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Translational Perspective on the Role of Testosterone in Sexual Function and Dysfunction

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Abstract

Introduction—The biological importance of testosterone is generally accepted by the medical community, however controversy focuses on its relevance to sexual function and the sexual response, and our understanding of the extent of its role in this area is evolving.

Aim—This article aims to provide scientific evidence examining the role of testosterone at the cellular and molecular levels as it pertains to normal erectile physiology and the development of erectile dysfunction, and to assist in guiding successful therapeutic interventions for androgen-dependent sexual dysfunction.

Methods—In this White Paper, the Basic Science Committee of the Sexual Medicine Society of North America assessed the current basic science literature examining the role of testosterone in sexual function and dysfunction.

Results—Testosterone plays an important role in sexual function via multiple processes: physiological (stimulates activity of NOS), developmental (establish and maintain the structural and functional integrity of the penis), neural (development, maintenance, function and plasticity of the cavernous nerve and pelvic ganglia), therapeutically for dysfunctional regulation (beneficial effect on aging, diabetes and prostatectomy), and PDE5i (testosterone supplement to counteract PDE5i resistance).

Conclusions—Despite controversies surrounding testosterone with regard to sexual function, basic science studies provide incontrovertible evidence for a significant role of testosterone in sexual function and suggest that properly administered testosterone therapy is potentially advantageous for treating male sexual dysfunction.

Summary Sentence

Basic science studies provide incontrovertible evidence for a significant role of testosterone in sexual function.

Keywords

testosterone; penis; development; morphology; autonomic input; prostatectomy; diabetes; aging; PDE5i

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Introduction: Arthur L. Burnett

Perhaps there is no other subject matter in sexual medicine today that garners as much attention, if not controversy, as testosterone. This commonly known “sex hormone” stirs interest because of its generally accepted importance in diverse aspects of male sexual prowess although it also is associated with a plethora of other masculine characteristics as well. Relevant to this topic is the concept of “testosterone deficiency” or “androgen deficiency”, in reference to a clinical entity of androgen-dependent biological dysfunctions including not just impaired sexual development and function but also impaired sense of well-being, sarcopenia, decreased strength, reduced bone mineral density, anemia and cognitive dysfunction. Recognizable too is the phrase “testosterone replacement therapy”, which denotes treatment for the condition of testosterone deficiency. These terms are now pervasive within both the lay and medical person’s lexicon, promoted by widespread mass media and reflected by a worldwide commercial industry surge in testosterone products and their prescriptive use in recent years [1,2]

The controversy surrounding testosterone derives from several sources. The biological importance of this hormone in sexual function, to begin with, suffices as a matter of debate. Although testosterone is generally accepted by the medical community to be involved in the sexual response (male and possibly female), its role and extent of effects in this arena continue to be defined. The physiology of the sexual response conventionally focuses on the vascular and neurologic systems, and the endocrine system is often assigned a secondary biological role. This role should not go unnoticed, however, since testosterone may well serve an essential factor in sexual biologic function and act critically to modulate multiple molecular mechanisms related to this field of study. Ongoing scientific study can be expected to elucidate these roles.

The clinical management of “testosterone deficiency” has historically been controversial, and recent epidemiologic observations have fueled the tempest. Although testosterone products have been approved by regulatory bodies in the United States for over 50 years and thus represent a known and accepted therapy in contemporary medical practice, concern exists that these products are being used inappropriately and are possibly over-utilized. Indications for testosterone replacement therapy appropriately specify the treatment of primary hypogonadism (testicular failure) and hypogonadotropic hypogonadism (congenital or acquired) in males, and so it is applicable to men having confirmed signs and symptoms of testosterone deficiency [3]. Dissent arises when determining appropriate candidates for therapy. Older men are often recipients of therapy in view of demonstrated testosterone deficiency in these men, which is consistent with a known decline in serum testosterone levels at a rate of 1% annually after age 40 years [4]. Arguable points are whether the hormone decline is a physiologic aspect of aging rather than a pathologic process and whether testosterone treatment is an enhancement rather than a necessary remedy of poor health. Men with adverse health conditions such as hypertension, hypercholesterolemia and diabetes mellitus are now recognized candidates for therapy based on evidence of testosterone decline in men with these comorbidities [5–8]. In addition to adverse health and lifestyle considerations, emerging environmental factors causing impairments in hormone

function may also be in play [9]. Accordingly, increased use of this therapy can be attributed at least in part to a host of adverse societal changes along with the fact that those affected by hypogonadism represent an expanding segment of the population.

With respect to the role of testosterone therapy in treating male sexual dysfunction specifically, the proven benefit of therapy has been questioned in the past although recent literature is supportive in this regard. Historical studies showing benefit often were confounded by many limitations such as inclusion of trial enrollees without definite biochemical evidence of testosterone deficiency, inclusion of trial enrollees lacking baseline sexual dysfunction, use of non-validated sexual dysfunction outcome instruments, and weak study design overall. Rigorously performed meta-analytic studies disputably support the positive impact of testosterone therapy [10,11]. In one recent analysis comprising 29 randomized controlled trials, testosterone therapy was demonstrated to improve several aspects of male sexual function in confirmed testosterone deficient patients to include erectile function, sexual desire, orgasmic function, and intercourse sexual satisfaction [11]. This level of evidence and ongoing appropriately designed studies lay the ground-work towards establishing that properly administered testosterone therapy benefits male sexual function.

A further area of concern surrounding testosterone therapy justly considers its possible health risks, prompting deliberation as to whether its harms outweigh its benefits [12]. Much of the concern pertains to potential cardiovascular morbidity and mortality arising from this therapy, stoked in part by recent publications investigating this possibility [13,14]. Longstanding reservations regarding other potential risks of therapy relating to prostate cancer, worsening lower urinary tract symptoms, worsening obstructive sleep apnea, and erythrocytosis are also frequently cited, although delineation of these risks await definitive clinical trials. Further investigation is also warranted to establish the scientific basis for real and theorized adverse clinical effects of this therapy particularly in the long term.

Despite controversies surrounding testosterone particularly with regard to sexual function, the incontrovertible current story line is that the hormone is relevant for sexual function and its appropriate therapeutic administration is potentially advantageous for treating sexual dysfunction. Ongoing investigation is clearly needed in several areas of this subject matter to refine understanding as to the particular roles of this hormone and establish fully the indications, benefits and risks of testosterone therapy. Scientific study at all levels of basic, clinical, translational and population science is envisioned to contribute to advancing the field, offering to evince testosterone as an important factor in sexual function.

This White Paper was conceived, acknowledging that basic scientific evidence is relevant in establishing testosterone's influence on sexual function. The *Basic Science Committee* of the *Sexual Medicine Society of North America* was commissioned to produce a report examining this subject area, with the principal aim of providing a basic scientific evidence-based assessment of the role of testosterone in sexual function and dysfunction. This endeavor was aimed secondarily to inform the clinical management of testosterone deficiency in sexual medicine and assist in guiding successful therapeutic interventions associated with testosterone therapy for androgen-dependent sexual dysfunction. This report

consists of sections covering select subject areas related to the main topic. A conclusion section serves to summarize and synthesize the content of information of the report followed by recommendations for conducting further basic scientific research in this field of study.

Role of Testosterone in Cellular Physiology: John Mulhall, Kelvin Davies

Testosterone is widely accepted as playing a role in male sexual anatomy and function [15, 16]. However, its physiological role in human erectile function remains a matter of some controversy [17–20]. Studies in animals provide strong evidence of a regulatory role for androgens in penile erectile physiology [21–23]. The majority of animal studies have focused on the role of testosterone in maintaining penile architecture, regulation of pathways involved in corporal smooth muscle tone and nitric oxide (NO) neurotransmission. The goal of this section is to provide a synopsis of the molecular and biochemical effects of testosterone, and their involvement in its physiological function.

Testosterone Affects Penile Architecture And Erectile Function

During embryonic development testosterone (T) and dihydrotestosterone (DHT) maintain the Wolffian duct system and promote growth and development of male sex accessory glands and external genitalia. In animal models it has been demonstrated that testosterone remains essential after birth for the maintenance of penile architecture and erectile function [24]. Castration of rodent animal models results in reduced erectile function, as evidence by lowered development of intracorporal pressure following cavernous nerve stimulation [25–29]. Several investigators have proposed that change in penile architecture is the main mediator of the effects of androgen deprivation which produces penile tissue atrophy concomitant with alterations in dorsal nerve structure, endothelial morphology, reduction in trabecular smooth muscle content, increased deposition of extracellular matrix and accumulation of fat-containing cells (adipocytes) in the sub-tunical region of the corpus cavernosum [30–36]. The androgen-dependent loss of erectile response is restored by androgen administration where there is some evidence that the mechanism may involve differentiation of progenitor cells into smooth muscle cells and inhibition of their differentiation into adipocytes [28, 29, 35, 37–38].

Testosterone Regulates Gene Expression Through Binding To The Androgen Receptor

Testosterone exerts its action by binding to the androgen receptor (AR). AR has been detected in the cavernosal tissue of all mammalian species where its expression has been investigated. Binding of either testosterone or dihydrotestosterone to the AR in the cytoplasm results in a conformational change in which heat shock proteins dissociate and the AR translocates to the cell nucleus. The primary function of AR in the nucleus is as a transcription factor, it binds to a specific sequence of DNA known as an androgen response element (ARE), resulting in up or down-regulation of specific gene transcription. Changes in gene expression could potentially account for the changes in penile architecture and several of the genes known to be regulated by testosterone could directly modulate the activity of pathways involved in erectile function, such as neuronal NOS (nNOS), α 1-adrenergic receptor, hemoxygenase and phosphodiesterase 5 activity.

Biochemical Actions of Testosterone

Regulation of NOS expression—Several lines of evidence suggest that testosterone acts to stimulate or maintain the activity of the enzyme NOS, and many investigators consider this to be the primary biochemical mechanism by which testosterone modulates erectile physiology [38–40]. The first evidence for an association between testosterone, NOS and erectile function came from the observation that in castrated rats intracorporal pressure following stimulation of the cavernous nerve is not enhanced by systemic administration of nitroglycerin, whereas in testosterone replacement rats given the same dose of nitroglycerin, there is increased intracorporal pressure [41]. Enzymatic assay of NOS activity show that in castrated animals there is decreased NOS activity compared to control or testosterone replacement animals and determination of NOS protein expression shows that in castrated animals there is less than half the quantity of NOS protein as in controls. In adult rats the decrease in NOS expression and activity that occur following castration are restored with androgen replacement, whereas no additional NOS activity is observed when intact animals were given additional testosterone.

Regulation of PDE5 activity—Experiments using the NOS inhibitor (L-NAME) suggest that androgens may also maintain the erectile response by alternate pathways independent of NO but involving the synthesis of cyclic GMP [42]. Therefore PDE5 expression and activity could represent another molecular target for androgenic regulation of PDE5. There is a putative androgen-response-element upstream of the human PDE5 gene, which would suggest the possibility of direct androgenic regulation [43], and some studies have shown that levels of expression in rat corporal tissue decrease with castration [44, 45]. Supporting a role for androgens in the positive regulation of PDE5 expression is the observation that during rat penile development increased levels of AR expression parallel that of PDE5 mRNA and protein expression [46]. Although it has generally been assumed that testosterone is the primary direct regulator of PDE5 expression, a recent paper by Vignozzi et al [47] suggests that estradiol levels can also regulate its expression. In cultured penile smooth muscle cells estradiol reduced expression of PDE5, and in diet induced obese rats where higher levels of aromatase result in elevated estradiol/testosterone levels, with the higher estradiol levels (rather than low testosterone) better associated with erectile dysfunction.

These observations raise an apparent dichotomy in androgenic treatment of ED in hypogonadal men. These treatments which would raise PDE5 levels, would lower the effectiveness of PDE5 inhibitors (which does not appear to be the case in clinical reports [31]). A possible explanation is that the effect of testosterone on PDE5 expression is not direct, but secondary to other effects on erectile physiology, such as reduced smooth muscle, which would result in reduction of total PDE5 levels [48]. Overall the evidence that testosterone, or its conversion to estradiol, directly regulates PDE5 expression is at present being reevaluated [49].

Regulation of contractile pathways—Release of norepinephrine from adrenergic nerves plays a major role in keeping the penis flaccid and for detumescence of the erect penis [50, 51]. There is evidence that testosterone can modulate the adrenergic

responsiveness of corporal cavernosal smooth muscle [52]. Of the adrenergic receptors identified in cavernosal tissue there are about 10-fold higher levels of the α -compared to β -receptors [53, 54]. Phenylephrine (PE) is an α -adrenergic agonist widely used to induce detumescence in priapism. PE, when administered to both castrated or testosterone-treated animals during erection, results in a dose-dependent decrease in the intracavernosal pressure, however the effective dose is significantly lower in castrated animal [52]. In a later study, isolated cavernosal smooth muscle strips from castrated rats were shown to be more sensitive to PE stimulation, an effect that was reversed by testosterone supplementation [55]. Overall these studies suggest that cavernosal smooth muscle in castrated animals displays increased reactivity to α -adrenergic stimulation as compared to the sensitivity in testosterone treated rats and that one of the mechanisms by which testosterone deficiency may lead to ED is enhanced responsiveness to α -adrenergic agonists resulting in increased sympathetic cavernosal smooth muscle tone.

Endothelin-1 (ET-1) is not only a potent vasoconstrictor, but also mediates contraction in a variety of smooth muscle preparations including urinary tract smooth muscle. Although the effect of castration on expression of ET-1 or its receptors in cavernosal tissue has not been reported, in the prostate of castrated rats there is a 2- to 4-fold increase in ET-1 receptor expression [56]. This has led to the suggestion that a similar mechanism may occur in corporal tissue [37]. There is evidence that the protein components of the Rho/Rho-kinase pathway, which is regulated by ET-1, are up-regulated following castration, and can contribute to a reduced erectile response after castration [55].

Direct regulation of biochemical pathways by testosterone—Androgens also have a “non-genomic” direct mode of action in which binding to androgen receptors can activate signal transduction proteins in the cytoplasm independent of changes in gene transcription [57, 58]. While this effect frequently has been observed in large arteries at micromolar concentrations, more recent studies have reported vasorelaxation of smaller resistance arteries at nanomolar (physiological) concentrations and has been shown to occur in isolated human corporal cavernosal strips [59]. The key mechanism underlying testosterone-induced vasorelaxation appears to be the modulation of smooth muscle ion channel function, particularly the inactivation of L-type voltage-operated Ca^{2+} channels and/or the activation of voltage-operated and Ca^{2+} -activated K^{+} channels [60–62]. Although these effects have not been studied in cavernosal smooth muscle cells, regulation of ion channels and intracellular Ca^{2+} is a key determinant of erectile function, and might be a component of testosterone regulation of erectile function that remains to be explored. In isolated corpora cavernosal strips there is evidence that testosterone activates adenosine triphosphate-sensitive $\text{K}(+)$ channels resulting in relaxation [63]. Studies employing testosterone analogs and metabolites reveal that androgen-induced vasodilation is a structurally specific non-genomic effect that is fundamentally different than the genomic effects on reproductive targets [64]. For example, 5α -dihydrotestosterone exhibits potent genomic-androgenic effects but only moderate vaso-relaxing activity, whereas its isomer 5β -dihydrotestosterone is devoid of androgenic effects but is a highly efficacious vasodilator.

Regulation of signal transduction pathways by cytoplasmic androgen receptors could potentially have a direct effect on pathways regulating corporal smooth muscle tone, or

indirectly by leading to changes in phosphorylation status of transcription factors. At least in the smooth muscle of the GI tract, androgens have been shown to induce a rapid activation of RhoA and its translocation to the plasma membrane to activate ROK [65]. The results demonstrate that androgens can induce sensitization of smooth muscle to calcium through activation of ROK, which in turn, activates PKC to induce CPI-17 phosphorylation. Activation of this pathway induces a potent steady stimulation of myosin through phosphorylation of the myosin Regulatory Chains (20 kDa) by inhibiting MLC phosphatase and displacing the equilibrium of the regulatory subunit towards its phosphorylated state. This further demonstrates a mechanism by which androgens modulate force generation of smooth muscle contractile machinery through non-genomic calcium sensitization pathways.

The role of testosterone in premature ejaculation—Although studies are limited in number, it has been suggested that in humans higher levels of testosterone are associated with premature ejaculation, whereas lower levels are associated with delayed ejaculation [66, 67]. The effects of testosterone on this physiological process may be exerted through regulation of the PDE5/cGMP pathway, as PDE5 was reduced in the vas deferens of hypogonadal animals [68] and there is at least some evidence that PDE5 inhibitors are useful in the management of premature ejaculation [69]. However, there are other potential peripheral and central ways in which testosterone may impact the ejaculatory response time, such as changes in sexual desire, effects on the CNS, and mechanical, such as effecting seminal volume. Compared to the studies of testosterone on the erectile physiology in animals, the mechanisms by which testosterone modulates the ejaculatory response is far less researched.

Conclusion—At least in animal models, testosterone plays a role in erectile physiology. At the molecular level its role in erectile function has best been defined through its regulation of protein synthesis, determining penile architecture, and the activity of pathways involved in smooth muscle tone (with the best evidence supporting a role in regulating NOS expression). Although there is good evidence that testosterone can have a “non-genomic” direct effect on erectile physiology, these mechanisms have not been investigated, even though research on other tissues suggests testosterone could directly affect pathways regulating corporal smooth muscle tone and thereby erectile physiology.

Testosterone Effects on Penile Development and Adult Penile Morphology: **Carol A. Podlasek, Christopher J. Wingard**

In this section we will examine the translational perspective on the role of testosterone in sexual function and dysfunction with particular focus on evidence linking testosterone to penile development and its continued function in the adult penis to maintain corpora cavernosal morphology. Gaps in our knowledge of how testosterone mediates penile structure/function will be identified.

Potential Mechanism Of Testosterone Impact

A plethora of studies demonstrate an effect of testosterone withdrawal on penile morphology, both in animal models and in hypogonadal patients. Aside from “testosterone

effects on libido, frequency of sexual activity and sleep related erections” [70], the literature supports two potential mechanisms of how testosterone may impact penile morphology and function. The first involves an indirect effect of testosterone and its metabolic product, dihydrotestosterone (DHT), on maintaining pelvic ganglia and cavernous nerve neurons. Decreased/inhibited testosterone abundance/signaling could negatively impact pelvic ganglia and cavernous neurons, resulting in decreased neurotransmitters and altered corpora cavernosal morphology. This indirect effect will be described in detail in the following section on autonomic input. In the second hypothesis, it is proposed that testosterone has additional direct effects on corpus cavernosal smooth muscle and endothelium via androgen receptor activity. In this section we will examine literature evidence supporting the direct effect of androgen on penile architecture. Additional effects of testosterone on the developing penis, molecular targets that may mediate these processes, and environmental influences on testosterone levels, are discussed.

Penile development—Testosterone is essential for embryonic and postnatal development of the penis. Decreased androgen in the neonatal period causes permanent infertility and a malformed penis, including smooth muscle replacement with fat cells [71]. Programming of reproductive organ size occurs during the embryonic period (E15.5 to 18.5) however, whether the growth potential is realized depends on androgen activity during the postnatal period after birth [72]. Reduced androgen levels in the neonatal period alter gene expression of smooth muscle differentiation markers and results in decreased smooth muscle.

Direct Effect Of Testosterone On Penile Architecture Via Androgen Receptor—

Testosterone may directly impact the corporal tissue. It has been shown that castration results in erectile dysfunction [31, 35] and reversibly alters corpora cavernosal architecture. Trabecular smooth muscle content decreases [29, 36] while connective tissue abundance increases [31, 36]. In addition, fat-containing cells accumulate in the corpora cavernosa [31], resulting from androgen deprivation effects on progenitor stromal cells causing them to differentiate into an adipogenic lineage [31]. Similar morphology changes (decreased smooth muscle and increased collagen) were shown in the penis after inhibition of testosterone conversion to DHT by 5 α -reductase inhibitors [73–75]. Additionally, in patients undergoing androgen deprivation therapy, decreased penile length was observed (~2.71 cm), which plateaued after 15 months of androgen deprivation [76]. In another study, testosterone deficiency in patients was associated with cavernosal fibrosis [77]. Stress also decreased testosterone abundance, resulting in decreased smooth muscle and increased penile collagen [78].

Several cell types in the penis respond to testosterone replacement after castration including myocytes, fibrocytes, endothelial cells and Schwann cells [79] and androgen receptors have been identified in the corpora cavernosal tissue (smooth muscle) [80, 81] although their abundance decreases with age [81]. In vitro, 10⁻⁵ mol/L testosterone increased proliferation of smooth muscle cells and fibroblasts while higher concentrations (10⁻⁴) inhibited their proliferation. This suggests a narrow range of testosterone impact on corpora cavernosal tissues and intricate regulation by testosterone [80]. Testosterone may impact corpora cavernosal tissues by decreasing neuronal and endothelial NOS in the corporal beds [82, 83].

Other studies have suggested that metabolic supplements may help maintain erectile response and mating behaviors in animal models of castration or aging through the actions of Tuarine and L-Citrulline focusing on the role of maintenance of the NO signaling pathway [84, 85].

Structural changes in the penis in response to testosterone deprivation or castration result from increased apoptosis in the corporal tissues [79]. Apoptosis increased 11-fold in castrated rats compared to testosterone supplemented rats and 16-fold in comparison to sham controls [86]. Penile tissue responds to testosterone supplement after castration through increased cellular proliferation and new DNA synthesis [79]. These findings support the idea that testosterone plays an important role in maintaining the structural and functional integrity of the penis [86, 75].

Testosterone Impacts Known Mediators Of Erectile Function—Castrated rats treated with L-NAME (NOS inhibitor) did not have an erectile response as measured by intracavernosal pressure however testosterone supplement allowed for erectile function, indicating the existence of testosterone dependent pathways that are not mediated by NO [87]. Several studies have shown that known mediators of erectile function are impacted by testosterone. Vcsa1 expression and its gene product Sialorphin, increase with testosterone supplementation after castration [28]. Vascular endothelial growth factor (VEGF) protein and mRNA decrease in the corpora cavernosa of castrated rats and androgen replacement returns VEGF to baseline expression [88]. RhoA and Rho-kinase protein increase with castration resulting in a depressed erectile response [55]. Erk1 and 2 increased with castration in the corpora cavernosa while PKB/Akt remained unchanged [89]. The apoptotic index may be impacted by testosterone since testosterone supplement increased Bcl2 and decreased Bax [90]. Testosterone induced relaxation in human isolated corpora cavernosal strips by activation of smooth muscle adenosine triphosphate-sensitive K⁺ channels [63]. Castration caused age dependent alterations in sonic hedgehog (SHH) protein and mRNA expression [91]. SHH protein and RNA increased when rats were castrated during puberty. Castration in the adult caused an inverse response with decreased *Shh* mRNA but not protein. Supra-physiological testosterone in the adult increased *Shh* mRNA and decreased SHH protein [91], reflecting earlier findings that a narrow range of testosterone may impact penile morphology and growth factors.

Environmental/Toxicology Exposures And Testosterone Mediate Erectile Responses—While there are various forms of environmental exposures some effort has focused on the pathogenic link between ED and metabolic syndrome and correlation to testosterone levels. In studies of animal models of diet or genetically induced metabolic syndrome, there are reports of reduction in free testosterone levels [92, 93]. Other evidence in animal models of diet influence on erectile function includes insulin resistance on methylation patterns targeting the androgen receptor promoter [94].

Investigations that are more toxicological include assessment exposure to Bisphenol A (BPA) that resulted in developed hypogonadism and related histological changes but no loss of EFS erectile function, and the prevalent and sustained use of phytoestrogen and thalates [95]. Exposure to di(2-ethyl-hexyl) phthalate (DEHP) in utero and during lactation causes

long-term pituitary-gonadal axis disruption in male and female mouse offspring [96]. Abnormalities of sexual development were observed in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate [97]. Relative sensitivity of developmental and immune parameters were observed in juvenile versus adult male rats after exposure to di(2-ethylhexyl) phthalate that mimics naturally occurring androgenic and estrogenic compounds [98]. There remains a largely unexplored aspect of environmental exposures on the sensitivity of erectile physiology.

Conclusions

Hormone replacement, even when instituted at a late stage, is effective in reversing the corpora cavernosa's structural alterations produced by castration [35]. Delayed testosterone replacement has no detrimental effect on the restoration of the erectile mechanism after castration [99]. This suggests that penile architecture has a great deal of plasticity and corpora cavernosal morphology has the potential capacity to regenerate in response to testosterone treatment in the adult. This may have potential ramifications to improve corpora cavernosa regeneration after prostatectomy, in diabetic men, or following environmental exposure. More detailed studies are required to define the mechanism (s) of how testosterone interacts with penile tissues to maintain and regenerate corpora cavernosal architecture. Study of the molecular pathways involved in these processes may be important for regeneration of tissues with disease or following surgical insult.

Autonomic Input and Testosterone: Johanna L. Hannan, Carol A. Podlasek, Trinity J. Bivalacqua

The autonomic nervous system is responsible for the activation of penile erection and subsequent ejaculation. In this section a potential mechanism of how autonomic input can be impacted by testosterone fluctuation is described. Fundamental gaps were identified in our understanding of how moderate to low physiological testosterone levels, both acute and chronic, impact pelvic ganglia, cavernous nerve and neuronal structure.

Autonomic Input

Spinal preganglionic neurons from the lumbar and sacral spinal cord lead to the major pelvic ganglia (PG; also known as the pelvic or hypogastric plexus). The PG is the primary autonomic supply to the urogenital organs and the lower bowel and is a mixed ganglia with both sympathetic and parasympathetic nerves and myelinated and unmyelinated axons [100]. The sympathetic nerves are adrenergic and release norepinephrine and neuropeptide Y, which help maintain the penis in a flaccid state and play a role in detumescence. The parasympathetic nerves release acetylcholine, nitric oxide (NO) and vasointestinal peptide and are responsible for the initiation of the erectile response.

Testosterone is Critical to PG Development—Testosterone plays an important role in the sexual dimorphism of the PG during pre- and postnatal development. Prenatal exposure to exogenous testosterone in mice increased the number of neurons in the PG [101]. Additionally, pregnant dams that were treated with the testosterone antagonist flutamide late during pregnancy (E15–21) had pups with markedly decreased sympathetic and

parasympathetic neurons compared to control pups [102]. Postnatal treatment with testosterone was unable to increase or normalize PG neuron number. Female mice administered testosterone between postnatal days 9–16 had an increase in neuronal number and size in the PG [103]. Postnatally, there is a critical period from birth to day 10 in which physiological levels of testosterone are required for normal neuronal development in male mice. When mice are castrated 12 hours after birth, there is a decreased number of neurons, smaller soma and less tyrosine hydroxylase activity. Delayed testosterone administration can restore soma size but not the number of neurites or tyrosine hydroxylase activity [102]. If castration occurs 10–11 days following birth, there is a large decrease in sympathetic neurons, a slight decrease in parasympathetic neurons and immediate or delayed testosterone supplementation can prevent the decline in soma size, neuron number and sympathetic and parasympathetic input [104]. These data suggest that testosterone plays an important role in PG development at late gestation and in the early postnatal period after birth.

Mechanism of Action of Testosterone in the PG—Testosterone primarily impacts neuronal function via androgen receptors that are located in the PG. They have been co-localized to VIP-positive neurons (parasympathetic) and tyrosine hydroxylase positive (sympathetic) neurons [105]. Androgen receptors are also located on both small and large primary afferent dorsal root ganglion nerves [106]. In addition to acting on androgen receptors, testosterone has also been converted to estrogen via aromatase present in the ganglionic neurons [107]. Estrogen receptors have been co-localized to PG neurons staining positive for neuronal nitric oxide synthase (nNOS) and tyrosine hydroxylase. Testosterone has also been demonstrated to have an indirect action on glial cells and can promote the expression of nerve growth factor (NGF), neurturin, and neuritin, which can play a role in the health and maintenance of the cavernous nerve and PG [108]. The expression of other neurotransmitters such as calcitonin gene related peptide (CGRP) may also depend on androgens [109].

Impact of Castration and Aging on NOS in the Cavernous Nerve and PG—Previous sections have demonstrated that castration results in dramatic structural changes to the morphology of the penis that contributes to erectile dysfunction. Similarly the cavernous nerve and PG are dependent on testosterone to maintain and preserve their structure and function. Cavernous nerves from castrated rats demonstrated decreased nerve fiber density and thinner myelin sheaths compared with intact rats or castrated rats supplemented with testosterone [22]. Evidence of neuronal degeneration, such as decreased myelin thickness, lower nerve density and smaller nerve cross-sectional area, is also seen in the dorsal nerve of the penis of castrated rats [110]. Testosterone impacts nitroergic nerves as castration revealed decreased abundance of nitric oxide synthase (NOS) in penile tissue [111, 70] and decreased NOS containing nerve fibers in the corpora cavernosa of castrated rat penis [99]. NOS mRNA expression was rescued with testosterone supplement [87] and delay in testosterone supplement did not impair regeneration of the erectile mechanism after castration [99]. Nicotinic acetylcholine receptors (nAChR) in the PG are also down-regulated following castration and were rescued with testosterone supplementation [112]. Additionally, whole – cell patch – clamp recording of nAChR channel activity in sympathetic and parasympathetic PG neurons projecting to the penile vasculature showed no change in sympathetic neurons

and a decrease in parasympathetic current density following castration that was prevented with testosterone. It has been proposed that testosterone targets are situated on postganglionic parasympathetic neurons rather than on sympathetic neurons and penile erectile tissue [100, 111, 113]. Androgen receptors have been identified on ~40% of PG neurons that innervate the penis [113] and 87% of these neurons also contain NOS [113]. These findings suggest that androgens regulate the erectile response by maintaining an adequate supply of NO and determine synaptic strength for parasympathetic transmission in the PG [87].

In addition to castration or androgen deprivation therapy, men experience lower testosterone levels as they age. Aging Sprague-Dawley rats demonstrated an age-related decrease in PG nNOS mRNA expression levels [114]. There is also an age-related decrease in the growth factor neurturin and its receptor, glial cell line derived neurotrophic factor family receptor alpha-2 (GFRa2) mRNA expression levels in the PG [114]. PG nNOS gene expression begins to decrease as early as 6 and 12 months of age. Furthermore, the co-expression of GFRa2 receptor and nNOS simultaneously decreased in old adult (24 months) rats. These changes in nNOS gene expression in 24-month old rats were comparable to young castrated rats. Almost all penis-projecting neurons in the PG are positive for neurturin and GDNF's GFRa2 receptors and it is hypothesized that these growth factors are retrogradely transported to the PG to maintain nNOS-positive neurons. Thus, changes in GFRa2 with age, castration and lower testosterone levels can impact the function and maintenance of the PG and its neural plasticity.

Testosterone and Nerve Regeneration—In vitro analysis of cultured PG neurons exposed to testosterone or DHT show increased neurite growth and length in noradrenergic and cholinergic NOS-expressing neurons, which can be attenuated by androgen receptor antagonists [107]. When cultured PG neurons were grown in the presence of nerve growth factor (NGF) there was a greater increase in the soma size and neurite length of tyrosine hydroxylase, VIP and vesicular acetylcholine transferase positive neurons compared to growth with testosterone alone [115]. Testosterone has been demonstrated to be neuroprotective and is required for nerve preservation and regeneration in animal models of sciatic nerve or spinal cord injury [116]. These findings have important implications for men suffering from ED due to nerve injury during radical prostatectomy who are hypogonadal or are undergoing androgen deprivation therapy. This will be further discussed in the next section.

Conclusions—Testosterone plays an important role in the development, maintenance, function and neuronal plasticity of the cavernous nerve and the PG. The role of testosterone in development and sexual function when it is intact, or completely inhibited in models of castration, has been elucidated. However, there is a lack of information on the impact of lower to moderate physiological levels of testosterone acutely and/or chronically on the structure and function of the cavernous nerve and PG. To fully understand the role of testosterone in the cavernous nerve and PG, studies are required to identify and characterize the cellular localization of androgen receptors and how its expression and function is

regulated in middle aged and old rodent models, to better replicate the clinical outcomes in older men.

Testosterone Impact/association with Prostatectomy, Diabetes, and Aging: Biljana Musicki, Mohit Khera

Aging and comorbidities such as diabetes, metabolic syndrome, and hypertension, are associated with both ED and reduced testosterone levels. While ED and testosterone deficiency have emerged as predictors of cardiovascular disease, basic science literature on the effect of testosterone on sexual function and the mechanism of testosterone action in the penis in aging and disease states is still emerging. In this section we will examine the basic science evidence describing the impact of testosterone on aging, diabetic, and prostatectomy models of ED.

Aging

Castration of young animals was used in many studies as a model for testosterone deprivation seen in aging, but the findings may not be fully applicable to natural aging [117]. Very limited basic science studies have evaluated the impact of aging-associated androgen deficiency on sexual function. Treatment of old (20–22-month) rats with testosterone and DHT for 1.5–2 months was found to improve sexual behavior (mount rate) [118] erectile function [117], and decrease collagen content in the penis [33]. On the other hand, no improvement in erectile function of old rats by testosterone replacement has been noted [119]. In addition to reduced androgen levels, another factor which contributes to decreased responsiveness to testosterone and aging-associated ED is downregulation of androgen receptors in the penis in old (24-month) rats [120]. These limited studies may indicate the protective role of androgens on erectile function with aging.

Diabetes And Metabolic Syndrome

Testosterone deficiency was demonstrated in animal models of ED associated with metabolic syndrome [92, 121], type 2 diabetes mellitus (T2DM) [122–124], and some [122, 125–130], but not all [131–134], animal models of type 1 DM (T1DM). In T1DM hypogonadal rats and rabbits, testosterone supplementation preserved erectile function and endothelial function in the penis by normalizing downregulated nNOS and PDE5 and upregulating ROCK1 mRNA and protein expression [128, 129]. In old T1DM rats, testosterone supplementation ameliorated cavernous oxidative stress, apoptosis, and corrected cGMP levels [90, 135]. In hypogonadal Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of T2DM, normalizing testosterone levels corrected erectile dysfunction and endothelial dysfunction in the penis by reversing abnormalities in the penis (decreased smooth muscle/collagen ratio, impaired mRNA expressions for eNOS, iNOS, and anti-inflammatory and NO-promoting molecule Sirt1, and proinflammatory molecules IL-6 and TNF- α); importantly, this effect of testosterone was associated with improvement of the metabolic parameters such as hemoglobin A1c and cholesterol levels, and decreased serum ADMA, a NOS inhibitor [124]. Similarly, in a hypogonadotropic hypogonadal rabbit model of metabolic syndrome, induced by high-fat diet, normalizing testosterone levels not only preserved erectile function and reversed abnormalities in the penis (endothelial dysfunction,

decreased mRNAs for eNOS and PDE5) [92, 121], but also partially ameliorated overall metabolic profile (glucose levels, glucose tolerance, and visceral obesity) [121]. Moreover, in a rabbit model of hypogonadal metabolic syndrome, nonalcoholic steatohepatitis (NASH) was found to play an active role in the pathogenesis of ED, likely via inflammatory TNF α derived from inflamed liver; testosterone supplementation exerted anti-inflammatory effects by decreasing TNF α levels in the liver and circulation, improving NASH, and reducing ED [92]. These findings are in line with non-ED-related basic science studies which suggest that androgen deprivation adversely affects carbohydrate, lipid and protein metabolism, thus contributing to oxidative stress, endothelial dysfunction, and increased production of pro-inflammatory factors [136, 137]. In a mouse model of high fat diet-induced insulin resistance, mRNA and protein expression of androgen receptors in the penis are downregulated, presenting an additional mechanism of impaired responsiveness to physiologic testosterone levels [94]. This, however, may not be the case in T1DM, which is not associated with reduced mRNA and protein expression of androgen receptors in the penis 4 and 8 weeks after the induction of diabetes [138]. These studies suggest that testosterone deficiency or non-responsiveness to testosterone are associated with diabetes and metabolic syndrome, and that testosterone supplementation has a beneficial effect on erectile function by maintaining structure and molecular signaling and suppressing inflammation in the penis.

Patients with metabolic syndrome and/or diabetes are at a significantly increased risk of having androgen deficiency. This is largely due to the fact that they share many of the same risk factors. Low testosterone levels represent a risk factor for insulin resistance and T2DM, and approximately 50% of diabetics are found to have androgen deficiency [139]. Low testosterone has been shown to result in elevated fasting insulin, glucose, and hemoglobin A1c (HbA1C) levels and possibly to predict the onset of diabetes [140]. The exact mechanism by which diabetes and insulin resistance impair testosterone production and how decreased testosterone levels increase the risk of diabetes and insulin resistance is poorly understood [140].

Radical Prostatectomy

Very limited studies evaluated the effect of testosterone on erectile function in animal models of radical prostatectomy. Bilateral cavernous nerve cut (to mimic radical prostatectomy-induced ED) resulted in testosterone deficiency in rats due to decreased Leydig cell function in the testis; testosterone supplementation for 3 months partially prevented penile structural alterations (reduced smooth muscle/fiber ratio) and PDE5 down-regulation, and restored eNOS (but not nNOS) mRNA expression and endothelium-dependent vasorelaxation [141]. In a rat model of bilateral cavernous nerve neurotomy followed by unilateral nerve graft, erectile function did not recover upon androgen ablation [142]. Further studies are needed to establish clinical relevance of these findings in humans and whether treating hypogonadism could be useful in post-prostatectomy penile rehabilitation.

Conclusions—Collectively, these basic science studies suggest a beneficial effect of testosterone on erectile function in animal models of aging, diabetes, and radical

prostatectomy. Continued basic science research is needed to confirm these findings and critically evaluate the molecular and cellular basis of this androgen action in the penis.

PDE5i and Testosterone: Nestor Gonzalez-Cadavid

It is widely accepted that androgens modulate erectile function at the central control of sexual arousal and desire in the brain cortex and hypothalamus, but also at the peripheral level of the erectile mechanism in the penis [143]. However, the efficacy of testosterone (T) to treat erectile dysfunction (ED), particularly for eugonadal men, and specifically its application to increase the response to PDE5 inhibitors (PDE5i) in patients who are refractory to them, is still inconsistent and controversial despite the animal experimentation data and biomedical rationale are solid. The purpose of this section is to provide basic science evidence on the relationship between T and PDE5i.

Testosterone Effects

During the last three decades a multiplicity of clinical and translational studies [143–151, 17] have confirmed and expanded the initial earlier evidence from animal models, and hypogonadal men (defined by a testosterone threshold of <12 nM T at baseline), that: a) the erectile mechanism is testosterone dependent, b) castration and/or extreme hypogonadal T levels down-regulate the NO/cGMP pathway increasing smooth muscle and endothelial apoptosis and lipofibrosis in the penile corpora cavernosa and leading to corporal veno-occlusive dysfunction (CVOD) [151], c) age and diabetes reduce serum total T in association with ED [145, 146], T also induces the vasodilation of penile arterioles and sinusoids [145], and d) that this is corrected by testosterone supplementation. This conceptually supports the use of testosterone supplementation for men with T deficiency or late onset hypogonadism, which is believed to be restricted to less than 5% of men with ED.

Since a large fraction of ED patients, particularly those with CVOD induced by diabetes, radical prostatectomy, or aging, become refractory to PDE5i treatment, in the last two decades there was a logical attempt to try to rescue the sensitivity to these agents in hypogonadal men by testosterone supplementation [17]. However, despite some success this evolved into a combination therapy applied to eugonadal, or moderately hypogonadal, men with ED and resistance to PDE5i, without a rationale for the sequence and objectives of the treatment, i.e., not a truly hormonal replacement but a synergistic approach based on a still controversial concept.

Castration Studies

Studies of testosterone supplementation in animal models of ED were conducted mainly in castrated animals, and they in general show restoration of the erectile response. One of the earliest reports [152] demonstrated two decades ago that finasteride blocked the restoration of the electrical field stimulation (EFS) response by T, and that DHT was as effective as T but with its effects not being decreased by finasteride. Nitric oxide synthase (NOS) activity in the penile cytosol was found to correlate with the EFS determinations, thus suggesting that DHT is the active androgen in the prevention of erectile failure seen in castrated rats, in a process mediated, at least partially, by changes in NOS levels in the penis. To our

knowledge, this DHT role has not been explored in erectile function in men. However, although androgen deprivation by surgical or medical castration reduces corporal smooth muscle content and impairs penile hemodynamics, and erectile function in rabbits, this did not modify NOS activity, illustrating a species difference [26].

Non-Castration Studies

Other early studies with long-term testosterone supplementation alone in non-castrated rat models of ED, specifically induced by aging [117], showed that aging-related ED in the intact rat may be responsive to androgen therapy and that this correction is not associated with an increase in the basal levels of penile NOS. This is in contrast to what occurs in castrated rats, implying a difference between processes associated with extreme and moderate hypogonadism that is pertinent to the clinical findings above. Serum T was found not just to be reduced by aging but also by diabetes in models of type 1 and 2 diabetes [122, 153] where this decrease was associated with ED and with a marked reduction of penile NOS activity and a lower decrease of penile nNOS content.

Beneficial/Controversial T Effects

Several reports show the beneficial effects of testosterone supplementation on erectile function and in particular on the responsiveness to PDE5i, such as to tadalafil in the rat corpus cavernosum [44], amelioration of ED and sildenafil responsiveness in rabbit models of diabetes [128] and metabolic syndrome [121], the protective anti-apoptotic role of T with sildenafil or tadalafil in aged diabetic rats [90], the reduction of corporal oxidative stress in diabetic rats [135], and the improvement by T of the relaxation response to sildenafil of corporal strips from diabetic and metabolic syndrome rabbits [37, 129]. This synergistic effect may result because tadalafil increases androgen receptor protein abundance [154].

Conversely, sildenafil stimulates Leydig cells and T secretion in the rat and mouse [155–157], thus reinforcing their therapeutic combination effects. However, as in the clinical setting there are still controversies, such as on the proposed paradoxical upregulation of corporal PDE5 expression in the rat corpora that would counteract PDE5i effects [121, 45]. A recent review [49] discussed opposite findings in the literature on up- and down-regulation, or no effects, on PDE5 expression, and on the presence or absence of androgen responsive elements in the promoter of the PDE5A gene, concluding that it is not directly regulated by androgens. This would resolve the T/PDE5 paradox and support the use of testosterone supplementation for PDE5i resistance.

In this context, an important novel concept on the action of PDE5i on the corporal histopathology that underlies CVOD has been proposed based on the demonstration that the continuous long-term administration of PDE5i in aged, diabetic, and cavernosal nerve damaged rat models of ED show correction of CVOD [158–163]. This occurs by an antifibrotic and smooth muscle/endothelial protective action, different from the on-demand PDE5i vasodilator effects to induce an erection. The correction of CVOD by continuous long-term administration of PDE5i is presumed due to their anti-fibrotic/apoptosis/oxidative stress effects.

Conclusions—Testosterone supplementation for eugonadal or moderately hypogonadal men with ED is often contradictory and the use of testosterone supplementation to counteract the resistance to PDE5i remains controversial, despite the promising basic science evidence. Although this may reflect the difficulty in extrapolating to men from animal models, the discrepancy between the human and animal data is more considerable than in many other topics of ED treatment or the use of testosterone supplementation. Since animal studies can easily be performed with multiple arms and adjusting for several conditions and variables, the problem may reside in the design and performance of the clinical trials. Some suggestions for future clinical trials of testosterone supplementation in eugonadal or moderately hypogonadal men with ED include: elimination of confounding comorbidities, exclusion of true hypogonadal patients (<12 nM T at baseline), characterization of PDE5i usage as “on demand” or “long-term daily” administration, increase trial duration, and increase the number of subjects. The consideration of these suggestions and their adaptation to the reality of recruitment, budget, effort, and duration constraints, may lead to a better approximation of the efficacy of testosterone supplementation in men, particularly in conjunction with PDE5i, towards the results achieved under the much easier and more viable conditions of animal experimentation.

Summary and Clinical Integration: John Mulhall

The preceding pages have outlined in exquisite detail the current state of knowledge regarding the role of testosterone at the cellular level as it pertains to sexual function. Our understanding of these mechanisms has evolved dramatically over the course of the past 50 years and is likely to continue to evolve over the next 50 years. It is possible that in the vein of personalized medicine we may be able to use genomics, proteomics and metabolomics to define with greater precision the exact nature of an individual's testosterone deficiency. Given the premise that understanding physiology and pathophysiologic mechanisms is the foundation for both the prevention and the treatment of illness, an appreciation of the basic science mechanisms involved in the testosterone regulation of an organ function is essential for any clinician treating men with testosterone deficiency and using testosterone therapy. There is no doubt that the approach to diagnosing and treating men with testosterone deficiency in the early part of the 21st-century is extremely crude. Because of this crudeness, it is quite a challenge for the practicing clinician to select the ideal patient for testosterone therapy. Indeed, in practice, much of what we do falls under the banner of trial and error. We are in dire need of better markers of true testosterone deficiency whether they be serum based, tissue based, or imaging based. Given our advances in basic science investigative technologies there is little doubt that the future is bright for our increased understanding of testosterone function at the cellular and sub cellular levels. As always, we should proceed with caution in our interpretation of animal based and in vitro based data. Particularly in the realm of hormones, where individual hormones rarely act in isolation from other hormones, the use of animal models is potentially fraught with errors in its translation to the human model. Likewise, in vitro experiments are limited in their extrapolation to humans. Saying this, much of the work performed over the past decade is elegant in its nature and has made a major contribution to the field of endocrine and sexual medicine. Clinicians are in dire need of assistance from our basic science brethren in the area of testosterone deficiency. Future

research will be ideally conducted in a collaborative partnership between our basic scientists and clinical researchers. We look forward to returning in a decade and rewriting this treatise demonstrating the advances that have been made between 2015 and 2025.

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