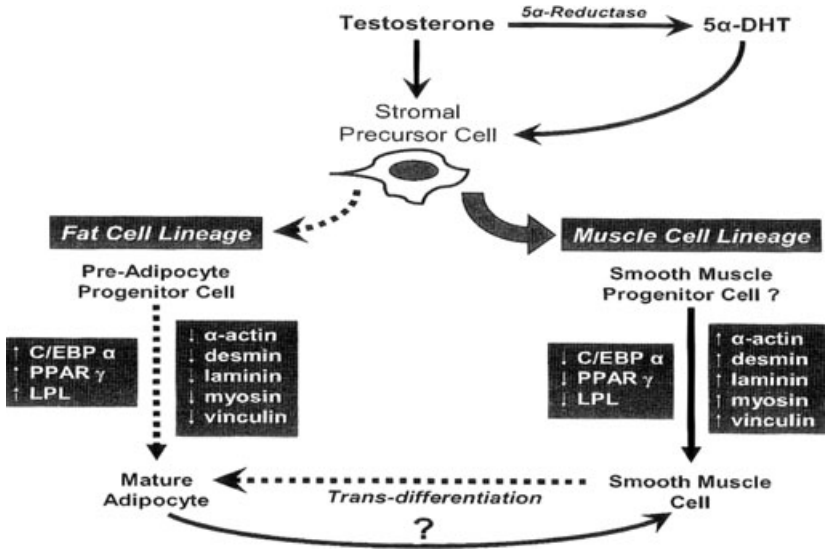

Figure 4 Action of testosterone on the differentiation of stromal precursor cells [65] (reproduced with authors' permission).

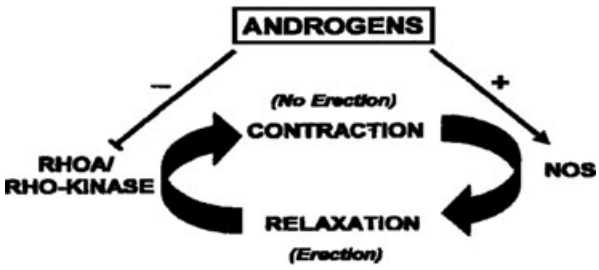

Figure 5 Effects of androgens on the balance of enzymic activity controlling erectile function [73] (reproduced with authors' permission).

to estrogen by aromatase enzymes. With age, and because of the action of 5α-reductase inhibitors, such as dutasteride and finasteride, DHT levels both in the circulation and in the cells can be decreased. Conversely, with age and obesity, aromatase levels can be increased, causing suppression of testosterone production via the hypothalamogonadal axis and antagonizing its action in the cells.

Within the cells, androgens also regulate the complex enzymatic machinery in endothelial and smooth muscle cells, which affects both the structure and function of their cytoskeleton. In the penis, for example, the RhoA/Rho kinase pathway, a mediator of cavernosal smooth muscle contraction, is inhibited by androgens, which stimulate the action of nitric oxide synthase (Figure 5) [73]. This at least partly explains the synergistic action of testosterone and PDE5 inhibitors [69,74,75], and why in diabetes, where of the two isoforms of Rho kinase only type 1 is increased in penile tissue, androgen treatment is effective in normalizing this pathway and restoring erectile function [76].

Metabolic Changes

Androgen deficiency has been shown to decrease lipid oxidation and resting energy expenditure, raising FFA, triglycerides, and cholesterol, and increasing insulin resistance [77]. These data indicated that low serum testosterone levels are associated with an adverse metabolic profile, erectile dysfunction, and increased cardiovascular risk [78,79], and suggest a novel unifying mechanism for the previously independent observations that androgen deficiency and impaired mitochondrial function promote insulin resistance in men.

Level 4—AR Activity

Genetic mutations in the AR have been shown to affect genital development, prostate tissue, spermatogenesis, bone density, hair growth, cardiovascular risk factors, psychological factors, insulin sensitivity, TT, SHBG, and FT levels.

CAG (Polyglutamine) Variations

CAG repeat lengths vary normally between 18 and 24—the greater the length, the more the androgen resistance, and in extreme cases, complete androgen insensitivity can cause complete loss of male phenotype in the androgen insensitivity syndrome. Longer mutations can also arise in prostate cancer, especially when it is metastatic or becomes hormone resistant [80].

Asian races with 22–23 CAG repeats have lower TT, SHBG, and FT, with greater insulin resistance, more diabetes and less prostate cancer than Afro-Caribbeans, with 18–20 repeats, higher TT, SHBG, and FT, and half the insulin resistance but more prostate cancer. White Europeans with 21–22 are intermediate in all these factors.

Strong positive correlations have been found between CAG repeat lengths, TT, FT, and LH, and are attributed to differences in androgen sensitivity and feedback set point [81].

GGN (Polyglycine) Variations

It has been shown both in vivo and in vitro that small differences in the length of the GGN codon can have marked effects on the activity of the AR, particularly when combined with longer CAG repeat lengths.

A study of infertile men in Sweden showed that those with 24 GGN repeats had lower testicular volumes, decreased seminal prostate specific antigen (PSA) and zinc, compared with those with 23 repeats, and concluded that the former was associated with relative androgen resistance [82].

The same Scandinavian group also found that unlike normal men, boys with hypospadias more often have AR gene with 24 rather than 23 repeats [83]. Longer GGN repeat lengths can also be linked to androgen resistance and may be the cause of "TDS," which includes testicular maldescent, hypospadias, testicular cancer, and infertility. This is sometimes summarized as "a bad testis" and attributed to the greater sensitivity of this genome to adverse environmental influences, ranging from maternal smoking to organochloride pollutants.

It has been shown in vitro that ARs with other glutamine numbers than 23 have lower transactivating capacity in response to both testosterone and DHT, and it is suggested that these could be linked to congenital malformations and other signs of a lower AR activity [84].

In these ways, minor variations in the AR gene can have major consequences in deciding the structure and function of androgen-responsive tissues throughout life. Referring to the variations in the CAG repeats, Zitzmann and Nieschlag state that "this modulation of androgen effects may be small but continuously present during a man's lifetime and, hence, exerts effects that are measurable in many tissues as various degrees of androgenicity and represents a relevant effector of maleness" [85]. With the inclusion of variations in glycine residues, this leads to a theory of the overall genetic regulation of androgen action within a particular individual.

Other Factors Affecting the AR

While the number of ARs increases with puberty, with age there is a decrease, especially in genital tissue. Upregulation and downregulation of ARs are known to occur with sustained decreases and elevations of androgen levels. A wide variety of xenoestrogens and antiandrogens are known to occur especially in agrochemicals, and antiandrogenic drugs are used in the treatment of prostate cancer.

There are two zinc fingers on the binding domain of each AR, and clinical zinc deficiency may impair binding. Zinc is also reported to inhibit the activity of the aromatase enzymes in the cell, limiting the conversion of testosterone to estrogen [86].

Level 5—Transcription and Translation Factors

Over 50 different transcription factors are known to bind to the promoter/enhancer or repressor sites for the steroid hormone receptors and affect their ability to activate RNA polymerase. The stability and availability of these proteins is largely regulated by heat shock proteins (HSP) grouped into families according to their molecular size.

HSP as ARs

HSP 90 is required for the maintenance of an active conformation in hormone-bound AR to regulate nuclear transfer, nuclear matrix binding, and transcriptional activity.

Pure antiandrogens block the transconformational change of AR in an intermediary complex, unable to acquire the active conformation and to dissociate the HSP 90.

Proteins that interact with both HSP 90 and 70 families lead to a large decrease in AR activity by slowing their rate of synthesis and reducing their rate of breakdown [87].

Coactivators and Corepressors

AR function is specifically modulated by transcriptional coregulators or corepressors that interact with a host of other transcription factors to either activate or repress the transcription of specific genes. These coactivators/corepressors act by modifying the chromatin structure/function and making the associated DNA either more or less accessible to RNA polymerase transcription. One major class of transcription coregulators modifies the chromatin structure through covalent modification of histones, the histone acyltransferases. A second, adenosine-triphosphate (ATP)-dependent class remodels the chromatin structure.

The complex functions of many coregulators of transcription are under intensive investigation because of their possible role in a wide range of disease processes, including both male and female reproductive aging, and associated pathophysiologic processes such as prostate cancer [88].

Post-Translational Factors

At the final step of androgen action after transcription by RNA polymerase, within the DNA spiral, histone-regulated acetylation, ubitylation, and sumoylation all play important roles in modulating AR function. The acetylation of the AR is induced by dihydrotestosterone and by histone deacetylase inhibitors [89].

Conclusions

Etiology of TDS

The many parallels and interactions between maturity onset diabetes and TDS suggest that a combination of lack of testosterone and its metabolites, combined with resistance to its action at multiple levels, underlies the pathology of androgen deficiency. Just as insulin resistance is thought to vary between tissues, so is androgen resistance, and therefore, different organs may respond functionally or metabolically with differing consequences.

As in diabetes, there can be genetic predispositions to androgen deficiency, both racial and familial, which interacts with lifestyle and disease-related factors. Similarly, after a period of compensation, the ability of the testis to over-

come the androgen resistance may fail, with structural changes in the Leydig cells, and signs and symptoms of endocrine failure developing.

In particular, there is a similarity between the changes observed in the testis with aging and with the pancreatic islets in maturity onset diabetes. Type 2 diabetes is associated with raised and then lowered insulin levels, combined with insulin resistance. This is due to the failure of beta-cell compensatory hypertrophy or hyperplasia. Prolonged stimulation of the beta-cells depletes the insulin granule stores and causes amyloid deposition in the islets (glucotoxicity). Beta-cells become unable to secrete pulses of insulin and are then "blind" to changes in glucose concentration. Hyperglycemia also contributes to insulin resistance as a result of downregulation, with decreased numbers of glucose transporters (GLUTS) in peripheral tissues.

Similarly, Leydig cell hyperplasia is often found in patients with testicular atrophy, androgen insensitivity syndrome [90], and in chronic exposure to toxic chemical agents [91]. Contributory factors in relation to this pathology are reduced testosterone synthesis and impaired regulation of the hypothalamo-gonadal axis with aging (Figure 2), decreased sensitivity and numbers of AR, and inhibition of 5α-reductase and aromatase activity.

Such Leydig cell micronodules have been associated with significantly increased total Leydig cell volume, and showed evidence of functional Leydig cell failure, shown by vacuolization and a decreased T/Leydig cell volume ratio. The T/LH and T/FSH ratios were also significantly decreased, indicating an impaired testicular function at the endocrine as well as the spermatogenic level [90].

Lifestyle factors in metabolic syndrome and alcoholism cause fibrosis and damage to both pancreatic islets and Leydig cells, and can be modified with benefit to both conditions [17].

New Definition of Androgen Deficiency

This hypothesis leads to a new definition of androgen deficiency in the adult male in accordance with that of diabetes mellitus:

> **An absolute or relative deficiency of testosterone or its metabolites according to the needs of that individual at that time in his life [5].**

Terminology

In the light of this information, terms like idiopathic hypergonadotrophic hypogonadism cease to convey meaningful information. Late onset hypogonadism seems similarly inappropriate, because although its symptoms most commonly appear around the age of 50, it can occur in men in their 20s and 30s, and the gonads may be functioning normally but working against high levels of androgen resistance.

It is suggested that as with diabetes mellitus, the terms "juvenile testosterone deficiency" and "maturity onset testosterone deficiency" would be more appropriate, and using the term "testosterone deficiency syndrome" for the characteristic symptom pattern of androgen deficiency appearing in adult life.

Diagnosis

Like most consensus statements on androgen treatment, the recent Endocrine Society guidelines [23] recommend "making a diagnosis of androgen deficiency only in men with consistent symptoms and signs and unequivocally low serum testosterone levels" and suggest "the measurement of morning total testosterone level by a reliable assay as the initial diagnostic test." However, lower limits of the reference ranges quoted by laboratories in the Eastern United States vary by 350%, from 4.5 to 15.6 nmol/L (130–450 ng/dL) [92], which is likely to cause confusion in the minds of clinicians trying to establish a definite diagnosis of androgen deficiency.

Because of the high sensitivity but low specificity of questionnaires to detect TDS, the complexity of factors involved in androgen resistance, and the invalidity of androgen assays [24], it seems logical to adopt the suggestion endorsed by Black et al. [93], which where typical symptoms or conditions known to be related to androgen deficiency occur, that a 3-month therapeutic trial of testosterone treatment be given.

This coincides with the emerging view that "An emphasis and reliance on serum T alone hinders the clinician's ability to manage testosterone deficiency syndromes (TDS)" [94]. Low total testosterone is just the tip of the iceberg of androgen deficiency.

Treatment

Lacking the equivalent of blood glucose in diabetes to regulate treatment, it is proposed that the sustained remission of symptoms be the guiding factor in regulating treatment, with three to six monthly androgen assays to ensure that physiological levels are maintained, along with safety measurements of PSA and hemoglobin.

In conclusion, this evidence-based review of the cellular and molecular mechanisms involved suggests that if a choice has to be made between symptomatology, as a whole-body bioassay, and standard endocrine measurements in the diagnosis and treatment of androgen deficiency, the seemingly infinite complexities of the actions of these hormones would seem to indicate that the former should take precedence for practical clinical purposes.

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