

# Differing levels of testosterone and the prostate: a physiological interplay

S. Larry Goldenberg, Anthony Koupparis and Michael E. Robinson

**Abstract** | The controversies surrounding testosterone replacement therapy (TRT) have been addressed in the past few years. Although the androgenic effects of TRT on normal and malignant prostate cells have been studied for over 70 years, little clinical prospective research exists on the physiological responses of prostate tissues to a wide range of serum testosterone levels. The prostate is both an androgen-dependent and an androgen-sensitive organ; active processes are triggered at a ‘threshold’ or ‘saturation’ level of testosterone. Despite decades of research, no compelling evidence exists that increasing testosterone beyond this threshold level has a causative role in prostate cancer, or indeed changes the biology of the prostate. Testosterone deficiency has marked physiological and clinical effects on men in middle age and beyond. With subnormal testosterone levels, the potential positive benefits of TRT on factors such as muscle mass, libido or erectile function are likely a dose–response phenomenon, and should be considered differently than the threshold influence on the prostate. This Review will re-examine classic androgen research and reflect on whether testosterone actually stimulates prostatic cellular growth and progression in a ‘threshold’ or a ‘dose–response’ (or both) manner, as well as discuss the influence of testosterone on prostate cells in the hypogonadal and eugonadal states.

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## Introduction

Late-onset hypogonadism (also known as age-associated testosterone deficiency syndrome), is a syndrome associated with advancing age and characterized by a spectrum of symptoms and a biochemical deficiency in serum testosterone levels below the young healthy adult male reference range (9.8–10.4 nmol/l, this range may vary in different laboratories).<sup>1</sup> The decrease in serum testosterone levels seems to be a gradual, age-related process resulting in an approximate 1–2% annual decline after age 30 years,<sup>2</sup> with a steep decline in bioavailable and free testosterone levels. Findings from the Baltimore Longitudinal Study of Aging demonstrated that 30% of men in their eighth decade have total testosterone values in the hypogonadal range (that is, 6.9–10.4 nmol/l), and 50% have low free testosterone values (0.17–0.31 nmol/l).<sup>2</sup> An estimated 500,000 new cases of late-onset hypogonadism occur annually in the USA,<sup>2,3</sup> with similar levels reported worldwide.<sup>4</sup>

Given that the number of men aged 65 years and older will increase dramatically by 2020, testosterone deficiency is becoming an increasingly discussed area of men's health. Testosterone has a ubiquitous role in the male body and the importance of a decline in testosterone levels has a wide-ranging influence on: regulation of gonadal function, prostate development and growth, libido, cerebral function, behavior, mood, muscle mass, liver function, lipid regulation, bone formation, atherogenesis,

erythropoiesis, hair growth and immune function (Box 1). What the minimum required level of serum testosterone for the optimal health of each of these areas, nor whether each organ system's biological response to increasing or decreasing testosterone levels follows a ‘dose–response’, ‘threshold’ or other behavior is unclear. Despite this general paucity of knowledge, current discussion regarding testosterone replacement therapy (TRT) safety is centered on its possible effects on the initiation, promotion, aggressiveness, and progression of prostate cancer.<sup>5</sup>

The original work by Huggins and colleagues<sup>6,7</sup> in the 1940s would advise caution in using testosterone supplementation in patients with prostate cancer owing to the apparent central role of testosterone in cancer regression. However, even though androgen withdrawal is the basis for the treatment of advanced prostate cancer, the development and progression of prostate cancer is not a straightforward relationship between serum androgen levels and prostate cellular physiology. Even though the primary signal for the onset of DNA synthesis and cell division in the prostate is androgen, it is likely that other extracellular (such as epigenetic factors) and intracellular factors related to late gene expression mediate the effect of androgen. Factors such as gene polymorphisms, sexually transmitted agents (viral), diet, environmental carcinogens and nonsteroidal hormones likely have a role in carcinogenesis. In studying androgen action on the prostate, one must carefully differentiate between proliferation or differentiation (androgen sensitivity) and apoptosis (androgen dependence).

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## Competing interests:

The authors declare no competing interests.

**Key points**

- The risks and benefits of testosterone replacement therapy (TRT) need to be considered through the physiological mechanisms of proliferation, differentiation, and apoptosis studied in *in vivo* experiments over the past 70 years
- The etiology of prostate cancer reflects a complex interplay between the domains of genetic determinants, endocrine milieu and environmental exposure—aneecdotal cases of cancer in men on TRT demonstrate association, not causation
- Multiple studies all conclude that testosterone level does not correlate with prostate cancer incidence; low (not absent) testosterone levels may actually be a marker of more-aggressive prostate cancer
- It is likely that occult cancers or premalignant cells in hypogonadal men have adequate testosterone levels for healthy homeostasis and TRT does not provide any additional stimulus to growth
- Maintaining a normal testosterone level throughout life may be beneficial from a survival point of view
- To determine whether TRT increases *de novo* tumors, an appropriately powered prospective study would require 10,000 men randomized for 13 years, questions remain as to whether this type of study would be feasible

**Box 1 | Relationship between health status and declines in serum testosterone**

- Drugs reported to reduce testosterone secretion and/or its effects include: opiates, glucocorticoids, gonadotropin-releasing hormone agonists and antagonists, estrogen, spironolactone and ketoconazole
- Clinically significant comorbid diseases include: chronic kidney disease, chronic liver disease, obesity, type 2 diabetes mellitus, rheumatoid arthritis, HIV-associated weight loss and Alzheimer disease
- Medical conditions such as increased BMI, hypertension, hyperlipidemia, diabetes mellitus, hypothyroidism, hemochromatosis, and asthma or chronic obstructive pulmonary disease are also important

Guidelines from the Endocrine Society, published in 2010, advise the avoidance of TRT in patients with prostate cancer, a palpable prostate nodule or induration, or PSA >4 ng/ml (or >3 ng/ml in men at high risk of prostate cancer).<sup>8</sup> The advice from the urological community is less stringent, but still advises caution. The current European Association of Urology guidelines<sup>1</sup> state that, at present, no conclusive evidence exists that TRT increases the risk of prostate cancer or benign prostatic hyperplasia, nor that TRT will convert subclinical prostate cancer to clinically detectable prostate cancer. However, these European guidelines do acknowledge evidence that testosterone can stimulate growth and aggravate symptoms in some men with locally advanced and metastatic prostate cancer.

As we study the safety of TRT, we need to consider the relationship between different serum testosterone levels and intraprostatic androgen receptor fluxes, signaling efficiency and downstream physiological responses. The concepts of ‘threshold androgen level’ or ‘saturation of androgen receptors’ should result in clear biological differences when considering testosterone replacement in the castrate versus the noncastrate hypogonadal, the eugonadal, or the suprphysiological state (Box 2). This review will re-examine the decades of experimentation that addressed androgen action within the prostate to guide our insights into understanding the risks

**Box 2 | Reference ranges for serum testosterone levels**

These values are approximations only and reference range levels will vary between different laboratories.

- Castrate level: <2 nmol/l
- Noncastrate hypogonadal: 2–10.4 nmol/l\*
- Eugonadal: 10.4–28 nmol/l\*
- Suprphysiological: >28 nmol/l

\*The exact cut-off for ‘hypogonadal’ in an individual male may vary between 6.9 nmol/l and 10.4 nmol/l.

associated with TRT in general, or in a particular patient with prostate cancer. We will explain the normal homeostatic mechanisms in prostate physiology and discuss the influence of differing levels of endogenous testosterone as well as TRT on prostate cancer development, progression and regression.

**Threshold implications for TRT safety  
A historical perspective**

Much of the science behind our understanding of TRT is not new and past science should always be reassessed to gain new insights. To quote Ambrose Bierce (1842–1914): “there is nothing new under the sun, but there are lots of old things we don’t know”. Knowledge relating to the cellular effects of androgens on the prostate began as far back as the 1890s.<sup>9</sup> In 1930, Moore *et al.*<sup>10,11</sup> demonstrated that androgen administration induces cytological changes characteristic of secretory activity in the accessory sex tissues of castrated animals. In a 1942 study of the regulation of androgen-induced cellular proliferation, Burkhart<sup>12</sup> measured the mitotic activity in the prostate and seminal vesicles of castrated rats after the injection of increasing doses of testosterone propionate. She demonstrated a wave of mitotic activity in the prostates of rats castrated 40 days previously by even small amounts of androgen administration. Between 1947 and 1967, Huggins and colleagues<sup>6,7,13</sup> described the decline of biochemical processes that occur after castration. Taken together, these observations resulted in the understanding of a critically important phenomenon: that androgen sensitivity (proliferation of prostate cells) and androgen dependence (apoptosis) may be mutually exclusive aspects of prostate glandular homeostasis.

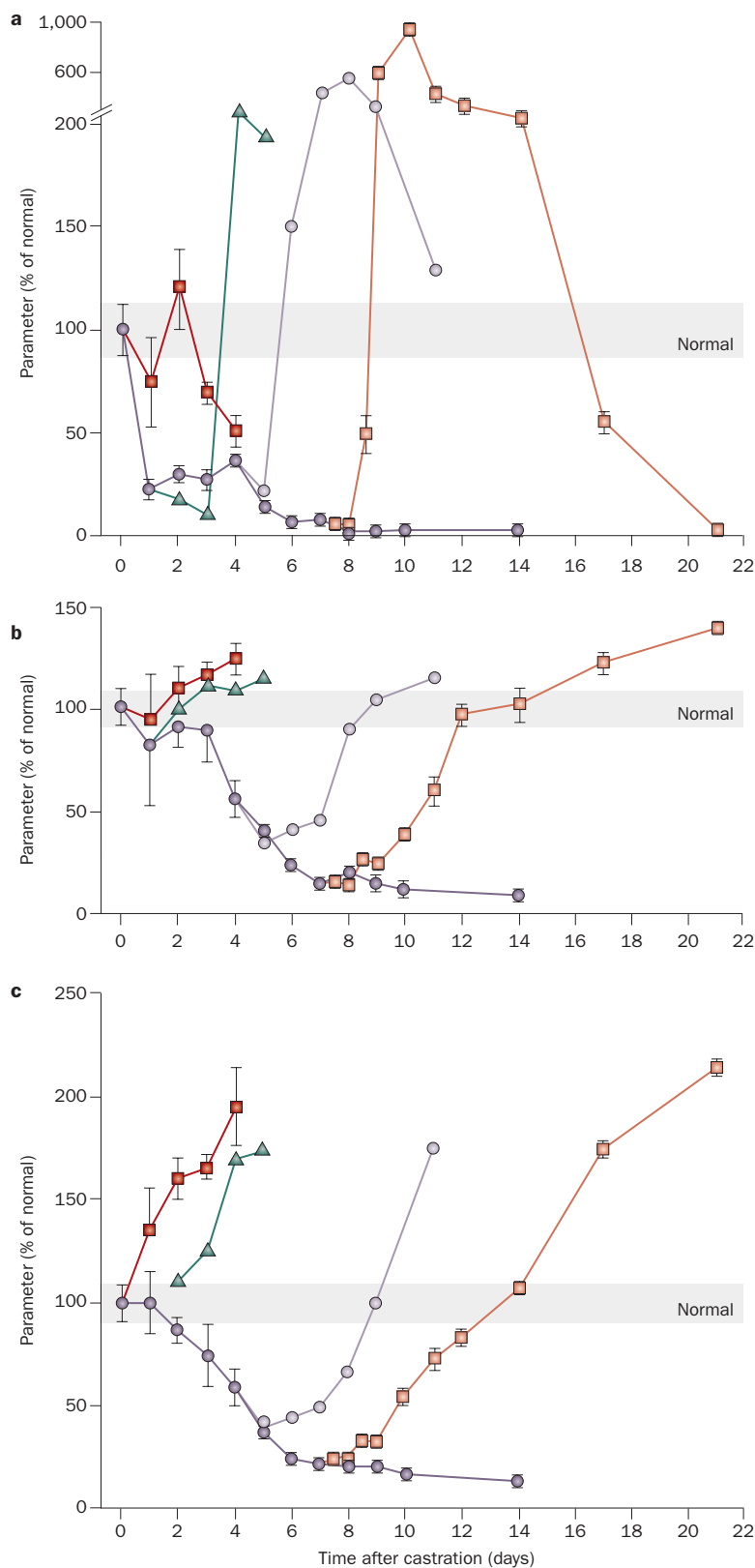
Investigators in the 1960s studied DNA synthesis to assess the effect of androgens on prostate cells. Sheppard *et al.*<sup>14</sup> measured a transient, but dose-dependent, increase in the levels of RNA, DNA, and protein of the ventral rat prostate, levator ani and seminal vesicles after the administration of androgens. In the first suggestion of a ‘threshold effect’, Coffey *et al.*<sup>15</sup> demonstrated that the stimulatory action of androgens on DNA synthesis of prostatic cells was limited at some point in previously castrated rats, beyond which increasing androgen administration had no further effect. Furthermore, administering suprphysiological doses of androgen had little stimulant effect on DNA synthesis in prostates of rats with normal testicular function, suggesting a situation in which androgen receptors are saturated. Lesser and

**Figure 1** | Response of rat prostate to 5 $\alpha$ -dihydrotestosterone administration. Groups of three to seven rats were castrated on day 0 and animals were left untreated (dark purple circles), or treated with daily doses of dihydrotestosterone commenced immediately (dark red squares), or 1 day (green triangles), 4 days (light purple circles) or 7 days (light red squares) after castration. The values for normal rats are shown at zero timepoints.

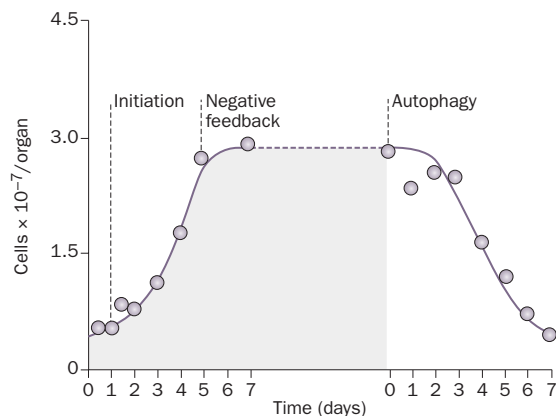
**a** | Rate of DNA synthesis; **b** | number of nuclei per prostate; **c** | prostatic wet weight. Treatment with 5 $\alpha$ -dihydrotestosterone results in threshold response in proliferative parameters and dose–response in differentiation (secretory) activity. Error bars, mean  $\pm$  SEM. Permission obtained from The Biochemical Society © Lesser, B. & Bruchovsky, N. *Biochem. J.* **142**, 429–431 (1974).

Bruchovsky<sup>16</sup> confirmed these findings in 1973, discovering large, but transitory, elevations in DNA polymerase activity of prostate extracts as a result of daily dihydrotestosterone treatment of castrated rats; however, with no effect of dihydrotestosterone administration observed in normal intact rats. In another elegant study, Lesser and Bruchovsky<sup>17</sup> demonstrated that dihydrotestosterone administration stimulated DNA synthesis in rat prostates, which contained fewer than the normal number of nuclei 4 days or 7 days after castration, but no marked effects on prostates of normal animals or those that had been castrated within 24 h, in which the number of nuclei had not yet fallen below normal (Figure 1). This study provided further evidence of a saturation or threshold phenomenon in response to testosterone administration.

These studies of homeostatic constraint mechanisms, as applied to the tightly coordinated anabolic and catabolic processes that are activated or potentiated by steroid hormones, provide an insight into the interaction between serum androgens and prostatic biology. In the Lesser and Bruchovsky experiment,<sup>17</sup> once the normal number of cells had regenerated, some form of negative feedback occurred and the proliferative activity switched off. The wet weight of tissue, however, continued to increase in the presence of dihydrotestosterone, suggesting that differentiation (stimulation of cellular secretory activity by transcriptional or post-transcriptional processes) was ongoing despite the curtailing of proliferation (DNA synthesis and production of nuclei). Organ homeostasis seems to be achieved by balancing the functions of two cellular constraint mechanisms, one that initiates DNA synthesis and cell proliferation, the other causing suppression of these processes. These study authors concluded that the response of rat prostate to changes in hormonal status of the animal occurs in three phases (Figure 2).<sup>18</sup> In the first, DNA synthesis and cell proliferation are initiated by the administration of androgen when the number of cells in the prostate gland is below normal. In the second phase, once the number of cells is restored to normal, DNA synthesis is curbed and cell proliferation is decreased, although secretory activity continues to be stimulated by the presence of androgens.<sup>17,18</sup> Clearly, this negative feedback is not solely the result of the loss of ability to respond to hormone, as hormone can stimulate



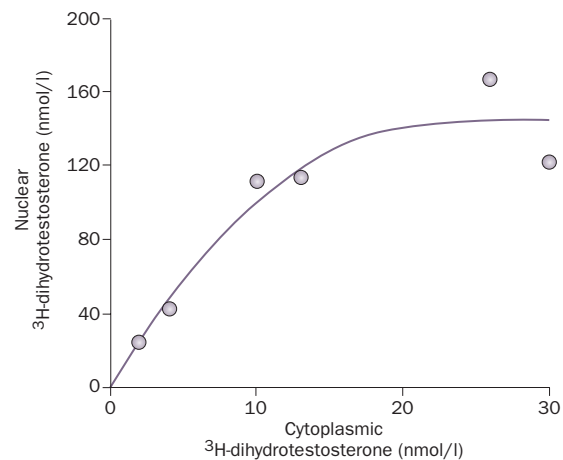
secretory activity in the absence of cell division. Thus, nonsteroidal factors likely contribute to achieving homeostasis within the prostate cell. The third phase is apoptosis or autophagy of prostate cells induced by the withdrawal of androgens from the cellular milieu.



**Figure 2** | Basic homeostatic responses of a hormone-sensitive organ. The shaded area under the curve indicates the period of hormone replacement administered to groups of mice castrated 7 days prior. Cessation of treatment (recastration) is followed by a reduction in the number of cells per prostate to the basal level. Three basic responses are observed: initiation of DNA synthesis with cell proliferation, negative feedback and autophagy. Reprinted from Bruchofsky, N. *et al. Vitam. Horm.* **33**, 61–102 (1975), with permission from Elsevier ©.

In considering testosterone deficiency and replacement in humans, we need to also understand the differences between serum and intraprostatic levels of androgens. Testosterone entering prostate tissue is rapidly reduced to dihydrotestosterone by 5 $\alpha$ -reductase and, as a result, the dihydrotestosterone concentration in the prostate is approximately 10 times higher than that of testosterone. Both the formation of dihydrotestosterone and the balance between synthesis and metabolism are critical to maintain constantly the required level of androgen in the prostate cell. In low levels of serum testosterone, the cell upregulates 5 $\alpha$ -reductase activity to maximize intranuclear dihydrotestosterone levels.<sup>19</sup> In an animal study, 2 weeks after castration, male Sprague Dawley rats were given testosterone implants of varying sizes in the presence or absence of finasteride. The presence of active 5 $\alpha$ -reductase enzyme enabled the prostate cell to concentrate dihydrotestosterone and proliferate even at very low levels of serum testosterone; this effect is negated in the presence of a 5 $\alpha$ -reductase inhibitor (finasteride). A key role of 5 $\alpha$ -reductase in the prostate is, therefore, to enhance growth at low circulating testosterone concentrations. Experiments in rats reveal that the threshold of androgen concentration that has to be exceeded for induction of prostate growth is two to three times lower for dihydrotestosterone than for testosterone.<sup>20</sup>

The precise level of dihydrotestosterone necessary to restore the normal weight of the prostate in a castrated animal would define a 'threshold'. As shown in Figure 3, small changes in the cytoplasmic concentration of dihydrotestosterone in rats, within the physiological range of 0–10 nmol/l, will produce much greater changes in the nuclear concentration of dihydrotestosterone, spanning the range of 0–120 nmol/l.<sup>18</sup> In the castrated animal,



**Figure 3** | Relationship between nuclear and cytoplasmic molar concentrations of dihydrotestosterone in rat prostate. Changes in the cytoplasmic concentration between 0 nmol/l and 10 nmol/l results in steady increases in nuclear dihydrotestosterone concentration to a ceiling of approximately 150 nmol/l. Further increases in plasma testosterone or dihydrotestosterone, and subsequent cytoplasmic concentration do not increase intranuclear levels (saturation point) and thus will have little further effect on the prostate cell. Reprinted from Bruchofsky, N. *et al. Vitam. Horm.* **33**, 61–102 (1975), with permission from Elsevier ©.

circulating levels of testosterone are  $\leq 10\%$  of normal, which would not sustain a cytoplasmic concentration of dihydrotestosterone  $> 1\text{--}2$  nmol/l. This decline would be accompanied by a decline in the concentration of nuclear dihydrotestosterone to a level insufficient to maintain the cell in a state of full differentiation. In the noncastrated animal receiving increasing doses of androgen, once the plasma androgen concentration exceeds a threshold somewhere between 10 nmol/l and 20 nmol/l, the prostate cell will no longer respond to small fluctuations in circulating levels of testosterone, and regulation of the nuclear concentration of dihydrotestosterone may require substrate changes  $> 100$ -fold. Above a certain threshold level, the prostate cell is, clearly, well adapted to provide itself with intranuclear androgen despite fluxes in the supply of hormone, and increasing availability of androgens will not result in increased intranuclear levels of active androgen.

### Implications for a clinical setting

The early, well-designed *in vivo* studies formed the basis of the concept that testosterone has a threshold or saturation level in all types of androgen-dependent prostate cells. That is, the stimulatory effects of androgens on the prostate reach a point within physiological serum levels above which they no longer have any proliferative effect and serum levels of testosterone and dihydrotestosterone can decrease substantially in both the eugonadal and hypogonadal states without affecting the amount of androgen within the nucleus of the cell. At a certain threshold level ('castrate' level), the intranuclear level of androgen will begin to decrease and the appropriate

physiological changes will be triggered. Questions remain as to whether results from experimental studies in the rat can be extrapolated to the situation in humans. Is the human prostate subject to the same homeostatic constraints as have been so well defined in animal experiments, and if so, what is the threshold or saturation level for maximal intracellular androgens and physiological responses in man?

This 'saturation theory' was first suggested in the clinical setting by Fowler and Whitmore<sup>21</sup> who concluded a quarter century ago that "normal endogenous testosterone levels may be sufficient to cause near maximal stimulation of prostatic tumors". In 2009, Morgentaler and Traish<sup>22</sup> proposed an upper limit to the stimulating activity of androgens on the growth of prostate cancer, owing to the fact that maximal androgen-receptor binding occurs at a relatively low serum testosterone level. In 2011, Morgentaler and colleagues<sup>23</sup> reported on a series of 13 testosterone-deficient men who received TRT for a median of 2.5 years while undergoing active surveillance for low-risk prostate cancer. The fact that none of the men demonstrated increases in PSA levels, local or systemic progression, and 54% of the follow-up biopsies showed no cancer, is strong support for the saturation concept.

The actual value for testosterone threshold level was studied recently by Khera and co-workers<sup>24</sup> in a review of 461 hypogonadal men enrolled in a national registry. At baseline, a statistically significant difference in serum PSA level between men with testosterone levels above versus below 200 ng/dl (approximately 6.94 nmol/l) was observed. When these men were treated with TRT, serum PSA level increased substantially (by 0.32 ng/ml) in patients with baseline serum testosterone level <250 ng/dl (approximately <8.68 nmol/l), while those with serum testosterone levels >250 ng/dl (approximately >8.68 nmol/l) at baseline had no change in PSA, suggesting these individuals had already exceeded a testosterone threshold or saturation point. As discussed in the next section, this finding would explain the paradox of cancer regression occurring with castration levels of testosterone, while testosterone replacement in hypogonadal men is not a cause of prostate cancer development or growth.

## Testosterone and prostate cancer

### Effects of endogenous serum testosterone

Although exposure of the prostate to androgens has always been considered a prerequisite for later development of prostate cancer, not all data from published prospective studies support this hypothesis.<sup>25</sup> As a disease of the aging male, prostate cancer actually presents at a biological time when testosterone levels are in decline, which would be considered counterintuitive if testosterone truly promoted carcinogenesis in a dose-response fashion, in which case one would actually expect young men with high levels of testosterone and microscopic disease to undergo more-rapid progression of prostate cancer.<sup>26</sup> Obviously, factors other than androgen levels are implicated in the development of

cancer, such as genetic or epigenetic determinants and environmental exposures.

Banach-Petrosky and colleagues<sup>27</sup> studied the relationship between androgen levels and prostate tumorigenesis in a genetically engineered mouse model of human cancer (*Nkx3.1;Pten* mutant mouse). The researchers showed that prolonged exposure to low levels of testosterone ('hypogonadal') promoted a more-aggressive phenotype of cancer and accelerated tumor progression than normal or castrate levels of testosterone. In a 2008 review of 18 prospective studies, the study authors found no association between the risk of prostate cancer and serum concentrations of sex hormones including: testosterone, free testosterone, dihydrotestosterone, dehydroepiandrosterone sulfate, androstenedione, androstanediol glucuronide, estradiol, or free estradiol.<sup>28</sup> In a large, longitudinal, pooled prospective study of >200,000 men from Finland, Norway, and Sweden, a modest, yet statistically significant, decrease in risk of prostate cancer was reported among men with increased levels of circulating testosterone.<sup>29</sup> In a study of a large prospective cohort of 10,049 men, Raynaud *et al.*<sup>30</sup> suggested that high levels of testosterone (within the reference range of androgens, estrogens and adrenal androgens) decrease the risk of aggressive prostate cancer. Sher and co-workers<sup>31</sup> found no relationship between circulating steroid hormone levels and Gleason score in 539 patients with prostate cancer, while others report a trend for high levels of testosterone and adrenal androgens being associated with a reduced risk of aggressive prostate cancer.<sup>32</sup>

In a review of 673 consecutive men undergoing radical prostatectomy at a single institute, 181 men were found to be hypogonadal on the morning of the day before their surgery.<sup>33</sup> Both high-grade disease and seminal vesicle invasion were markedly increased in men with low testosterone levels compared with men with normal testosterone levels. Multivariate analysis did not find mild to moderate hypogonadism (defined as approximately 3.4–10.4 nmol/l or 100–300 ng/dl) to be an independent predictor of high-risk pathological outcomes; however, a testosterone level <3.47 nmol/l (<100 ng/dl) did predict for seminal vesicle involvement with an odds ratio of 3.11.<sup>32</sup> Several other cross-sectional studies have found that a low testosterone level at the time of prostate cancer diagnosis is associated with more-aggressive disease.<sup>34–36</sup> Moreover, testosterone may be suppressed in men with prostate cancer,<sup>30,37</sup> and men who undergo radical prostatectomy for organ-confined disease experience an increase in their serum testosterone level after the surgery.<sup>38</sup>

Some evidence does exist to the contrary.<sup>39,40</sup> In the prospective Physicians' Health study,<sup>40</sup> the study authors found that high levels of circulating testosterone and low levels of sex hormone-binding globulin (both within normal endogenous ranges) were associated with an increased risk of prostate cancer, but no association between circulating levels of dihydrotestosterone and prostate cancer risk. The clinical implications of this finding are uncertain because of the statistical method by which the study authors arrived at their conclusion.<sup>30,41</sup>

Furthermore, serial serum androgen measurements were examined from 794 individuals participating in the Baltimore Longitudinal Study of Aging.<sup>42</sup> The study authors found that high levels of calculated serum levels of free testosterone were associated with an increased risk of prostate cancer, and an increased risk of aggressive prostate cancer among older men. In one further study, a substantially higher concentration of dihydrotestosterone and testosterone was found in tissue samples taken from patients with prostate cancer ( $n = 75$ ) than those with benign prostatic hyperplasia ( $n = 51$ ).<sup>43</sup> These researchers did not, however, study the relationship between tissue androgen levels and the aggressiveness of the cancers.

These contradictory observations herald a word of caution: serum levels of androgens do not reflect the intraprostatic hormonal environment, signaling pathways, transcription activity, and downstream events.<sup>43</sup> We evidently need more information to explain the complex relationship between serum and tissue levels of endogenous androgens and the risk of prostate cancer.

### The protective effect of testosterone

Why is there no clear relationship to prostate cancer above a certain threshold of serum androgen level? The evolutionary pathway of human prostate cancer development is complex and likely involves genetic alterations, polymorphisms, abnormal growth response, decreased or diminished apoptosis, and the combined effects of all of these factors, under some form of androgenic control.

#### Genetic factors

The androgen receptor gene is located on the X-chromosome so that only one allele is responsible for gene expression in males. Results from population studies have indicated that this region is highly variable and that normal men may have anywhere between 11 and 31 CAG repeats in the androgen receptor gene, with a corresponding number of glutamines in the androgen receptor protein.<sup>45</sup> Findings from *in vitro* studies demonstrate that androgen-induced transcriptional activity is dependent on the number of CAG repeats in exon 1, which encodes the glutamine residues located in the middle of the receptor's transactivation domain.<sup>46</sup> It seems that reduced ligand-induced transcriptional activity is present in androgen receptors with an increased number of CAG repeats, while the deletion of CAG repeat regions results in increased transcriptional activity<sup>47</sup> and risk of developing prostate cancer. Giovannucci *et al.*<sup>48</sup> reported on 587 patients with prostate cancer and 588 individuals without prostate cancer (controls) in the Physicians' Health Study using a nested case-control design.<sup>48</sup> Men with CAG repeat lengths of <19 had a 1.5-fold relative risk of prostate cancer compared with men with a CAG repeat length of >25. The CAG repeat length was also inversely related to prognosis, in that men with shorter lengths had increased incidence of lymph-node positive prostate cancer. Stanford and colleagues,<sup>49</sup> in a study of 281 patients with prostate cancer and 266 healthy individuals as controls, reported that men with <17 GGC repeats have a 1.6-fold relative risk of prostate

cancer compared with men with  $\geq 17$  repeats. They also concluded that some form of interaction between GGC and CAG repeats seems to exist, in that men with both GGC <17 and CAG repeats of <22 were at increased risk of prostate cancer compared with men with longer GGC and CAG repeats.<sup>49</sup> These types of polymorphisms may help to explain discrepancies between serum testosterone levels and the degree of symptoms related to different androgen target tissues, as well as the varying risks of carcinogenesis among men with similar serum testosterone levels.

The *CYP3A5* gene encodes the drug metabolizing cytochrome P450 3A5 protein, an enzyme that is known to metabolize a number of drugs and steroids (for example, nifedipine or progesterone) and to inactivate testosterone by catalyzing the 6 $\beta$ -hydroxylation of testosterone, producing a metabolite that is less biologically active and more-readily eliminated. *CYP3A5* has been identified as an androgen-regulated gene in human prostate tissue. Moilanen and colleagues<sup>50</sup> have shown that the *CYP3A5* promoter region contains an androgen response element and theorized that androgen can induce an autoregulatory feedback loop controlling *CYP3A5*<sup>50</sup> and, thus, prostate cell exposure to androgens. As a result, in the presence of low or absent serum androgens there may actually be more testosterone available at the cellular level, explaining why men with lower testosterone levels may have higher stage and grade of cancer. This hypothesis supports the concept that maintenance of normal androgen levels in the aging male is important.

#### Growth factors

Understanding that carcinogenesis is a multistep process involving hyperplasia, dysplasia and, ultimately, carcinoma is important. In the presence of testosterone and other steroids, other growth factors, including transforming growth factor  $\alpha$ , transforming growth factor  $\beta 1$ , heparin-binding growth factor 2 (also known as basic fibroblast growth factor), vascular endothelial growth factor and insulin-like growth factor I (IGF-I) also have an effect. Initially, many of these growth factors may act in a paracrine manner as part of the epithelial-stromal interaction, but later on, during carcinogenesis, could become involved with autocrine steroidogenesis, which would override the levels that are present in serum. This fundamental change in the mechanism for androgen-stimulated growth was studied in the androgen receptor null nude male mouse model,<sup>51</sup> in which androgen-stimulated growth occurred identically in wild-type nude male mice and those lacking androgen receptor—highly suggestive of a direct autocrine mechanism.

IGF-I has been linked to prostate cancer risk by stimulating androgen-receptor-mediated gene transcription in the absence of exogenous androgen.<sup>52-56</sup> Animal experiments demonstrate that tumor growth can be inhibited by disrupting IGF-I signaling pathways<sup>53</sup> and in a nested case-control study using data derived from the Physicians' Health Study cohort it was demonstrated that increasing plasma levels of IGF-I were directly linked to increasing prostate cancer risk.<sup>52</sup> After

adjusting for IGF-binding proteins (such as IGFBP3), men with a serum PSA level >4 ng/ml in combination with the highest quartile of IGF-I had a 17.5-fold risk of prostate cancer.<sup>52</sup> Autocrine upregulation of IGF-I may become more important as we further understand the association between diabetes mellitus and prostate cancer risk; an area of extensive research as insulin and insulin receptors may have a role in progression to castration-resistant cancer.

Many research groups are studying the effects of growth hormone and/or sex steroid administration on the local tissue production of IGF-binding proteins and are trying to determine whether they influence the autocrine or paracrine actions of IGF-I. Munzer *et al.*<sup>54</sup> demonstrated that hormone administration increased IGFBP3 and IGFBP5 levels. IGFBP3 is the most abundant circulating IGF-binding protein that inhibits cell growth and induces apoptosis by both IGF-I-dependent and IGF-I-independent pathways in many cancers, including prostate cancer. Peng and colleagues<sup>55</sup> explored the relationship between IGFBP3 and androgens *in vitro* in human LNCaP cells. The researchers demonstrated that the *IGFBP3* promoter sequence responded to androgen treatment and identified a putative androgen response element in the *IGFBP3* promoter region. Increasing doses of androgen, particularly when combined with calcitriol, led to increased inhibition of LNCaP cell growth. This stimulation by androgens of an antiproliferative and proapoptotic protein seems to be paradoxical; however, it may explain why normal androgen levels have a putative role in suppressing carcinogenesis and tumor promotion.<sup>55</sup> In other *in vitro* experiments, secreted PSA was shown to catalyze IGFBP3 fragmentation.<sup>56</sup> After PSA induced cleavage, IGFBP3 has a markedly reduced IGF-I binding affinity, thereby increasing the amount of IGF-I available to interact with its cell surface receptor. Basic and clinical data, therefore, support the concept that increasing plasma IGF-I levels are associated with a substantially increased prostate cancer risk in men and that this effect can be blunted by androgen-stimulated production of IGF-binding proteins.

The relationship between obesity and increased risk of death from prostate cancer may be explained by altered serum concentrations of testosterone, estradiol, insulin, IGF-I and leptin. As obese men have low free testosterone levels and increased serum estradiol levels, owing to peripheral conversion of testosterone by aromatase in adipocytes, it is possible that an altered testosterone:estradiol ratio may enhance growth of prostate cancer. In one study, mice with genetically-altered aromatase or estrogen receptor expression had increased proliferation of prostate cells when estradiol was combined with testosterone.<sup>57</sup> Obese men have insulin resistance and high insulin levels that inhibit production of IGFBP1 and 2, which in turn increase bioactive IGF-I concentrations. Study results suggest that high insulin states may be related to increased prostate cancer risk, while low insulin states may be protective.<sup>58,59</sup> Leptin is a polypeptide hormone produced by adipocytes and findings have indicated that human prostate cancer may

express leptin receptors,<sup>60</sup> generating the hypothesis that leptin may mediate some of the effects of obesity on prostate cancer.

## Biological influence of TRT

### The castrate state

The clinical situation of medical castration may provide insight into the biological effects of testosterone above and below a 'threshold point' in men. Injection of a luteinizing-hormone-releasing hormone (LHRH, also known as gonadotropin-releasing hormone) agonist results in a transient increase, or flare, in testosterone for 1–2 weeks before dropping to castrate levels. The concern with this initial 'flare' is that the acute increase in serum testosterone level may lead to a rapid progression of prostate cancer, resulting in urinary retention, spinal cord compression, or exacerbation of bony pain.<sup>61</sup> This type of complication is actually very rare and may reflect unfortunate coincidences owing to the natural history of advanced disease rather than androgen-induced cellular proliferation. In addition, PSA, known to correlate well with progression of prostate cancer,<sup>62</sup> does not show notable increases in levels after patients with prostate cancer commence treatment with an LHRH agonist.<sup>63,64</sup> The lack of clinical or biochemical changes in millions of men on LHRH agonists would support the lack of a dose–response to the flare in testosterone within the normal serum testosterone range and saturated receptors in prostate cells. After testosterone has been suppressed into the castrate range, subsequent injections of LHRH agonist cause 'mini flares', which do not cause detectable physiological changes, possibly because they are not high enough to reach the 'threshold' level.

Experience with intermittent androgen suppression provides another insight into prostatic cellular responses as testosterone increases out of the castrate range, past the 'threshold level', and into the normal range during the off-treatment cycle. The basic theory of intermittent androgen suppression is that the replacement of androgens, even in small amounts, would have a conditioning effect on surviving cells enabling these cells to conserve or regain desirable traits of differentiation and forestall tumor progression. In clinical trials, during the off-treatment phase, the serum PSA level also increases alongside the rise in testosterone levels; however, a delay in the increase of PSA until testosterone levels pass a certain threshold is almost always observed.<sup>65</sup> The pattern of PSA response to rising testosterone levels does vary between individuals, and even between treatment cycles for each individual, which brings to light the vexatious question of whether androgen-sensitive PSA secretory activity is an appropriate surrogate for cellular proliferation in the presence of androgens. If the human situation recapitulates the observations seen in castrate mice, then both cellular processes occur up to the point of negative feedback, and then secretory activity may or may not continue while proliferation slows or ceases, depending on the biology of the particular cancer.

Testosterone recovery during intermittent androgen suppression may influence organs other than the prostate

in more of a dose–response manner. Several researchers have reported that the return of serum testosterone from castrate into the hypogonadal, and eventually the eugonadal, state is associated with a marked improvement of general well-being, recovery of anemia, and urinary and sexual function.<sup>66</sup> The degree of these and other measures continuously improve in proportion to the level of testosterone recovery.

#### The hypogonadal (noncastrate) state

In terms of benign tissue, administering testosterone has no effect on PSA serum level or prostate volume in normal men.<sup>67,68</sup> In hypogonadal men, PSA levels are often low, which normalize after TRT, but do not continue to increase with continued therapy,<sup>21,69</sup> and prostate volume returns essentially to that of eugonadal men but no higher.<sup>70</sup> In Swerdloff's study<sup>71</sup> of 166 hypogonadal men, 3 years of TRT resulted in a mean serum PSA increase of 0.37 ng/ml and a 1.8% incidence of biopsy-proven prostate cancer (three men). The study authors noted that the PSA level increase occurred in the first 6 months of therapy and remained stable afterwards, supporting the concept of 'saturation'. Khera and co-workers<sup>24</sup> studied TRT in men with different levels of hypogonadism and identified a testosterone threshold level of approximately 8.68 nmol/l (about 250 ng/dl) for PSA response; that is men with testosterone level >8.68 nmol/l no longer had increases in serum PSA level.<sup>24</sup>

In a prospective study, Marks *et al.*<sup>44</sup> demonstrated that exogenous testosterone administration does not substantially increase intraprostatic androgen levels in hypogonadal noncastrate men. A series of 44 hypogonadal men (testosterone level <10.41 nmol/l or 300 ng/dl) were randomized to receive either testosterone enanthate ( $n = 21$ ) or placebo ( $n = 20$ ) for 6 months. All had a negative 14-core biopsy and 41 men completed treatment and underwent post-treatment biopsy with detailed histology, biomarker studies for cell proliferation (antigen KI-67), stromal:epithelial ratio, androgen receptor, and angiogenesis (CD34), and gene-expression profiling using a microarray for prostate complementary DNAs. The patient groups were comparable at baseline and results showed that, despite increased serum testosterone and dihydrotestosterone levels in men receiving TRT, intraprostatic levels of testosterone and dihydrotestosterone did not change from baseline in either group. PSA, prostate volume, tissue biomarkers, and histological indices for percentage atrophy and inflammation were also unchanged and gene expression profiles of prostate epithelial cells before and after testosterone replacement showed no treatment effect. Findings from this elegant study strongly suggest that the prostate is buffered against a wide range of circulating testosterone levels above a minimum threshold or saturation point.

Administering testosterone to a large population of men will undoubtedly uncover some incidences of cancer,<sup>72–77</sup> as the background prevalence of subclinical disease is high. A number of papers from the past decade have sought to clarify the relationship between exogenous testosterone and prostate cancer<sup>30,40,78–86</sup> and all study authors found

no evidence to date of testosterone having a causative role in initiating *de novo* prostate cancer or promoting occult prostate cancer. In addition, no evidence has been found that TRT resulted in an increased Gleason score for prostate cancer detected in men receiving TRT, or any consistent negative effect of TRT on serum PSA levels.<sup>87</sup> The mode or duration of testosterone administration had no influence on increasing the risk of prostate cancer.<sup>86,88–102</sup> In a study of 2,200 men,<sup>100</sup> 1,500 with a biochemical diagnosis of hypogonadism were treated and followed up with digital rectal examinations and serum PSA measurements on a 3–6 monthly basis for up to 15 years. 10 cases of prostate cancer, all clinically localized, were detected over this time frame of 2,100 man-years of testosterone treatment (0.48% cases per annum). The incidence of prostate cancer in men treated with testosterone was identified as 1.1% by Rhoden and Morgentaler,<sup>41</sup> and a meta-analysis by Calof *et al.*<sup>103</sup> of 19 randomized placebo-controlled trials of TRT found no statistically significant difference in the rate of prostate cancer between hypogonadal men receiving TRT or placebo.

Rhoden *et al.* investigated the relationship between TRT and prostate cancer in men at increased risk of prostate cancer.<sup>101</sup> The study authors examined 75 hypogonadal men who underwent prostate biopsy before initiation of TRT, of whom 20 had high-grade prostatic intraepithelial neoplasia (PIN), a possible precursor to prostate cancer,<sup>104,105</sup> whereas the remaining 55 had negative biopsies. At the end of 1 year of TRT, neither a substantial increase in serum PSA level, nor an increased risk of cancer between men with PIN-positive biopsies and men with PIN-negative biopsies was observed. Rhoden and colleagues<sup>101</sup> suggested that presence of PIN should not be a contraindication to TRT—although this view is not yet widely accepted.

Although, individually, none of the aforementioned studies are sufficiently powered to confirm the safety profile of TRT with respect to prostate cancer initiation or progression, they represent a large body of indirect evidence to support the safety of TRT. Interestingly, no researchers have documented even a trend towards an increased risk of prostate cancer associated with administration of exogenous androgen in the hypogonadal man.

The discrepancies between studies reflect that the probability of finding cancer is dependent on the diligence of the search, as well as the population under study, and, thus, supports the importance of prospective trials to ensure the safety of TRT. To demonstrate a 30% difference in incidence of prostate cancer between testosterone-treated and placebo-treated men, a trial of 6,000 men aged 65–80 years, randomized to both placebo and TRT arms, and followed for at least 5 years would be required.<sup>80,81</sup> This study type obviously presents quite an undertaking, but one whose value is underscored by the increasing number of men receiving TRT.

#### Known prostate cancer

Many health-care providers and patients remain concerned about the dangers of TRT in men with active

or treated prostate cancer. In Huggins' original publications,<sup>6,7</sup> of the eight men with advanced prostate, exogenous androgen was administered to only three men. Data are provided for only two of these men, one of whom had previously been castrated. The progression of disease for the single man who received androgen injections before castration was inferred by measuring serum acid phosphatase, which varied considerably both during and after administration of exogenous androgen. Thus, the basis for the perpetuated belief that testosterone activates prostate cancer in untreated men derives from the equivocal acid phosphatase levels of this one individual. Perhaps most notable in this regard is a retrospective review on the response of 67 men with metastatic prostate cancer to exogenous testosterone, published in 1981.<sup>21</sup> In this study, Fowler and Whitmore<sup>21</sup> did report adverse outcomes in previously-castrated men given exogenous testosterone, suggesting that androgen-sensitive cells remain after castration. They also described the influence of testosterone administration to four men who had not undergone prior androgen deprivation therapy. An unfavorable response was seen within 1 month from testosterone treatment in only one patient, suggesting that the other cancers were already in an androgen milieu 'above a saturation point'. A confounding issue in all these studies is the problem of distinguishing an 'unfavorable response' from symptoms owing to the natural history of metastatic prostate cancer.

Contrary to the decade of literature that seemed to confirm Huggins' observation that high serum testosterone levels lead to progression of prostate cancer,<sup>106</sup> the belief has been challenged by several overlooked biological and medical publications from the second half of the 20<sup>th</sup> century. These early clinical reports from the 1950s and 1960s failed to show a deleterious effect with testosterone administration in men with prostate cancer.<sup>107-109</sup> Brendler *et al.*<sup>107</sup> found an exacerbation of symptoms in only a fraction of patients administered testosterone. This finding led Rawson, in his 1953 review of the hormonal control of neoplastic growth, to suggest that investigators should search for an alternative mechanism for the "established therapeutic effects of castration on prostate cancer".<sup>110</sup> Pearson's discussion of Huggins' paper in 1957 included a case of a previously untreated patient with advanced prostate cancer with severe bone pain from osseous metastases, who was treated with daily injections of testosterone.<sup>109</sup> The patient found a prompt relief of pain after testosterone administration, which continued for a further 9 months of treatment. Prout and Brewer<sup>108</sup> examined the effect of daily testosterone injections in 26 men with locally advanced prostate cancer who had not undergone castration or other hormonal treatment. They found that many patients experienced an improved sense of well-being and a diminution in pain with TRT. Furthermore, the researchers reported that the acid phosphatase response to testosterone injection was extremely variable, which again casted doubt on Huggins' original conclusions. These studies, which were carried out over 50 years ago, show that testosterone

administration in men with locally advanced disease did not lead to rapid cancer progression and support the concept of a saturation point in prostatic cells, both normal or malignant.

Several case studies examine TRT in the hypogonadal man previously treated for prostate cancer with radical prostatectomy or brachytherapy.<sup>111-115</sup> Kaufman and Graydon<sup>111</sup> treated seven patients for hypogonadism, all of whom had previously undergone radical prostatectomy with a postoperative PSA <0.1 ng/ml. They reported no local or metastatic recurrence during a follow-up of 12 years. In a similar group of 10 patients, Agarwal and Oefelein<sup>112</sup> used TRT over a median duration of 19 months. No PSA recurrence occurred and patients experienced an actual improvement in their quality of life. A 2009 retrospective review of 57 patients given TRT after radical prostatectomy documented no biochemical recurrence at a mean follow-up of 13 months.<sup>114</sup>

TRT after brachytherapy has been suggested to be more risky than after radical prostatectomy given the presence of residual prostate tissue with this approach.<sup>115</sup> However, the only study investigating TRT in men undergoing brachytherapy did not come to the same conclusion.<sup>113</sup> Sarosdy<sup>113</sup> treated 31 hypogonadal patients with TRT after brachytherapy, nearly one-third of whom had been treated for intermediate or high-risk disease, for anywhere from 0.5 to 4.5 years after radioactive seed implantation, for a median duration of 4.5 years. Median follow-up was 5 years, and no patient discontinued TRT owing to disease recurrence or documented disease progression.

Strong evidence from a small, but important, trial supports the hypothesis that raising serum testosterone levels from the hypogonadal to the eugonadal level has no biological influence on established prostate cancer. Morgentaler and colleagues<sup>23</sup> administered TRT for a mean of 2.5 years to 13 hypogonadal men who were under active surveillance for low-risk prostate cancer. They noted no adverse progressive event in any patient and, in fact, only one man underwent a radical prostatectomy and his surgical pathology did not show any sign of high-risk disease.

### Nonprostate tissues

In nonprostatic androgen-dependent tissues, evidence exists that the threshold or saturation effect remains biologically relevant, but at a higher level of serum testosterone than for prostate tissue. Zitzman *et al.*<sup>116</sup> observed that the loss of libido and vigor became prominent when serum testosterone level dropped below 15 nmol/l, while hot flushes and erectile dysfunction did not become notable until serum testosterone levels were below 8 nmol/l. Wu and colleagues<sup>117</sup> reported a 'threshold' for erectile dysfunction at 8.5 nmol/l serum testosterone level. Bhasin and colleagues<sup>67</sup> demonstrated that androgenic activity reaches tissue-specific plateaus at different levels, with some effects only seen when serum testosterone level is increased to supraphysiological levels. As a result, testosterone replacement in hypogonadal men will have quite variable effects between individuals. Some of

**Box 3** | Examples of research questions for TRT and the prostate

- Interactions between testosterone and growth hormone and IGF-I need to be further elucidated. Should IGF-I be routinely measured in elderly men with LOH syndrome? The putative relationship between IGFs, testosterone and prostate biology and physiology, and development of BPH and cancer should be investigated as well as the relationship between adult-onset growth hormone deficiency and LOH and prostate disease.
- What can be considered the 'normal' level of testosterone in a given individual? Is low testosterone levels a pathological condition or response to an associated underlying condition? Research is needed to determine whether changes in testosterone levels or the rate of change of testosterone levels over time is important. Additionally, when replacing testosterone, the testosterone level needed for optimal therapeutic effect on symptomatic patients is unclear.
- At what level of serum testosterone replacement does PSA production switch from reflecting proliferation and differentiation, to reflecting differentiation alone? Moreover, do proliferation and differentiation have different saturation points?
- How do threshold or saturation effects differ for BPH and prostate cancer? Measurement of polymorphisms in individuals could help predict the target testosterone level required in a given man.
- Is TRT dangerous in the elderly man or does it improve survival? Moreover, is there a point at which risks of TRT outweigh the benefits of this therapy?
- How does testosterone level influence the operating characteristics of PSA in screening for prostate cancer? Is serum testosterone level a predictor of prostate cancer susceptibility, incidence, or aggressiveness? Should testosterone be measured at the same time as PSA determination and should it be a parameter to be considered in treatment-decision algorithms?

Abbreviations: BPH, benign prostatic hyperplasia; IGF-I, insulin-like growth factor I; LOH, late-onset hypogonadism; TRT, testosterone replacement therapy.

these differences may be accounted for by the biology of the involved tissue, CAG repeat length polymorphisms of the androgen receptor gene,<sup>118</sup> androgen receptor co-activators and co-repressors (not expressed equally in all androgen-sensitive tissues), and by the duration of the hypogonadal state (which may cause recruitment of more androgen receptors). Varying the testosterone level from low-normal to high-normal levels does not change libido, mood, sexual function, cognition and bone density ('androgenic effects'). That is, a threshold or plateau in response occurs once testosterone levels increase through the low-normal range.

Some information suggests that overall survival may also be linked to testosterone levels in aging men. In Shores' study of 858 male veterans in the Puget Sound area—after controlling for age, illness and other comorbidities—the risk of dying over the course of 8 years of follow-up decreased with increasing levels of serum testosterone.<sup>119</sup> Laughlin *et al.*<sup>120</sup> conducted a prospective, population-based study of 794 men, aged 50–91 years who had low testosterone levels at baseline. After approximately 12 years, 538 deaths had occurred. Men whose serum testosterone levels in the lowest quartile (<8.36 nmol/l) were 40% more likely to die than those with higher levels, independent of age, adiposity, and lifestyle. In case-specific analysis, low serum testosterone level predicted increased risk of death from cardiovascular and respiratory disease, but was not related to cancer death. Results were similar for bioavailable testosterone.<sup>120</sup>

**Conclusions**

A question that arises when considering the equivocal data regarding testosterone effect on the prostate is: how can TRT have a positive effect on a man's sense of well-being (anabolic, steroidal) while not stimulating continual proliferation of the prostate (androgenic), and why is this influence of testosterone so variable between different men with prostate cancer? Answers may be found in some of the older literature. Huggins and Hodges landmark, and later Nobel Prize winning work,<sup>6,7</sup> showed that prostate cancer ceases to grow in the absence of testosterone and extrapolated from this finding that testosterone administration would lead to enhanced growth. However, multiple studies from the past 60–70 years that examined the interaction between sex steroids and prostate tissue at different degrees of prostate involution suggest that testosterone, whether in serum or intracellular, stimulates proliferation of prostate cells up to a point, beyond which differentiation continues, but cellular growth ceases. The sensitivity of an individual to varying levels of testosterone is also influenced by his genetic makeup, particularly polymorphisms in the androgen receptor, and other upstream signaling and downstream metabolic events, including diabetes mellitus and obesity. Notwithstanding this concept of a saturation point<sup>24</sup> or threshold level above which testosterone has limited effect, the reluctance to utilize testosterone replacement has been incorporated into urological dogma and is largely responsible for the FDA's continuing caution about the relationship between therapy and initiation or progression of prostate cancer.<sup>121</sup>

The risks and benefits of TRT continue to be hotly debated. Although the need for a large randomized clinical trial examining this area is indisputable, the balance of evidence does not demonstrate an increased risk of prostate cancer with TRT, whether considering causation, promotion, aggressiveness, or recurrence. In fact, a growing body of literature demonstrates that testosterone supplementation in the hypogonadal noncastrate range does not lead to any prostate growth. Prostate homeostasis is maintained by a relatively low level of androgenic stimulation, and a dose response beyond a certain serum or intraprostatic level is unlikely, as demonstrated by many early physiological experiments. On the other hand, a dose-response state likely underlies the physiological health of many other nonprostate tissues. The importance of normalized testosterone levels on the overall well-being of an aging male is demonstrated by a number of published studies,<sup>119,120</sup> including Malkin *et al.*<sup>122</sup> who found an increased prevalence of androgen deficiency in a cohort of men with coronary artery disease, and an inverse relationship between mortality and the level of bioavailable testosterone. A cautionary note that TRT is not for every man, or that the selection of patients may influence outcome, was sounded by Basaria *et al.*<sup>123</sup> in 2010 who reported an increased risk of cardiovascular complications in frail, older (≥65 years) men treated with TRT.

In conclusion, definitive studies on the safety of TRT are needed, but until they are completed, the past 70 years of

experimentation should support giving the benefit of doubt for use of this therapy. The field of androgen replacement remains a fertile area for research (Box 3). The hypogonadal older (>45 years) man should be counselled on the potential risks and benefits of testosterone replacement before treatment, and should be carefully monitored for safety during treatment, but it seems logical to treat the symptomatic hypogonadal man with gradually increasing doses of exogenous testosterone to the point of symptomatic relief. This step would provide improved quality and quantity of life without the danger of increased risk of prostate cancer, because of the threshold or saturation level associated with testosterone. Potentially, men who have been successfully treated for prostate cancer and who suffer from biochemically-confirmed, symptomatic hypogonadism are suitable candidates for TRT after a prudent interval as long as no

clinical or laboratory evidence exists of residual active cancer. The testosterone threshold phenomenon in the prostate has been confirmed in the literature—let us not ignore 70 years of research and observation.

#### Review criteria

We performed a comprehensive PubMed search to identify original articles, review articles and editorials addressing the relationship between testosterone and the risk of prostate cancer development and progression. We also scanned the older literature for experiments relating to the influence of testosterone administration on prostate tissues. Selected papers were restricted to the English language, with preference given to full-text articles published within the past 70 years. Reference lists of retrieved articles were also searched for additional relevant peer-reviewed articles.

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#### Author contributions

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