AGE-RELATED CHANGES IN THE MALE REPRODUCTIVE SYSTEM

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ABSTRACT

In male mammals, changes at all levels of the hypothalamic-pituitary-testicular axis, including alterations in the GnRH pulse generator, gonadotropin secretion, and testicular steroidogenesis, in addition to alterations of feed-forward and feed-back relationships contribute to the age-related decline in circulating testosterone concentrations. The rate of age-related decline in testosterone levels is affected by the presence of chronic illness, adiposity, medication, sampling time, and the methods of testosterone measurement. Epidemiologic surveys reveal an association of low testosterone levels with changes in sexual function, body composition, physical function and mobility, and increased risk of diabetes, late life persistent depressive disorder (dysthymia), unexplained anemia of aging, osteoporosis and bone fractures. Age-related decline in testosterone should be distinguished from classical hypogonadism due to known diseases of the hypothalamus, pituitary, and the testis. In young hypogonadal men who have a known disease of the hypothalamus, pituitary, and testis, testosterone therapy is generally beneficial and has been associated with a low frequency of adverse events. However, neither the long-term benefits in improved health outcomes nor the long-term risks of testosterone therapy are known in older men with age-related decline in testosterone levels. Well-conducted randomized trials have found that testosterone replacement of older men with unequivocally low testosterone levels improves sexual desire, erectile function, and overall sexual activity; lean body mass, muscle strength and some measures of physical function and mobility; areal and volumetric bone density and bone strength; depressive symptoms; and corrects anemia of aging. Testosterone treatment does not worsen lower urinary tract symptoms but the effects of long-term testosterone treatment on the risk of prostate cancer and major adverse cardiovascular events remain unknown. Although testicular morphology, semen production, and fertility are maintained up to a very old age in men, there is clear evidence of decreased fecundity with advancing age and an increased risk of specific genetic disorders related to paternal age among the offspring of older men. Thus, reproductive aging of men is emerging as an important public health problem whose serious societal consequences go far beyond the quality-of-life issues related to low testosterone levels.

INTRODUCTION

Aging of male mammals is a very recent evolutionary event observed mostly in humans and animals in captivity. Most animal species in the wild with few exceptions [e.g., short-finned pilot whales, killer whales and some fish (1)] do not live beyond their reproductive years; during periods of food deprivation, many small animals may not even live beyond puberty. Even among humans, only the men and women of the past three generations have enjoyed a life expectancy of greater than fifty years. With increasing life expectancies of human populations across the globe, today, most men and women can expect to spend a substantial proportion of their lifespan past their procreative years.

The historical transition towards aging of human populations has profoundly influenced the health and wellbeing of older adults in their post-reproductive years as well as the size, health, vitality, and economies of human societies (2). At an individual level, many conditions related to reproductive aging, including sexual dysfunction, subfertility or infertility, conditions related to sex-steroid deficiency, genitourinary disorders, pelvic floor disorders, and cancers of the reproductive and accessory organs motivate middle-aged and older men and women to seek medical care. At a societal level, reproductive aging poses a potential threat to the reproductive capacity, health, and welfare of the current and future generations (2,3). Birth-rates in the United States, which had been declining since the turn of the nineteenth century - except during a short baby boom period after World War II - have trended below replacement levels since 1971 (Figure 1) (3-6). Several factors have contributed to this trend, including a growing proportion of couples having their first child after age 30, and an increasing proportion postponing pregnancy beyond age 35 (Figure 1) (4,7). Societal developments underpinning these trends include the availability of contraceptives that enable couples to separate their sexual and procreative lives; increased work force participation and changing career expectations of women; and a higher age of the male and female partners at reproductive union (2,3). Postponement of childbearing to an older age increases the risk of involuntary childlessness because of the adverse effects of advanced maternal and paternal age *per se* on fecundity, increased risk of comorbidities associated with advancing age that may indirectly affect fecundity, and the age-related changes in reproductive behaviors (4,8-11).
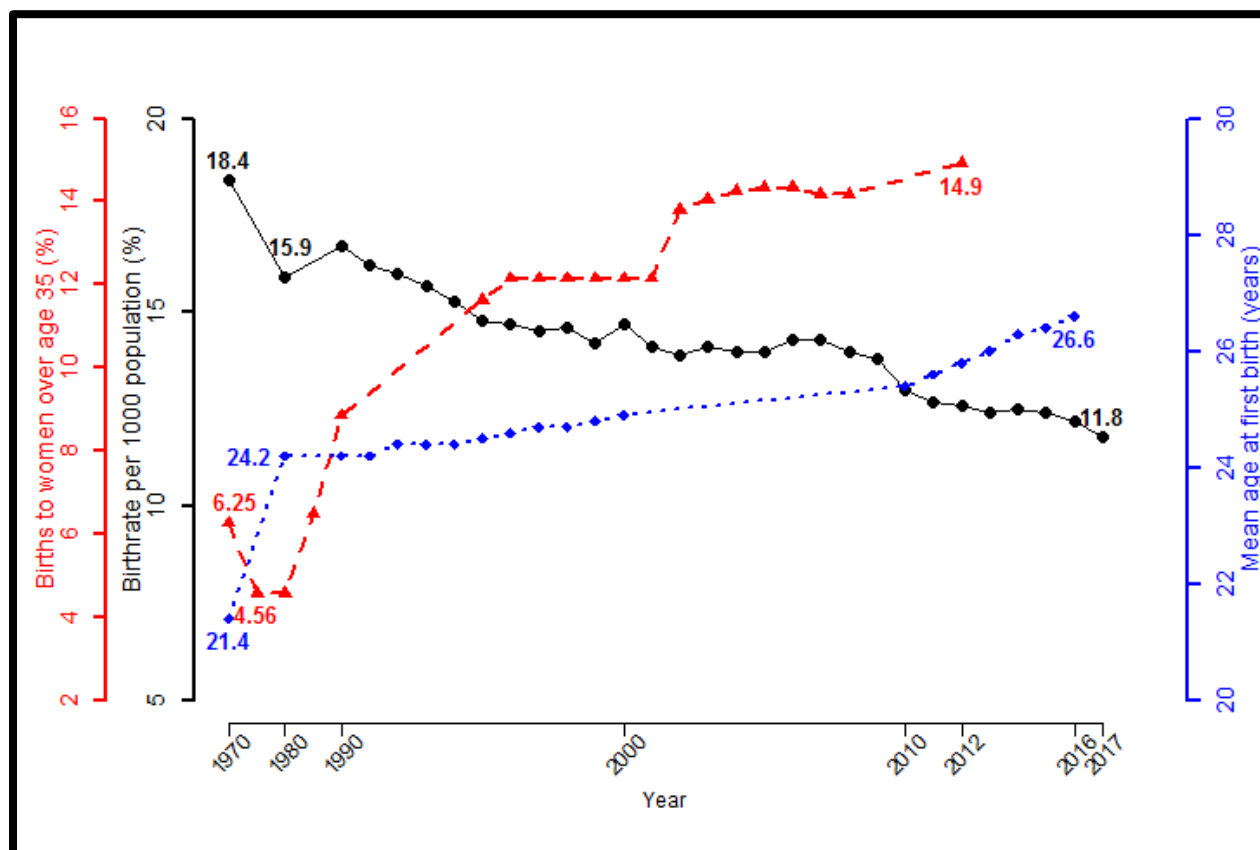
Figure 1. Birth rates, mean age of mother at first childbirth, and the proportion of infants born in the United States to women >35 years of age since 1970. Legend. The birth rate per 1000 population declined from 18.4 in 1970 to 11.8 in 2017. The mean age of mothers at first child birth increased from 21.4 years in 1970 to 26.6 in 2016. The proportion of all infants born in the USA to mothers > 35 years increased from 4.6% in 1970 to 14.9% in 2012. Birth rates are per 1,000 population estimated as of July 1 for each year except in 1970 and 1980, which were estimated as of April 1. Reproduced with permission from Bhasin S, Kerr C, Oktay K, Racowsky C. The Implications of Reproductive Aging for the Health, Vitality and Economic Welfare of Human Societies. J Clin Endocrinol Metab. 2019 Apr 16:jc.2019-00315. doi: 10.1210/jc.2019-00315. Epub ahead of print. PMID: 30990518. The original figure was based on data derived from: Centers for Disease Control and Prevention. National Vital Statistics System: birth data. Available at: www.cdc.gov/nchs/nvss/births.htm. Accessed 24 June 2021.

The health issues related to reproductive aging of women have been the subject of intense research for nearly 50 years and are covered in other sections of this textbook (12-15). This chapter focuses only on the reproductive aging of men, which has recently begun to garner considerable attention as reflected by the opening of hundreds of men's health clinics across the United States, and in the growing sales of testosterone and erectile dysfunction products.

The aging of men is associated with functional alterations at all levels of the reproductive axis that affect both the steroidogenic and gametogenic compartments (16-19). As discussed in this chapter, there is agreement that serum testosterone levels decline with age, a decline that is exacerbated by the accumulation of comorbidities (20,21); however, the long-term effects of testosterone supplementation on

health-related outcomes in older men have not been fully examined. Long-term safety data on the effects of testosterone supplementation on the risk of prostate cancer and major adverse cardiovascular events are also lacking. The recent publication of several well-conducted placebo-controlled trials of testosterone in middle-aged and older men has greatly advanced our understanding of the effects of testosterone treatment on sexual function, mobility, vitality, lower urinary tract symptoms and atherogenesis progression (22-28). However, in the absence of long-term, adequately-powered randomized trials of the effects of testosterone on hard patient-important health outcomes – fractures, falls, physical disability, progression from prediabetes to diabetes, remission of depressive disorders, wellbeing, and progression to dementia - the risks and benefits of long-term testosterone replacement in older men remain incompletely understood. The first section of this chapter reviews the pathophysiology and health consequences of age-related decline of testosterone levels and offers a patient-centric individualized approach to the treatment decisions. The second section describes the age-related alterations in the gametogenic compartment of the testes.

CHANGES IN THE STEROIDOGENIC COMPARTMENT OF THE TESTIS

Age Related Changes in Circulating Concentrations of Reproductive Hormones

Many studies suggest that aging *per se* affects the gonadal axis independently of the co-morbidities that accrete with aging, but there remains controversy about the relative contributions of the aging and the accumulation of co-morbidities to the age-related decline in testosterone levels. A few studies of older men have reported preservation of normal testosterone concentrations and its circadian rhythm in healthy older men (29,30). However, many other cross-sectional studies have shown that even after accounting for the potential confounding factors such as time of sampling, concomitant illness and medications, and technical issues related to hormone assays, serum total testosterone levels are lower in older men in comparison to younger men (31-52). Several longitudinal studies (31-34) also have confirmed a gradual but progressive decrease in serum testosterone concentrations from age 20 to 80. Adiposity, chronic illness, weight gain, lifestyle factors, medications, and genetic factors affect testosterone levels and the trajectory of the age-related decline in testosterone levels in men (29,32,35,53-56). The rate of age-related decline is greater in older men with chronic illness and adiposity than in healthy, non-obese older men (35,53,54). In the European Male Aging Study, adiposity and comorbidities were more strongly associated with low testosterone levels than age (57).

In contrast to the sharp reduction in ovarian estrogen production at menopause, the age-related decline in men does not start at a discrete coordinate in old age; rather, total testosterone concentrations, after reaching a peak in the second and third decade, decline inexorably throughout a man's life (Figure 2). Because of the absence of an identifiable inflection point at which testosterone levels begin to decline abruptly or more rapidly, many investigators have questioned the validity of the concept of "andropause", which misleadingly implies an abrupt cessation of androgen production in men (39,58). The term 'late-onset hypogonadism' has been proposed to reflect the view that in some middle-aged and older men (> 65 years), the age-related decline in testosterone concentration is associated with a cluster of symptoms and signs in a syndromic constellation which resembles in some aspects that observed in men with classical hypogonadism (47,59).
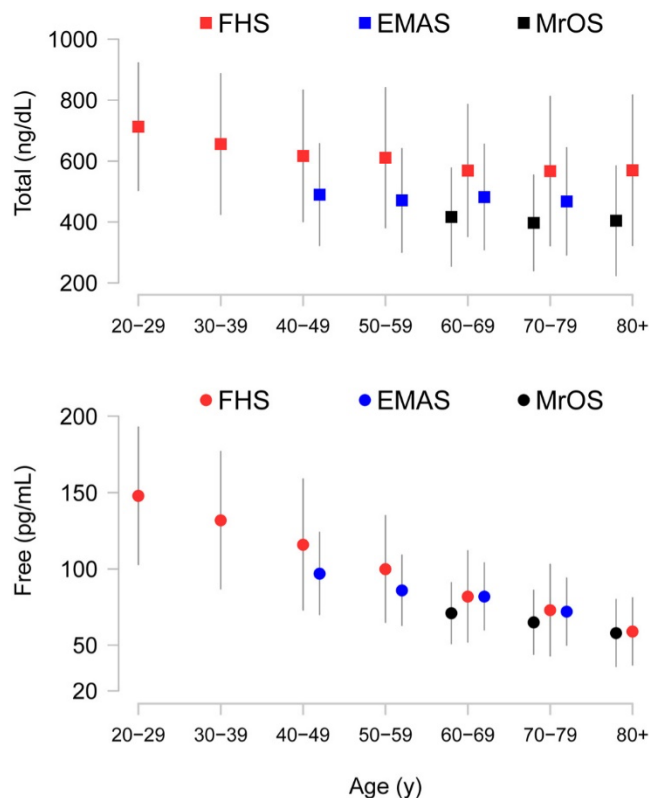
Figure 2. The distribution of total and free testosterone levels by decades of age in male participants of the Framingham Heart Study, the European Male Aging Study (EMAS) and the Study of Osteoporotic Fractures in Men (MrOS). Means and standard deviations are shown. To convert total testosterone from ng/dL to nmol/L, multiply concentrations in ng/dL with 0.0347. To convert free testosterone from pg/mL to pmol/L, multiply concentrations in pg/mL with 3.47. Reproduced with permission from Bhasin et al, J Clin Endocrinol Metab. 2011 Aug;96(8):2430-9.

Sex-hormone binding globulin concentrations are higher in older men than younger men (32,43,48). Thus, the age-related decline in free testosterone levels is of a greater magnitude than that in total testosterone levels. Similarly, there is a greater percent decline in bioavailable testosterone concentrations (the fraction of circulating testosterone that is not bound to SHBG) than in total testosterone concentrations.

An Expert Panel of the Endocrine Society defined androgen deficiency as a syndrome resulting from reduced production of testosterone and characterized by a set of signs and symptoms in association with unequivocally low testosterone levels (16). Many epidemiologic studies have defined androgen deficiency solely in terms of serum testosterone concentrations below the lower limit of the normal range for healthy, young men leading to inaccurate estimates of the prevalence of androgen deficiency in older men. Additionally, serum testosterone levels in most studies were measured using direct immunoassays, whose accuracy in the low range has been questioned. Not surprisingly, the estimates of the prevalence of androgen deficiency in older men have varied greatly among different studies. In the Baltimore Longitudinal Study of Aging (BLSA) (31), 30% of men over the age of 60 and 50% of men over the age of 70 had total testosterone concentration

below the lower limit of normal range for healthy young men (325 ng/dL, 11.3 nmol/L). The prevalence was even higher when these investigators used a free testosterone index to define androgen deficiency (31). In contrast, more recent studies that have used liquid chromatography tandem mass spectrometry found the prevalence of androgen deficiency to be significantly lower than that observed in the MMAS and BLSA (39,40,47-50). Although 10–15% of men aged ≥65 years have low total testosterone levels (Table 1) (47-50), the prevalence of late-onset hypogonadism defined by symptoms and a total testosterone level <8

nmol/L in the EMAS was 3.2% for men aged 60–69 years and 5.1% for those aged 70–79 years (47). The Healthy Man Study in Australia found no significant age-related decline in testosterone or dihydrotestosterone in men who reported being in good health (60). The authors of the Health Man Study have argued that ill health, rather than aging itself, is the major contributor to androgen deficiency in older men. A Finnish cross-sectional study also demonstrated very low prevalence of low serum testosterone concentrations in older men who were healthy (39).

Table 1. Percent of Community-Dwelling Older Men with Unequivocally Low Testosterone Level in Population Studies			
Study	**Principal Investigator**	**Number of Men with Age > 65 years**	**% Men with Testosterone <250 ng/dL**
Framingham Heart Study (FHS)	Bhasin	1870	12.1%
Osteoporotic Fractures in Men Study (MrOs)	Orwoll	2623	10%
European Male Aging Study (EMAS)	Wu	1080	7.3%
Cardiovascular Health Study (CHS)	Hirsch	639	14.3%

Data derived from Bhasin et al, JCEM 2011; Orwoll et al, JCEM 2009; Wu et al, NEJM 2010; Hirsch et al, JCEM 2009.

Mechanisms of Age-Related Decline in Testosterone Levels

Circulating testosterone concentrations are a function of testosterone production and clearance rates; the age-related decline in serum testosterone concentrations is primarily a consequence of decreased production rates in older men (20,21,43-45,48). Plasma clearance rates of testosterone are, in fact, lower in older men than in younger men (54,55). The decline in testosterone production in older men is

the result of abnormalities at all levels of the hypothalamic-pituitary-testicular axis (42-44,60-71).

GONDADOTROPIN-RELEASING HORMONE SECRETION AND REGULATION IN OLDER MEN

Pulsatile GnRH secretion is attenuated in older men. In addition, there are disturbances of the feedback and feed-forward relationships between testosterone and LH secretion (63,71,72). Thus, the sensitivity of pituitary LH secretion to androgen-mediated *feedback*

inhibition is increased; in addition, the ability of LH to stimulate synchronously testicular testosterone secretion (*feedforward*) is attenuated (63,71,72). Veldhuis has shown that the orderliness of LH pulses and the synchrony between LH and testosterone pulses are decreased in older men (63,71,72); in addition, there is greater variability in LH pulse frequency, amplitude, and secretory mass in older men, in comparison to younger men (71,72).

GONADOTROPIN SECRETION AND REGULATION IN OLDER MEN

There is considerable heterogeneity in circulating LH and FSH concentrations in individual older men; both hypogonadotropic and hypergonadotropic hypogonadism have been reported (54,59). As a group, serum LH and FSH concentrations are higher in older men than in young men (32,33). However, serum LH concentrations do not increase in proportion to the age-related decline in circulating testosterone levels, due to the impairment of GnRH secretion and alterations in gonadal steroid feedback and feedforward relationships (60-71).

In the EMAS, secondary hypogonadism (low testosterone and low or normal LH) was more prevalent (nearly 12%) than primary hypogonadism (low testosterone and elevated LH, 2%) (57). Secondary hypogonadism was associated with obesity and comorbid conditions, while primary hypogonadism was associated predominately with age (57). Nearly 10% of men in EMAS had normal testosterone levels and elevated LH; these men with elevated LH tended to be older and in poor health and were at increased risk of developing low testosterone and other comorbid conditions (73).

The data on LH response to GnRH are somewhat inconsistent across studies. Urban et al (65) used an interstitial cell bioassay to measure serum

concentrations of bioactive LH and found that although basal bioactive LH concentrations were similar in this sample of young and older men, older men demonstrated diminished LH response to GnRH administration. However, in a subsequent study, Zwart et al (66) found greater gonadotropin responsiveness to GnRH in older men than younger men; the maximal and incremental LH and FSH secretory masses in response to graded doses of GnRH were significantly higher in healthy, older men than in younger men. The estimated half-lives of LH, FSH, or alpha-subunit did not significantly differ between young and older men (66).

The Brown Norway rat has been widely used as a model of reproductive aging. In this experimental model, the prepro-GnRH mRNA content and the number of neurons expressing prepro-GnRH mRNA are lower in older male rats in comparison to young rats (67,68). The GnRH content of several hypothalamic areas is also lower in intact older rats than younger rats (67). Older Brown Norway rats exhibit significant reductions in glutamate and γ-aminobutyric acid (GABA) levels in the hypothalamus compared to young rats (68). These observations suggest that the decreased hypothalamic excitatory amino acid expression and the reduced responsiveness of GnRH neurons to N-methyl-D-aspartate may contribute to the altered LH pulsatile secretion observed in old rats (68).

Infusions of testosterone and DHT are associated with greater reductions in mean serum LH and FSH levels and the frequency of LH pulses in older men in comparison to young men (69). Winters et al (64) reported that the degree of LH inhibition during testosterone replacement of older, hypogonadal men was significantly greater than in young, hypogonadal men suggesting that older men are more sensitive to the feedback inhibitory effects of testosterone on LH. Deslypere et al (69) also found decreased LH pulse frequency and a greater degree of LH inhibitory

response to estradiol administration in older men than young controls. Age-related increase in FSH levels is not associated with a progressive or proportionate decrease in inhibin B levels (70). Thus, the mechanistic basis of FSH increase with advancing age is not fully understood, although the lack of change in inhibin B levels suggests that Sertoli cell function is relatively preserved in older men.

TESTICULAR TESTOSTERONE PRODUCTION IN OLDER

Testosterone secretion in healthy, young men exhibits a diurnal rhythm characterized by higher concentrations in the morning and lower concentrations in the late afternoon. The diurnal rhythm of testosterone secretion is dampened in older men (41,51). Testosterone response to LH and human chorionic gonadotropin is decreased in older men, compared to younger men (42-44).

Physiological and Clinical Correlates of Age-Related Decline in Circulating Testosterone in Epidemiological Studies

Many physiological changes that occur with advancing age, such as the loss of bone and muscle mass, increased fat mass, impairment of physical and sexual functions, loss of body hair, and decreased hemoglobin levels, are similar to those associated with androgen deficiency in young men. Aging is associated with loss of skeletal muscle mass (Figure 3), muscle strength and power, and progressive impairment of physical function (74-98). Epidemiological studies of older men have reported associations between low testosterone levels and some age-related conditions, although these associations are weak. For instance, in a number of epidemiologic studies, such as the St. Louis Inner City Study of Aging Men (77), the Olmsted County Epidemiological Study (76), and the New Mexico Elderly Health Study (79,80), low bioavailable testosterone levels (unbound and albumin-bound testosterone) were associated with low appendicular skeletal muscle mass. Low bioavailable testosterone levels also have been associated with decreased strength of upper as well as lower extremity muscles (77,78) and decreased performance in self-reported as well as performance-based measures of physical function (99-103). Low free testosterone levels have also been associated with the development of mobility limitation and the frailty syndrome (104-107).
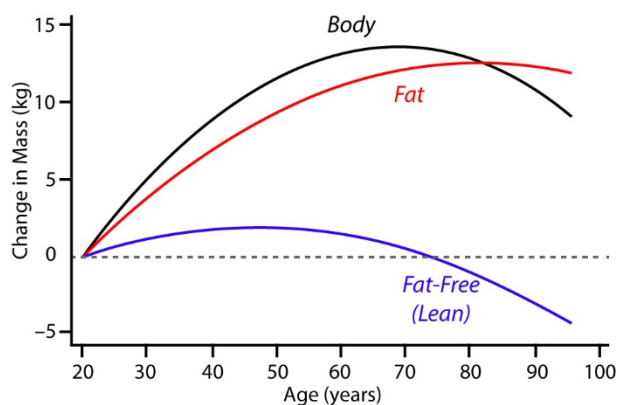

Figure 3. A schematic diagram of the age-related changes in body composition in 7265 men. Lines represent the longitudinal changes in body weight (black line), fat mass (red line) and fat-free. mass (blue line) components from age 20 years. The estimated mass values at age 20 years were as follows: body

mass, 72.72 kg; fat mass, 9.14 kg; fat-free mass, 64.09 kg. Figure adapted with permission from Jackson et al. Br J Nutr. 2012;107(7):1085-91.

The association of testosterone levels with sexual dysfunction has been inconsistent across studies because of the heterogeneity and variable quality of instruments used to assess sexual dysfunction, problems of testosterone assay quality, and failure to distinguish among various categories of sexual dysfunction (108-113). Androgen deficiency and erectile dysfunction are two independently distributed clinical disorders and because both disorders are prevalent in middle-aged and older men, they can often co-exist (112,113). Low testosterone levels were associated with low sexual desire in the MMAS (108). Among men enrolled in the testosterone trials, free and total testosterone levels were independently associated with sexual desire, erectile function, and sexual activity scores (114).

In the EMAS, total and free testosterone levels were associated with overall sexual function in middle-aged and older men (47). This relationship was observed more robustly at testosterone concentrations <8 nmol/L, but not at higher testosterone concentrations (115). Men deemed to have low total and free testosterone levels in EMAS were more likely to report decreased morning erections, erectile dysfunction, and decreased frequency of sexual thoughts than those with normal testosterone levels (48). In another study of men over the age of 50 who had benign prostatic hyperplasia, sexual dysfunction was reported only by men with serum total testosterone levels less than 225 ng/dL (110).

Aging of humans is attended by a decline in several aspects of cognitive function; of these multiple domains of cognition that decline with aging, declines in verbal memory, visual memory, spatial ability, and executive function are associated with the age-related decline in testosterone (109-113,115-124).

The relationship of testosterone levels with depression has been inconsistent across epidemiologic studies (125-129). Low testosterone levels in older men are associated more with late-onset low grade persistent depressive disorder (dysthymia) but not with major depression (128-130). In general, testosterone levels are lower in older men with dysthymic disorder than in those without any depressive symptoms (129).

Several epidemiologic studies of older men (131-135), including MrOS (131), Rancho Bernardo Study (132), Framingham Heart Study (133), and the Olmsted County Study (134) - have found bioavailable testosterone levels to be associated with bone mineral density, bone geometry, and bone quality (135); the associations are stronger with bioavailable testosterone and estradiol levels than with total testosterone levels. In the MrOS Study, the odds of osteoporosis in men with a total testosterone less than 200 ng/dL were 3.7-fold higher than in men with normal testosterone level (131); free testosterone was an independent predictor of prevalent osteoporotic bone fractures (136).

Several studies have evaluated the association of testosterone levels and mortality (137-141). Some, but not all, studies found higher all-cause mortality and cardiovascular mortality in men with low testosterone levels than in those with normal testosterone levels. In a meta-analyses of epidemiologic studies of community-dwelling men, low testosterone levels were associated with an increased risk of all-cause and CVD death (Figure 4) (142,143). However, the strength of the inferences of these meta-analyses was limited by considerable heterogeneity in study populations; it is possible that effects may have been

driven by differences in the age distribution and the health status of the study populations (142-146).

All-Cause Mortality

Primary author and Year		Relative Risk (95% CI)
Araujo, 2007		1.04 (0.83–1.31)
Haring, 2010		3.62 (1.64–7.98)
Khaw, 2007		1.58 (1.20–2.11)
Laughlin, 2008		1.37 (1.10–1.69)
Lehtonen, 2008		2.52 (1.00–5.45)
Menke, 2010		1.08 (0.82–1.42)
Shores, 2006		2.09 (1.41–3.09)
Smith, 2005		1.05 (0.86–1.29)
Szulc, 2009		1.00 (0.72–1.41)
Tivesten, 2009		1.72 (1.46–1.99)
Vikan, 2009		1.02 (0.80–1.29)
Random−effects model		1.35 (1.13–1.62)

$I^2 = 77.9\%$, $p < 0.001$

Relative Risk (95% CI)
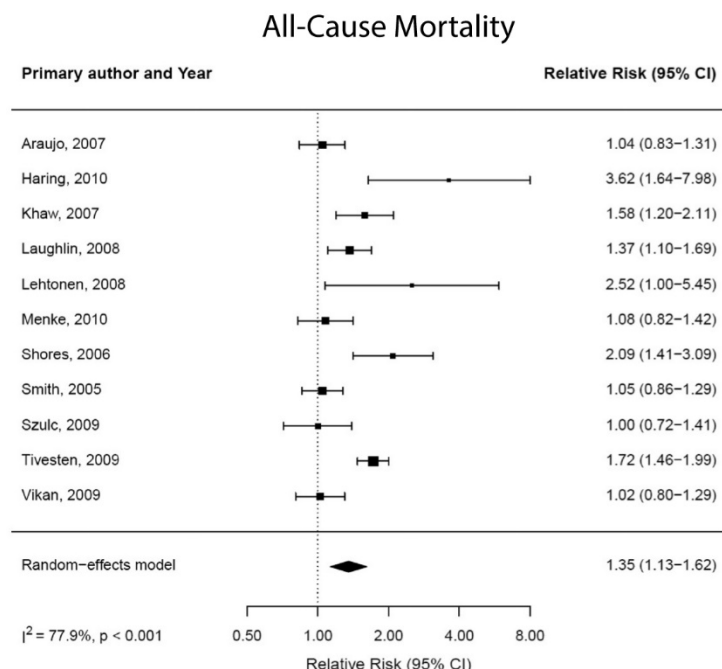0.50 1.00 2.00 4.00 8.00

Figure 4. The relationship of low testosterone level with all-cause mortality in a meta-analysis of epidemiologic studies of community-based men. Eleven studies which enrolled 16,184 subjects were included in this meta-analysis. There was considerable heterogeneity of the age distribution, health status, and other subject characteristics. Reproduced with permission from Araujo et al, J Clin Endocrinol Metab 2011;96:3007-19.

Testosterone levels are not correlated with aging-related symptoms assessed by the Aging Male Symptom (AMS) score or with lower urinary tract symptoms assessed by the IPSS/AUA prostate symptom questionnaire (144). Some cross-sectional studies found no difference in serum testosterone levels between men who had coronary artery disease and those who did not have coronary artery disease; other studies have reported testosterone levels to be lower in men with coronary artery disease than in men without coronary artery disease (145-150).

Epidemiologic studies, especially cross-sectional studies, can only demonstrate associations; causal relationships are difficult to establish from these studies. Furthermore, the associations between testosterone levels and health-related outcomes are generally weak. The inferences are further confounded by the co-linearity of aging-related co-morbid conditions, low testosterone levels, and age-related changes in body composition and inflammatory markers. Although epidemiologic studies have reported associations between the age-related changes in circulating testosterone levels and skeletal muscle mass, muscle strength and physical function; sexual and cognitive functions; areal and volumetric bone density and fracture risk; and mood, long-term randomized trials are needed to determine whether these relations are causal.

Potential Beneficial Effects of Testosterone Treatment in Older Men with Low Testosterone Levels

It has been hypothesized that increasing serum testosterone concentrations in older men with low testosterone levels into a range that is mid-normal for healthy, young men would improve physical function and mobility, some domains of sexual and cognitive functions, energy and sense of wellbeing, and reduce the risk of falls and fractures, and improve overall quality of life. A number of randomized trials have demonstrated improvements in measures of sexual function, lean and fat mass, and areal and volumetric bone mineral density; however, there has been a paucity of long-term, placebo-controlled, randomized trials that are adequately powered to detect clinically meaningful changes in health outcomes such as fracture rates, physical disability, progression to dementia, remission of late onset low grade persistent depressive disorder (dysthymia), progression from prediabetes to diabetes, and overall quality of life. Furthermore, none of the previously published studies had sufficient power to address the long-term risks of prostate and cardiovascular disease.

The following section describes the effects of testosterone supplementation on multiple organ systems focusing on physical function, sexual function, vitality, bone health, mood, wellbeing, and depression, and cognitive function.

EFFECTS OF TESTOSTERONE SUPPLEMENTAION ON MUSCLE MASS AND PERFORMANCE AND PHYSICAL FUNCTION IN OLDER MEN WITH LOW TESTOSTERONE LEVELS

Age-Related Changes in Muscle Mass and Performance

Sarcopenia, the loss of muscle mass and function, is an important consequence of aging (75-79). The principal component of the decrease in fat-free mass is the loss of muscle mass; there is little change in non-muscle lean mass (81-87). Between 20 and 80 years of age, the skeletal muscle mass decreases by 35-40% in men (85), in part due to decreased muscle protein synthesis (92). Although there is a loss of both type I and type II fibers, there is a disproportionate decrease in the number of type II muscle fibers that are important for the generation of muscle power (93,94). In spite of the significant depletion of skeletal muscle mass, body weight does not decrease, and may even increase because of the accumulation of body fat (81-87) (Figure 3).

The loss of skeletal muscle mass that occurs with aging is associated with a reduction in muscle strength (95-98). There is a substantial decrease in muscle strength and power between 50 and 70 years of age, primarily due to muscle fiber loss and selective atrophy of type II fibers (93-98). The loss of muscle strength is even greater after the age of 70; 28% of men over the age of 74 could not lift objects weighing more than 4.5 kg (97). With increasing age, there is a progressive reduction in muscle power (151,152), the speed of strength generation, and fatigability, the ability to persist in a task.

Loss of muscle mass and strength leads to impairment of physical function, as indicated by the impaired ability to arise from a chair, climb stairs, generate gait speed, and maintain balance (151-154). The impairment of physical function contributes to loss of independence, and increased risk of physical disability, falls and fractures in older men.

Anabolic Effects of Testosterone in Humans: Testosterone Trials in Healthy, Hypogonadal Men, Men with Chronic Illness, and Older Men

The anabolic effects of testosterone on the muscle have been a source of controversy for over sixty years. The athletes and recreational bodybuilders use large doses of androgenic steroids with the belief that these compounds increase muscle mass and strength. Until recently, the academic community was skeptical about such claims because of the problems of study design. However, a large number of studies in healthy young men, healthy hypogonadal men, men with chronic illness, and in healthy older men have established that testosterone administration improves skeletal muscle mass, maximal voluntary strength, leg power, aerobic capacity, and some measures of physical performance and mobility (154-165). In a systematic review of testosterone trials in healthy, hypogonadal men, testosterone therapy increased fat-free mass and body weight (Figure 5) (154-161).
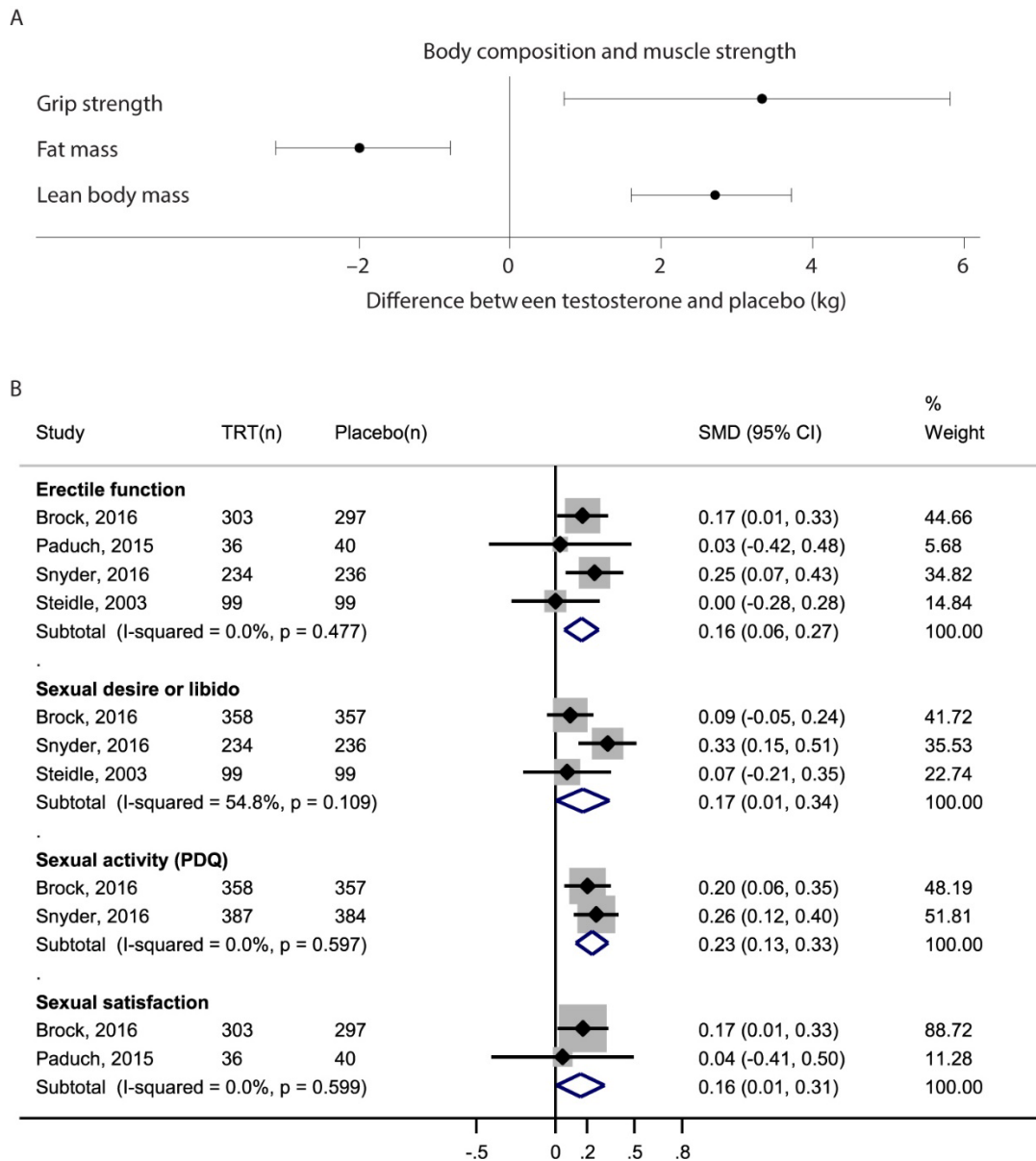
A

Body composition and muscle strength

Grip strength	
Fat mass	
Lean body mass	

−2   0   2   4   6

Difference between testosterone and placebo (kg)

B

Study	TRT(n)	Placebo(n)		SMD (95% CI)	% Weight
Erectile function					
Brock, 2016	303	297		0.17 (0.01, 0.33)	44.66
Paduch, 2015	36	40		0.03 (-0.42, 0.48)	5.68
Snyder, 2016	234	236		0.25 (0.07, 0.43)	34.82
Steidle, 2003	99	99		0.00 (-0.28, 0.28)	14.84
Subtotal (I-squared = 0.0%, p = 0.477)				0.16 (0.06, 0.27)	100.00
.					
Sexual desire or libido					
Brock, 2016	358	357		0.09 (-0.05, 0.24)	41.72
Snyder, 2016	234	236		0.33 (0.15, 0.51)	35.53
Steidle, 2003	99	99		0.07 (-0.21, 0.35)	22.74
Subtotal (I-squared = 54.8%, p = 0.109)				0.17 (0.01, 0.34)	100.00
.					
Sexual activity (PDQ)					
Brock, 2016	358	357		0.20 (0.06, 0.35)	48.19
Snyder, 2016	387	384		0.26 (0.12, 0.40)	51.81
Subtotal (I-squared = 0.0%, p = 0.597)				0.23 (0.13, 0.33)	100.00
.					
Sexual satisfaction					
Brock, 2016	303	297		0.17 (0.01, 0.33)	88.72
Paduch, 2015	36	40		0.04 (-0.41, 0.50)	11.28
Subtotal (I-squared = 0.0%, p = 0.599)				0.16 (0.01, 0.31)	100.00

-.5   0 .2 .5 .8

Figure 5. The effects of testosterone therapy on body composition, muscle strength, and sexual function in intervention trials. The point estimates and the associated 95% confidence intervals are shown. Panel A shows the effects of testosterone therapy on, grip strength, fat mass and lean body mass in a meta-analysis of randomized trials (data derived from Bhasin et al. Nat Clin Pract Endocrinol Metab. 2006;2(3):146-59; figure reproduced with permission from Spitzer et al. Nat Rev Endocrinol. 2013;9(7):414-24). Panel B shows the effects of testosterone therapy on sexual function in a meta-analysis of randomized trials (figure adapted with permission from Ponce et al. J Clin Endocrinol Metab. 2018;103(5):1745-54).

The anabolic effects of testosterone on fat-free mass, muscle size, and maximal voluntary strength are related to the administered testosterone dose and the circulating testosterone concentrations (166-168) (Figure 6). The administration of supraphysiologic doses of

testosterone in eugonadal men increases fat-free mass, muscle size, and maximal voluntary strength (166-169).
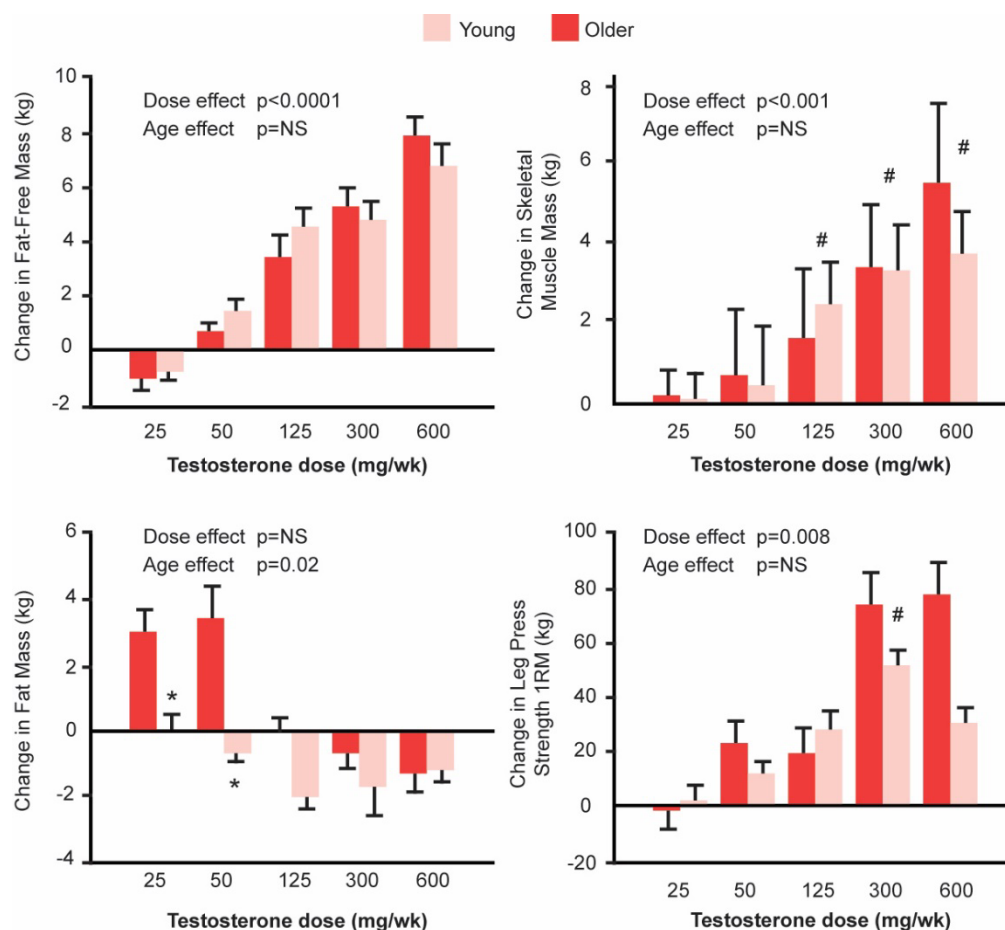

Figure 6. Testosterone Dose Response Relationship in Young and Older Men. In this study, healthy, young men (18-34 years of age) and healthy older men (60-75 years of age) were treated with a long-acting GnRH agonist plus graded doses of testosterone enanthate for 20 weeks. Shown are mean (±SEM) changes from baseline in fat free mass (upper left), skeletal muscle mass (upper right), fat mass (lower left), and leg press strength (lower right) in young (black bars) and older (lightly shaded bars) men. Adapted with permission from Bhasin et al. J Clin Endocrinol Metab. 2005 Feb;90(2):678-88.

Testosterone effects on muscle performance are domain-specific: testosterone administration increases maximal voluntary strength and leg power, but does not affect fatigability or specific tension (167). The gains in maximal voluntary strength during testosterone administration are proportional to the increase in muscle mass; unlike resistance exercise training, testosterone does not improve the contractile properties of the human skeletal muscle (167).

Resistance exercise training augments the anabolic response to androgens; thus, men receiving testosterone and resistance exercise training together experience greater gains in fat-free mass and muscle strength than those receiving either intervention alone (169). The anabolic effects of testosterone are also augmented by concomitant recombinant growth hormone administration (170). Although it has been speculated in the sports medicine literature that increasing the protein intake can enhance the muscle mass and strength gains in response to anabolic stimuli such as resistance exercise training or androgens, the evidence supporting such speculation is weak. In a recent controlled feeding study, increasing the daily protein intake to a level (1.3 g/kg/day) higher than the recommended daily allowance (0.8 g/kg/day) for six months did not increase lean body mass or maximal muscle strength more than that associated with the daily intake of the recommended daily allowance of 0.8 g/kg/day (171) . The higher level of daily protein intake (1.3 g/kg/day) also did not augment the gains in lean body mass and muscle strength in response to testosterone administration above that observed in participants eating the recommended dietary allowance for protein (171,172).

Testosterone replacement of young, hypogonadal men has been reported to increase muscle protein synthesis (158,173,174). The effects of testosterone replacement on muscle protein degradation need further investigation.

Systematic reviews (155,175,176) of randomized, placebo-controlled trials in HIV-infected men with weight loss (176-181) have revealed that testosterone therapy for 3 to 6 months was associated with greater gains in lean body mass than placebo administration (difference in lean body mass change between placebo and testosterone arms 1.22 kg, 95% CI 0.23-2.22 for the random effect model). In two (176,180) out of three trials that measured muscle strength (176,180,181), testosterone administration was associated with significantly greater improvements in maximal voluntary strength than placebo. Testosterone therapy had a moderate effect on depression indices (-0.6, 95% CI -1.0, -0.2) (182) and fatigue (183), but did not improve overall quality of life (182,183). Changes in CD4+ T lymphocyte counts, HIV copy number, PSA, plasma HDL cholesterol, and adverse event rates were not significantly different between the placebo and testosterone-treatment groups (176-183). Overall, short-term (3-6 months) testosterone use in HIV-infected men with low testosterone levels and weight loss can induce modest gains in body weight and lean body mass with minimal changes in quality of life and mood. This inference is weakened by inconsistency of results across trials, and heterogeneity in inclusion and exclusion criteria, disease status, testosterone formulations and doses, treatment duration, and methods of body composition analysis (155). Data on testosterone effects on physical function, risk of disability, or long-term safety in HIV-infected men are limited.

Testosterone administration increases fat-free mass and decreases fat mass in older men with low testosterone levels. Meta-analyses (155,183) of randomized trials (184-188) that included middle-aged and older men with low or low normal testosterone levels, and that used testosterone or its esters in replacement doses for >90 days, have confirmed that testosterone administration is associated with a significantly greater increase in whole body and appendicular fat-free mass and a greater reduction in whole body and appendicular fat mass than placebo (Figure 5). The average gains in fat-free mass generally were greater in trials that used injectable testosterone esters than in those which used transdermal testosterone gel, presumably because of the higher doses of testosterone delivered by the injectable formulations than by transdermal gel formulations. The change in body weight did not differ significantly between the testosterone and placebo groups.

Testosterone administration improves stair climbing speed and power, and self-reported physical function, as assessed by the Medical Outcomes Study Short Form 36 (MOS SF36) questionnaire. Testosterone' Effects on Atherosclerosis Progression in Aging Men Trial (The TEAAM Trial), a randomized trial conducted in healthy community-dwelling older men without functional limitations and low to low-normal testosterone levels, showed that testosterone replacement for 3-years was associated with modest improvements in leg-press and chest-press power and the stair-climb power (163). Changes in gait speed generally have been modest and inconsistent across randomized trials (25,185,188,189). Testosterone administration is associated with small improvements in aerobic capacity and attenuation of the age-related decline in VO_{2peak} (Figure 7) (164,165).
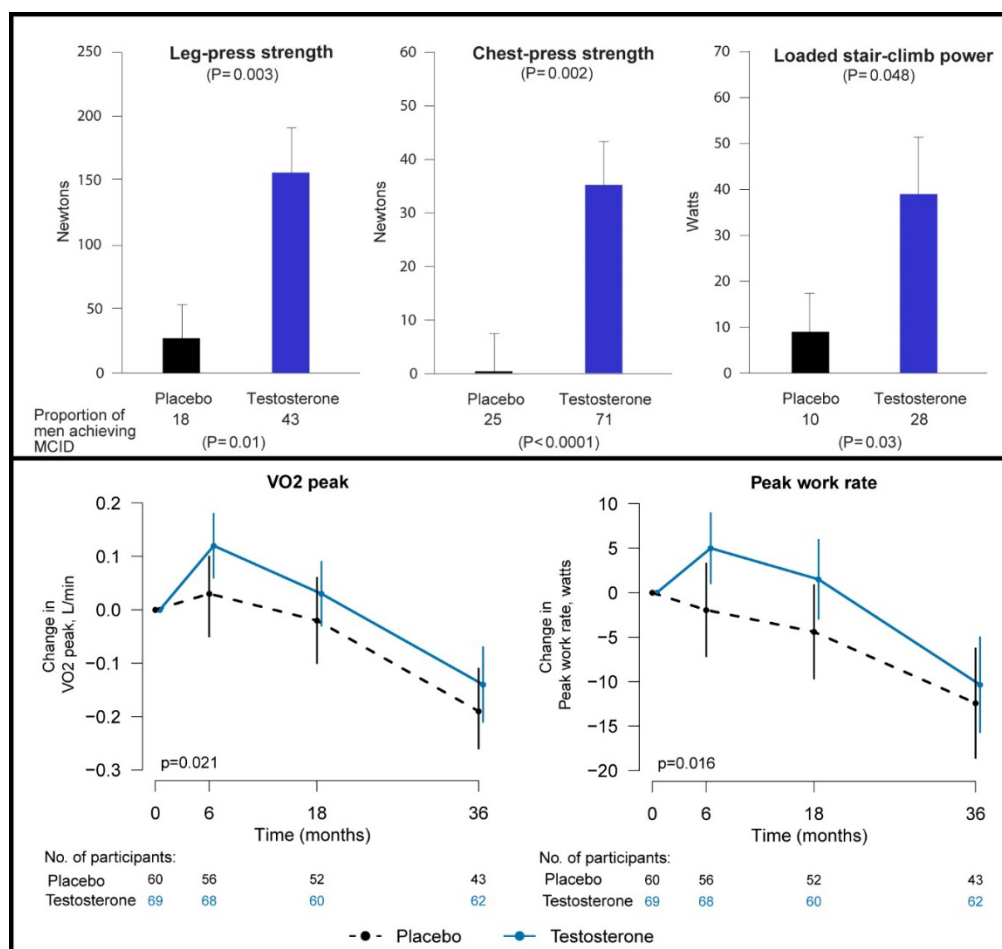
Figure 7. Effects of testosterone administration on measures of muscle performance and physical function in randomized testosterone trials in older men. Panel A shows the mean (SD) change from baseline to maximal voluntary strength in the leg press and chest press exercises and on loaded stair climbing power at either the end of the intervention period or at the last measurement performed in who dropped out before study completion in the testosterone in older men with mobility limitation (The TOM Trial). The minimal clinically important difference (MCID) for each outcome was determined using an anchor-based method within the trial. The proportion of men (percent) whose change from baseline either equaled or exceeded the MCID is shown below the figure along with the P-value for the comparison of placebo and testosterone groups (figure adapted with permission from Spitzer et al. Nat

Rev Endocrinol. 2013;9(7):414-24). Panel B shows the long-term effects of testosterone administration on aerobic capacity in older men participating in the TEAAM trial. Data points represent mean changes from baseline and error bars are 95% CI in VO2peak (L/min) and in peak work rate. P values indicate the overall effect of the testosterone intervention over time (figure reproduced with permission from Traustadóttir et al, J Clin Endocrinol Metab. 2018;103(8):2861-2869).

One reason for the variable improvements in physical function in various testosterone trials is that the measures of physical function used in previous studies had low ceilings. Another confounder of the effects of anabolic interventions on muscle function is the learning effect. For instance, subjects who are unfamiliar with weightlifting exercises often demonstrate improvements in measures of muscle performance simply because of increased familiarity with the exercise equipment and technique. Because of the considerable test-to-test variability in tests of physical function, it is possible that previous studies did not have adequate power to detect meaningful differences in measures of physical function between the placebo and testosterone-treated groups. It is also possible that neuromuscular adaptations needed to translate strength gains into functional improvements require a lot longer than the 3 to 6-month duration of most of the previous trials. The measures of physical function that are more robustly related to lower extremity muscle strength, such as stair climbing speed and power, have shown more consistent improvements in testosterone trials than walking speed (22,23,190).

Only a few testosterone trials have been conducted in older men with functional limitations (22,26,28,190-192). In a trial of pre-frail or frail men (28), administration of 50 mg testosterone gel daily for 6 months induced greater improvements in lean mass, knee extension peak torque, and sexual symptoms than did placebo gel (28). Performance-based measures of physical function did not differ significantly between groups, but they improved in the subgroup of frail elderly men (28). In Testosterone in Older Men (TOM) Trial, older men with mobility limitation were randomly assigned to either placebo or 10 g testosterone gel daily for 6 months (22,190). The

testosterone dose was adjusted to achieve testosterone levels between 17.4 nmol/l and 34.7 nmol/L (500 to 1000 ng/dL). The improvements in leg-press strength, chest-press strength and power, and loaded stair-climbing speed and power were significantly greater in men assigned to testosterone arm than in those receiving placebo (Figure 7). A greater proportion of men in the testosterone arm improved more than the minimal clinically important difference for leg-press and chest-press strength and stair-climbing speed than that in the placebo arm. Because of a higher frequency of cardiovascular-related events in the testosterone arm compared with the placebo arm, the trial's data and safety monitoring board stopped further administration of study medication (22,190). The findings of the TOM trial and other epidemiologic studies have heightened the concern that frail elderly men with a high burden of chronic co-morbidities may be at an increased risk of adverse events (22), providing the impetus to develop, strategies to achieve increased selectivity and a more favourable risk to benefit ratio (22).

The Testosterone Trials were a coordinated set of seven randomized double-blind, placebo-controlled trials designed to determine the benefits of testosterone therapy in older men 65 years and older with low testosterone levels and clinical symptoms of androgen deficiency on a variety of androgen-dependent outcomes (192). To participate in these trials, the men had to be eligible for at least one of the three main trials (the Sexual Function Trial, the Physical Function Trial, or the Vitality Trial). The men were assigned to testosterone or placebo gel for 1 year and the dose was adjusted to maintain testosterone concentrations within the normal range for healthy young men. The Physical Function Trial of the TTrials recruited older men with self-reported

difficulty walking or climbing stairs and walking speed less than 1.2 m/s and an average of two morning fasting testosterone levels less than 275 ng/dL (162). The 6-minute walking distance improved significantly more in the testosterone than in the placebo group among all men in the TTrials, but not in those who were enrolled in the PFT (162). The self-reported physical function assessed using the physical component of the Medical Outcomes Study Short Form-36 questionnaire, improved more in the testosterone group than in the placebo group in all men in TTrials and in men enrolled in the PFT (162). The men in the testosterone group were more likely to report improvement in their walking ability than men in the placebo group. The changes in 6-minute walking distance were significantly associated with changes in testosterone, free testosterone, dihydrotestosterone, and hemoglobin levels, and to baseline gait speed and self-reported mobility limitation (162). Thus, testosterone treatment of older men with mobility limitation consistently improved self-reported walking ability, modestly improved 6-minute walking distance (162). The number of falls was similar in the testosterone and placebo arms (162).

Innovative strategies to translate gains in muscle mass and strength induced by testosterone into functional improvements are needed (18). Resistance exercise training augments the anabolic effects of androgens on muscle mass and performance and physical function (193). Thus, adjunctive exercise training might be required to induce the neuromuscular and behavioural adaptations that are necessary to translate the gains in muscle mass and strength into clinically-meaningful functional improvements (18). In addition, there is some evidence that the anabolic response of skeletal muscle to dietary protein is attenuated with age (194,195). These findings have raised the question whether the current recommended dietary allowance (RDA) for protein (0.8 g/kg/day) is adequate to preserve lean body mass and physical function in older adults. However, In a recent controlled feeding study in functionally-limited older men with usual

protein intake less than or equal to the RDA for protein (171) higher protein intake exceeding the RDA did not increase lean body mass, muscle performance or physical function nor augmented the anabolic response to testosterone. However, higher protein intake was associated with lower whole body and visceral abdominal fat, although no significant changes in metabolic biomarkers (fasting glucose, fasting insulin, HOMA-IR, leptin, adiponectin, IL-6, and hs-CRP) were observed (196). These findings suggest that the current RDA for protein is adequate to maintain lean body mass and higher protein intake above the RDA does not promote additional gains in muscle mass or physical function with or without testosterone supplementation.

Mechanisms of Androgen Action on Muscle

Testosterone-induced increase in muscle mass is associated with hypertrophy of both type I and II muscle fibers (197). The absolute number and the relative proportion of type I and type II fibers do not change during testosterone administration. Testosterone-induced muscle fiber hypertrophy is associated with dose-dependent increases in myonuclear number and satellite cell number (198), suggesting that testosterone administration increases the number of muscle progenitor cells.

Testosterone administration has been shown to increase fractional muscle protein synthesis and improve the reutilization of amino acids (173,174). The effects of testosterone on muscle protein degradation have not been well studied. However, the muscle protein synthesis hypothesis does not explain the reciprocal decrease in fat mass or the increases in myonuclear and satellite cell number that occur during testosterone administration (198). Testosterone promotes the differentiation of mesenchymal multipotent muscle progenitor cells into the myogenic lineage and inhibits the differentiation of these progenitor cells into the adipogenic lineage (199,200).

Thus, testosterone promotes the formation of myosin heavy chain II positive myotubes in multipotent cells and up-regulates markers of myogenic differentiation, such as MyoD and myosin heavy chain (199,200). Testosterone and DHT inhibit adipogenic differentiation and downregulate markers of adipogenic differentiation, such as PPAR-ϒ and C/EBP∝ (201).

Testosterone's effects on myogenic differentiation are mediated largely through its binding to the classical androgen receptor, which induces a conformational change in the androgen receptor protein, promoting its association with its co-activator, beta-catenin, causing the complex to translocate into the nucleus (200,202). The androgen receptor – beta-catenin complex associates with TCF-4 and activates a number of Wnt target genes (200,202), including follistatin. Follistatin cross-communicates the signal from the AR-beta-catenin pathway to the TGF-beta signaling pathway, blocking signaling through the TGF-beta / Smad 2/3 (201,203). Follistatin plays an essential role in mediating the effects of testosterone on myogenic

differentiation (203,204). Jasuja et al (204) found that the administration of recombinant follistatin selectively increased muscle mass and decreased fat mass but had no effect on prostate growth. Recombinant follistatin and testosterone each regulated the expression of a large number of common genes in the skeletal muscle, but they differed substantially in the expression profile of genes activated in the prostate (204). Among the genes activated differentially by testosterone but not by follistatin in the prostate, Jasuja et al (204) identified polyamine pathway as an important signaling pathway. The polyamine pathway has been known to be involved in regulating prostate growth. Administration of testosterone in combination with an inhibitor of ornithine decarboxylase-1, a key enzyme in the polyamine pathway, to castrated male mice restored levator ani muscle mass but not prostate mass, indicating that ODC1 plays an important role in mediating the effects of testosterone on the prostate (Figure 8) (204). Therefore, combined administration of testosterone plus ODC1 inhibitor provides a novel approach for achieving selectivity of testosterone's anabolic effects on the muscle while sparing the prostate (204).
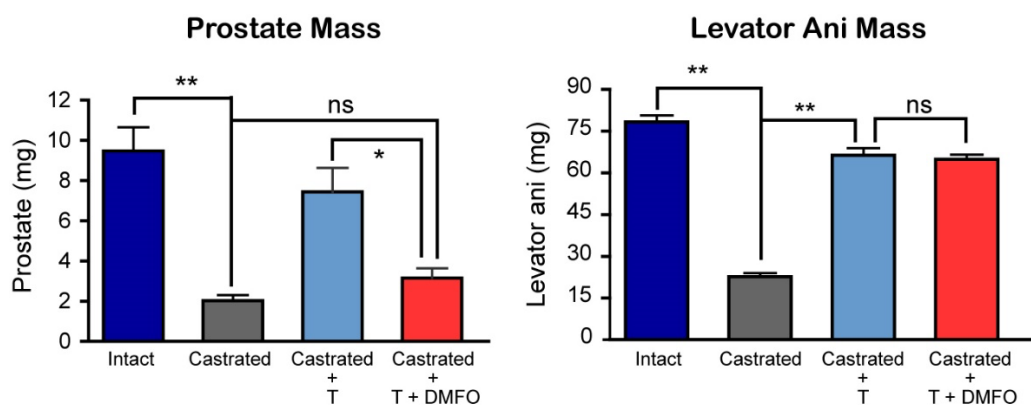

Figure 8. Testosterone plus ornithine decarboxylase 1 inhibitor as a selective prostate sparing anabolic therapy. Intact and castrated adult male mice were treated for 2-weeks with vehicle or testosterone with and without α-difluoromethylornithine (dfmo), a specific odc1 inhibitor, as follows: intact, castrated (cx), castrated + 15µg/day t (cx+t), castrated +15µg/day t+ 15µg/day dfmo (cx+t+dfmo). Levator ani weights (right panel) in mice treated with testosterone plus dfmo were similar to those in intact controls and testosterone-treated castrated mice. Prostate weights in castrated mice were lower than in intact controls and were restored by testosterone administration to levels seen in intact mice (left panel). Mice

treated with testosterone plus dfmo had significantly lower prostate weights than intact controls or castrated mice treated with testosterone alone, but not significantly different from those in castrated mice treated with vehicle alone. Thus, testosterone plus odc1 inhibitor could serve as prostate-sparing selective anabolic therapy. Reproduced with permission from jasuja et al. Aging cell. 2014 apr;13(2):303-10.

The Role of Steroid 5-Alpha Reductase and DHT in Mediating Androgen Effects in the Muscle

Although the enzyme steroid 5-alpha-reductase is expressed at low concentrations within the muscle (205,206), we do not know whether conversion of testosterone to dihydrotestosterone is required for mediating testosterone's effects on the muscle. Men with benign prostatic hypertrophy who are treated with a 5-alpha reductase inhibitor do not experience muscle loss (207). Similarly, individuals with congenital 5-alpha-reductase deficiency have normal muscle development at puberty (207). These data suggest that 5-alpha reduction of testosterone to DHT is not obligatory for mediating its effects on the muscle. However, all the kindred with steroid 5-alpha reductase deficiency that have been published to-date have had mutations of type 2 isoform of the enzyme. Similarly, finasteride is a weak inhibitor of only the type 2 isoform of the enzyme. The circulating concentrations of DHT in male patients with congenital mutation of type 2 steroid 5-alpha reductase enzyme or in men treated with finasteride are lower than eugonadal men; however, these patients still produce significant amounts of DHT and their circulating DHT concentrations are often in the lower end of the range in healthy young men. Long-term administration of dutasteride, a dual and potent inhibitor of both 5-alpha reductase isoforms, has not been associated with significant reductions in bone mineral density (207). This issue is important because if 5-alpha reduction of testosterone to DHT were not obligatory for mediating its anabolic effects on the muscle, then it might be beneficial to administer testosterone with an inhibitor of steroid 5-alpha reductase or to develop selective androgen receptor modulators that do not undergo 5-alpha reduction.

To determine whether testosterone's effects on muscle mass and strength, sexual function, hematocrit, prostate, sebum production, and lipids are attenuated when its conversion to DHT is blocked, we administered to healthy men, 21-50 years, a long-acting GnRH-agonist to suppress endogenous testosterone. We randomized them to placebo or dutasteride (dual inhibitor of steroid 5-alpha reductase type 1 and 2) 2.5-mg daily, plus 50, 125, 300, or 600-mg testosterone enanthate weekly for 20-weeks (208). Changes in lean and fat mass, leg-press and chest-press strength, were related to testosterone dose but did not differ between placebo and dutasteride groups (208). The relation between testosterone concentrations and the changes in lean body mass, maximum voluntary muscle strength, hematocrit, and sebum production was similar between dutasteride and placebo arms (Figure 9) (208). Changes in sexual-function scores, bone markers, prostate volume, and PSA did not differ between groups (208). These data indicate that testosterone's conversion to DHT is not essential for mediating its effects on muscle mass and strength, sexual function, hematocrit, or sebum production in men over the range of testosterone concentrations achieved in this trial (208). These data are consistent with studies that have reported that administration of steroid 5α-reductase inhibitors has little or no effect on muscle or bone mass (209-211). The isoforms of steroid 5α reductase enzyme also catalyze the 5α reduction of cortisol, progesterone, bile acids and other metabolites. In the central nervous system, 5α-reductase is the rate-limiting enzyme in the conversion of progesterone to allopregnanolone that serves as a positive allosteric modulator of gamma-aminobutyric acid (GABA) A receptors to modulate

neural pathways that regulate mood, affect, and cognition (212-214). Low levels of allopregnanolone have been implicated in the pathogenesis of some forms of depressive and anxiety disorders (215). An intravenous preparation of allopregnanolone was found to be efficacious and approved for the treatment of postpartum depression (216,217) and is being investigated for the treatment of other depressive disorders. Steroid 5α-reductase enzymes are also involved in cortisol metabolism and in the pathogenesis of metabolic disease (218).
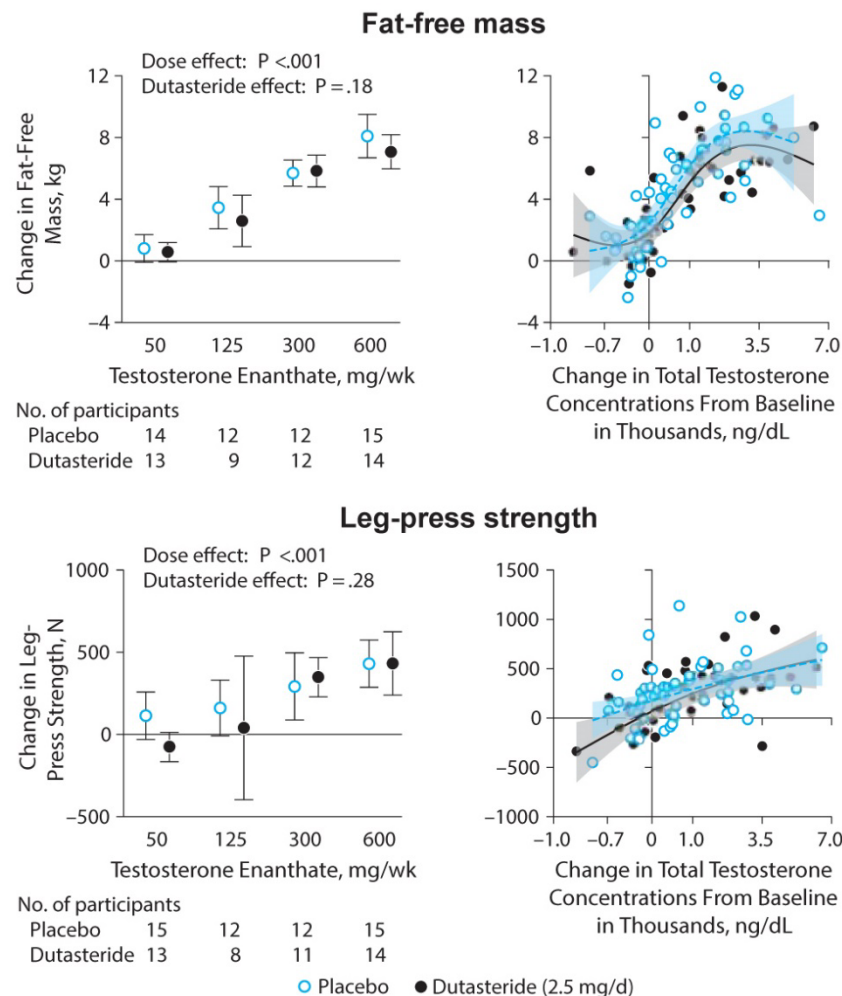
Figure 9. The Role of 5-alpha-Dihydrotestosterone in Men. In this randomized trial, healthy men, 18-50 years, received a long-acting GnRH-agonist to suppress endogenous testosterone. They were then randomized to either placebo or dutasteride (dual inhibitor of steroid 5-alpha reductase types 1 and 2) 2.5-mg daily, plus 50, 125, 300, or 600-mg testosterone enanthate weekly for 20-weeks (535). Changes in fat-free mass (upper panel) and leg-press strength (lower panel), were related to testosterone dose but did not differ between placebo and dutasteride groups (535). The relationship between change in total testosterone (TT) levels and change in fat-free mass and leg press strength (right panels) did not differ between men assigned to placebo or dutasteride arms. Reproduced with permission from Bhasin et al, JAMA. 2012 Mar 7;307(9):931-9.

The Role of CYP19A1 (Aromatase) in Mediating Testosterone's Effects on the Muscle

Studies of aromatase knockout mice have revealed higher fat mass and lower muscle mass in mice that are null for the P450-linked CYP19A1 aromatase gene (219). Similarly, humans with CYP19A1 mutations have decreased muscle mass and increased fat mass, and they exhibit insulin resistance (220). Data from these experiments of nature suggest that aromatization of testosterone to estradiol is important in mediating androgen effects on body composition. Finkelstein et al (221) have recently examined the relative roles of testosterone and estradiol in regulation of muscle and fat mass, and sexual function. These investigators found that testosterone's effects on lean mass, muscle size, and strength were not significantly attenuated when its conversion to estradiol was blocked by administration of an aromatase inhibitor (221).

REGULATION OF FAT MASS, FAT DISTRIBUTION, AND METABOLISM BY TESTOSTERONE

Testosterone is an important regulator of fat mass and distribution. Lowering testosterone concentrations by administration of a GnRH agonist increases fat mass, and testosterone administration in hypogonadal men decreases whole body fat mass (159,222-224). The loss of fat mass during testosterone administration occurs both in the appendices as well as the trunk and is distributed evenly between the superficial subcutaneous and deep intra-abdominal and intermuscular compartments (166,223). The effects of testosterone on whole body fat mass are related to the administered testosterone dose and the circulating testosterone concentrations (166,223).

Mechanisms of Testosterone's Effects on Fat Mass and Metabolism

The effects of testosterone on fat mass are mediated through its conversion to estradiol by the aromatase enzyme encoded by CYP19A1 (221). Men with inactivating mutations of CYP19A1 are characterized by increased fat mass, metabolic syndrome, hepatic steatosis, and insulin resistance (225-227). Estradiol replacement of male aromatase knockout mice reverses the adiposity and metabolic abnormalities associated with estrogen deficiency (228).

Testosterone regulates adipose tissue mass and metabolism through multiple mechanistic pathways. Androgens inhibit adipogenic differentiation of multipotent mesenchymal progenitor cells; these effects are blocked by androgen receptor blocker, bicalutamide (200,201,229). Testosterone regulates fat oxidation but does not appear to affect triglyceride secretion over short durations (230).

Testosterone, after its aromatization to estradiol, acts through the estrogen receptors in specific brain regions to regulate eating behavior, energy expenditure, and adipose tissue metabolism. The deletion of estrogen receptor α (ER-α) in specific brain regions is associated with adiposity, hyperphagia, and hypometabolism (231); estradiol acting through ER-α regulates eating behavior and energy expenditure differentially through actions on different hypothalamic neurons (231). Activation of estrogen receptor β (ER-β) by selective agonists inhibits weight gain, adiposity, increases energy expenditure and thermogenesis, and reverses hepatic steatosis in mice through direct effects on xenobiotic and bile acid receptors in the liver (232).

TESTOSTERONE AND SEXUAL FUNCTION IN OLDER MEN

Regulation of Sexual Function by Testosterone

Sexual function in men is a complex process that includes central mechanisms for regulation of sexual desire and arousal, and local mechanisms for penile tumescence, orgasm, and ejaculation (233). Primary effects of testosterone are on sexual interest and motivation (233-238). Testosterone replacement of young, androgen deficient men improves a wide range of sexual behaviors including frequency of sexual activity, sexual daydreams, sexual thoughts, feelings of sexual desire, attentiveness to erotic stimuli, and spontaneous erections (233-241). Kwan et al (237) demonstrated that androgen-deficient men have decreased frequency of sexual thoughts and lower overall sexual activity scores; however, these men can achieve erections in response to visual erotic stimuli. Hypogonadal men have lower frequency and duration of the episodes of nocturnal penile tumescence; testosterone replacement increases both the frequency and duration of sleep-entrained, penile erections (239-241). Although both orgasm and ejaculation are believed to be androgen-independent, hypogonadal men have decreased ejaculate volume and their orgasm may be delayed.

Although hypogonadal men can achieve erections, it is possible that achievement of optimal penile rigidity might require physiologic testosterone concentrations. Testosterone regulates nitric oxide synthase activity in the cavernosal smooth muscle (242). Testosterone administration in orchiectomized rats increases penile blood flow and has trophic effects on cavernosal smooth muscle (243-245).

In male rodents, all measures of mating behavior are normalized by relatively low testosterone levels that are insufficient to maintain prostate and seminal vesicle weight (246,247). Similarly, in men, sexual function is maintained at relatively low normal levels of serum testosterone (221,238,248). Testosterone's effects on libido are mediated through its conversion to estradiol (221).

Total and free serum testosterone levels are positively associated with sexual desire, erectile function and sexual activity in older men with unequivocally low testosterone levels and symptoms of sexual dysfunction (114). These findings suggest that low testosterone levels may contribute to impaired sexual functioning in older men.

Erectile dysfunction and androgen deficiency are two common but independently distributed, clinical disorders that sometimes co-exist in the same patient (112,113,233,249). Hypogonadism is a clinical syndrome that results from androgen deficiency (16); in contrast, erectile dysfunction is usually a manifestation of a systemic vasculopathy, often of atherosclerotic origin. Thus, androgen deficiency and erectile dysfunction have distinct pathophysiology. Eight to ten percent of middle-aged men presenting with erectile dysfunction have low testosterone levels (113,249-251).

Clinical Trials of the Effects of Testosterone Therapy on Sexual Function of Older Men with Low Circulating Testosterone Concentrations

In open-label trials, testosterone treatment has been shown to improve sexual function in young men with classical hypogonadism due to disorders of the hypothalamus, pituitary, or testes (159,252). However, previous trials evaluating the benefits of testosterone therapy in men 60 years and older with age-related decline in testosterone levels on sexual functioning have yielded inconsistent results (253), with some studies showing improvement (254,255), while others have suggested no clear benefit (23). The inconsistencies in these previous studies are due to several factors, including small sample sizes, inclusion of men who were not clearly hypogonadal or did not have sexual symptoms, inclusion of men with heterogeneous sexual disorders, variable treatment durations, and the use of outcomes assessment tools that had not been rigorously validated.

In a small number of placebo-controlled trials of testosterone that have been conducted in men with sexual symptoms and low testosterone levels (24,26,161), testosterone replacement has been associated with a small but significant increase in overall sexual activity, sexual desire, erectile function, and sexual satisfaction. A meta-analysis of these placebo-controlled trials found that testosterone replacement of hypogonadal men is associated with a small but significant increase in sexual desire [standardized mean difference (SMD): 0.17; 95% CI, 0.01, 0.34], erectile function (SMD: 0.16; 95% CI, 0.06, 0.27), and sexual satisfaction (SMD: 0.16; 95% CI, 0.01, 0.31) (256).

The Sexual Function Trial of the TTrials determined the efficacy of testosterone treatment for 1-year on sexual function in symptomatic, community-dwelling, older men ≥65 years with low testosterone levels (26). Testosterone administration for 1-year to raise testosterone concentrations into a range that is mid-normal for healthy young men was associated with significant improvements in sexual activity, desire, and erectile function (257). The treatment effects tended to wane over time, and the effect on erectile function was substantially smaller than that reported with phosphodiesterase 5 inhibitors (258). The magnitude of increase in testosterone levels was related to the improvements in sexual activity and desire, but not erectile function (257). There was no clear testosterone threshold level of effect.

Testosterone does not improve sexual function in middle-aged and older men who have normal testosterone levels and do not have any sexual symptoms (23). Testosterone replacement therapy does not improve ejaculatory function in men with ejaculatory disorder (259).

It had been speculated that testosterone administration might improve erectile response of men with ED to selective phosphodiesterase inhibitors (260-262). To determine whether the addition of testosterone to a phosphodiesterase-5-inhibitor improves erectile response, we conducted a randomized, placebo-controlled trial (263), in men, 40-to-70 years, with erectile dysfunction and low total testosterone< 11.5 nmol/L (330ng/dL) and/or free testosterone <173.5 pmol/L (50 pg/mL). All participants were initially started on sildenafil alone and the sildenafil dose was optimized based on their response during a 3 to 7-week run-in period (263). The participants were then randomized to 10-g testosterone or placebo gel for 14-weeks in combination with the optimized sildenafil dose (263). The administration of sildenafil alone was associated with substantial increases in erectile function domain (EFD) score and total and satisfactory sexual encounters (263). However, the change in EFD score in men assigned to testosterone plus sildenafil did not differ significantly from that in men assigned to placebo plus sildenafil (263). Changes in total and successful sexual encounters, quality-of-life, and marital-intimacy did not differ between testosterone and placebo groups. Even among the subsets of men with baseline testosterone <250 ng/dL or those without diabetes, there were no significant differences in EFD scores between the two arms (263). Another placebo-controlled trial of men with erectile dysfunction who were non-responders to tadalafil also did not show a greater improvement in erectile function in men assigned to the testosterone arm than in those assigned to the placebo arm (262). Thus, in randomized trials, the addition of testosterone to PDE5Is has not been shown to improve erectile function in men with erectile dysfunction (262,263).

Synopsis of The Effects of Testosterone on Sexual Function

In older hypogonadal men with low sexual desire, testosterone treatment improves sexual desire, erectile function, and overall sexual activity. Androgen deficiency is an important cause of low sexual desire disorder (233). Therefore, serum testosterone concentrations should be measured in the diagnostic

evaluation of hypoactive sexual desire disorder as well as erectile dysfunction, recognizing that low sexual desire is often multifactorial; systemic illness, relationship and differentiation (the ability of individuals in a relationship to maintain their distinct identities) issues, depression, and many medications can be important antecedents or contributors to low sexual desire and sexual dysfunction.

TESTOSTERONE EFFECTS ON BONE MINERAL METABOLISM

The Effects of Androgen Deficiency on Bone Mass

Testosterone deficiency is associated with a progressive loss of bone mass (264-267). In one study performed in sexual offenders (264), surgical orchiectomy was associated with a progressive decrease in bone mineral density of a magnitude similar to that seen in women after menopause. Similarly, androgen deficiency induced by the administration of a GnRH agonist, surgical orchiectomy, or an androgen antagonist for the treatment of prostate cancer leads to loss of bone mass (265-267) and an increase in fracture risk (268,269), which is related to the dose of GnRH agonist and the degree of testosterone suppression (270). In male rats, surgical orchiectomy or androgen blockade by administration of an androgen receptor antagonist is associated with loss of bone mass (271).

Androgen deficiency that develops before the completion of pubertal development is associated with reduced cortical and trabecular bone mass (272,273). During the pubertal years, bone accretion, and bone length and thickness is regulated by sex steroids. During puberty, sex hormones slow long bone growth and accelerate axial growth. Prepubertal sex hormone deficiency allows continued long bone growth and slows axial growth resulting in longer limbs and a shorter trunk (eunuchoidal proportions) (274). Sex differences in bone width are also established during pubertal development. Men increase bone width by periosteal bone formation and women mostly by endocortical apposition (275). Young men with constitutional delay of puberty have lower bone mineral density (276), which does not improve spontaneously 2 years later (277). Indeed, men with hip fractures have been shown to have smaller femoral head diameters, which may potentially be related to delayed puberty (278). Therefore, individuals with sex-steroid deficiency before or during peri-pubertal years may end up with suboptimal peak bone mass and increased lifetime fracture risk. Similarly, men with acquired androgen deficiency have lower bone mineral density than age-matched controls (155).

Clinical Trials on The Effects of Testosterone Therapy on Bone in Young, Hypogonadal Men

Testosterone therapy of healthy, young, hypogonadal men is associated with significant increases in vertebral bone mineral density (156,279-283). However, bone mineral density is typically not normalized after 1-2 years of testosterone replacement therapy (156). Some hypogonadal patients included in these testosterone trials had panhypopituitarism and also suffered from growth hormone deficiency. It is possible that concomitant GH replacement might be necessary for restoration of normal bone mineral density. Excessive glucocorticoid replacement might also contribute to bone loss in these patients. In addition, some participants had experienced testosterone deficiency before the onset and completion of pubertal development; the individuals who develop androgen deficiency during the critical pubertal developmental window of bone accretion, may end up with decreased peak bone mass, and testosterone administration may not be able to restore bone mass to levels seen in eugonadal age-matched controls. Many testosterone replacement trials were less than 3 years in duration, and it is possible that a longer period of testosterone administration might be necessary to achieve maximal improvements in bone mineral density. Indeed, Behre et al (279) reported that bone mineral density in some

hypogonadal men continued to increase even after many years of testosterone treatment using a scrotal transdermal patch and reached the levels expected for age-matched eugonadal controls.

Cross-Sectional Studies of the Relationship Between Sex-Hormone Concentrations and Osteoporosis in Older Men

The age-related decline in sex hormones is associated with age-related changes in bone mineral density and increased risk of osteoporotic fractures (131-136,284,285). Older men with hip fractures have lower testosterone levels than age-matched controls (286). Bioavailable testosterone levels have been found to be better predictors of fracture risk than total testosterone levels (287). Interestingly, a U-shaped association between endogenous testosterone concentrations and incident fractures was recently observed, with midrange plasma testosterone levels being associated with lower incidence of any fracture and with hip fracture compared to lower or higher testosterone (288). Men with osteoporosis have been found to have lower DHT levels than those without osteoporosis (289). In the Cardiovascular Health Study, in which testosterone and DHT levels were measured by liquid chromatography–tandem mass spectrometry, circulating DHT, but not testosterone, was found to be negatively associated with hip fracture risk in men (290).

In epidemiologic studies, estradiol levels are more strongly associated with bone mineral density of the spine, hip, and distal radius than total testosterone levels (132,134,135,285). Men with low bioavailable estrogen have increased risk of non-vertebral fracture which is increased further in those with low bioavailable estrogen, low bioavailable testosterone as well as high SHBG (287) suggesting a complex interplay of these hormones in fracture resilience. Mendelian randomization analysis have found that increased genetically determined estradiol levels are associated with increase in lumbar spine bone mineral density (291) and lower fracture risk (292). The *CYP19A1* alleles associated with higher estradiol levels are associated with higher bone mineral density (291).

Clinical Trials of the Effects of Testosterone Therapy on Bone of Middle-Aged and Older Men with Low Circulating Testosterone Concentrations

Earlier studies of testosterone replacement of relatively healthy older men that examined the effects of testosterone on bone mineral density reported inconsistent results (188,189,293,294). One study found greater increases in vertebral bone mineral density in the testosterone arm of the trial than in the placebo arm, while another study did not find any significant differences between the change in vertebral or femoral bone mineral density between testosterone and placebo groups (294). A meta-analysis of randomized trials found a significantly greater increase in lumbar bone mineral density but not in femoral bone mineral density in the testosterone arms of trials that used intramuscular testosterone than in placebo arms (Figure 10) (295); transdermal testosterone had no significant effect.
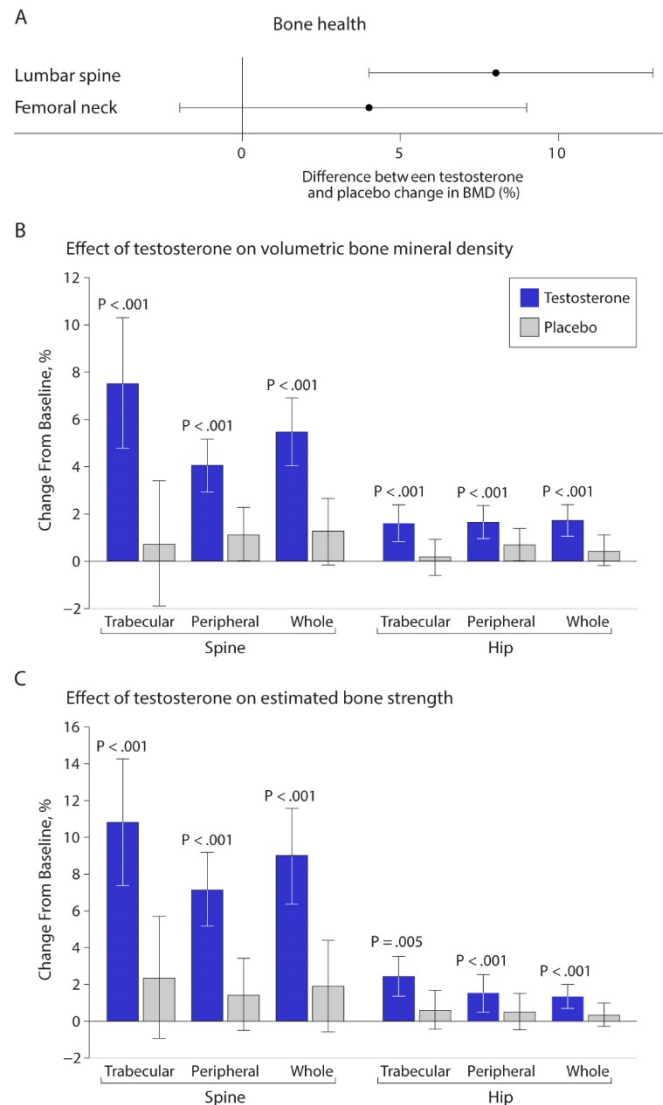
Figure 10. The effects of testosterone therapy on bone health in intervention trials. Panel A shows the effects of testosterone therapy on lumbar and femoral bone mineral density in a meta-analysis of randomized trials (data derived from a meta-analysis by Tracz et al, J Clin Endocrinol Metab. 2006;91(6):2011-6.; figure adapted with Spitzer et al. Nat Rev Endocrinol. 2013;9(7):414-24). Panels B and C show the effects of testosterone replacement for 12 Months on volumetric bone mineral density and estimated bone strength of trabecular, peripheral, and whole bone of the spine and hip, as assessed by quantitative computed tomography (figure reproduced with permission from Snyder et al. JAMA Intern Med. 2017;177(4):471-479).

The Bone Trial of the TTrials determined the effects of testosterone replacement for 1-year in men 65 years or older with low testosterone levels on volumetric bone mineral density and bone strength using quantitative computed tomography (296). This trial found significantly greater increases in volumetric bone mineral density and estimated bone strength in the testosterone arms compared to placebo; specifically, these increases were most prominent in the spine than hip and more in trabecular than peripheral bone (Figure 10). The treatment effects on volumetric bone density and bone strength observed

in the TTrials compare favorably with those reported in trials of bisphosphonates and some selective estrogen receptor modulators.

The T4Bone substudy of the T4DM trial determined the effect of 24 months of testosterone treatment on bone microarchitecture and bone mineral density of men aged 50 years or older enrolled in a community-based lifestyle program using high resolution-peripheral quantitative computed tomography (297). Compared to placebo, testosterone treatment increased cortical and total bone mineral density of the tibia and radius, as well as cortical area and thickness at both sites. Testosterone treatment also increased areal thickness at the lumbar spine (297).

Future studies are needed to determine whether these improvements from testosterone treatment are associated with reduced fracture risk in older men with low testosterone levels.

Mechanisms of Androgen Action on the Bone

Testosterone increases bone mass by several mechanisms (298). Short-term studies of androgen replacement have shown inconsistent increases in markers of bone formation, but a more consistent reduction in markers of bone resorption (283,298-300). These observations suggest that testosterone increases bone mineral density in part through its aromatization to estrogen, which inhibits bone resorption. Estrogen deficiency contributes to increased bone resorption and remodeling by multiple mechanisms. Estrogens regulate the activation frequency of bone functional basic multicellular units, the duration of the resorption phase and the formation phase, and osteoclast recruitment (301). The protective effects of estrogen on bone in both male and female mice during growth and maturation are mediated largely through estrogen receptor-alpha (302-308). 17β-estradiol has also been shown to increase connexin-43 based intracellular communication which may modulate the bone response to mechanical loading in osteocytes (309).

Dias and colleagues found that treatment with testosterone for 12-months improved lumbar spine bone mineral density compared to placebo but not in men treated concomitantly with anastrozole, suggesting that aromatization of testosterone to estrogen may be required for maintaining bone mineral density (310). Similarly, another recent study found significant reduction in spine bone mineral density in men treated with testosterone and anastrozole for 16-weeks that was independent of testosterone dose (311). In addition, treatment with lower testosterone doses were associated with greater increases in bone turnover markers; an effect that was significantly greater in combination with anastrozole. DHT suppresses osteoclast formation *in vitro* via NF-kB ligand (RANKL) mediated effects, comparable to estradiol (312); the clinical significance of DHT in suppressing bone resorption is incompletely understood.

Testosterone also directly stimulates osteoblastic bone formation. Androgen receptors have been demonstrated on osteoblasts and on mesenchymal stem cells (313). Testosterone stimulates cortical bone formation (314). Sclerostin is secreted by osteocytes and inhibits osteoblast differentiation. Sclerostin was found to be negatively related to total and free testosterone in men with idiopathic osteoporosis (312). Hypogonadal men have higher serum sclerostin levels than eugonadal men, and DHT directly suppresses sclerostin production in cultured human osteocytes through an AR-mediated mechanism (315). Testosterone also stimulates the production of several growth factors within the bone, including IGF-1; these growth factors may contribute to bone formation (316). Leydig cells in the testis secrete insulin-like peptide 3 (INSL3) in addition to testosterone. INSL3 has been reported to have a negative association with sclerostin in specific populations and INSL3 downregulates sclerostin protein expression in cultured osteocytes (317). Osteocalcin secreted by osteoblasts acts on Leydig cells through the GPRC6A receptor, suggesting a possible feedback mechanism for bone-testis

crosstalk (318). Testosterone increases muscle mass, which may indirectly increase bone mass by increased loading. Testosterone might inhibit apoptosis of osteoblasts through non-genotropic mechanisms (319,320). In addition to its effects on bone mineral density, testosterone might reduce fall propensity because of its effects on muscle strength and reaction time.

We have shown that testosterone has dose dependent effects on erythropoiesis (321) possibly through increased erythropoietin and reduced hepcidin (322). Hematopoietic cells and bone cells are interdependent and support each other at different stages in development (323,324). Androgen deficiency seems to favor hematopoietic precursor differentiation to an osteoclast fate (312), which is consistent with the decreased bone resorption observed with testosterone supplementation (275,287-289). We have shown that older men in the MrOS study with accelerated bone density loss (>0.5%/year) have increased risk of anemia (325), and that anemia increases the risk of non-spine fractures independent of bone density (326). In a prospective analysis of the Cardiovascular Health Study, we have recently shown that men with anemia and, separately, men with decreasing hemoglobin were at increased risk of hip fracture (327). Low endogenous testosterone levels have been associated with lower hemoglobin (328), which is reversible with testosterone supplementation (329). It is possible that testosterone sufficiency is required for a healthy hematopoietic niche in men, which is then able to support a favorable microenvironment for bone health.

In men androgens and estrogens both play independent roles in regulating bone resorption (301). Estradiol levels above 10 pg/ml are generally believed to be sufficient to prevent increases in bone resorption and decreases in BMD in men (311).

Synopsis of The Effects of Testosterone on Bone

Testosterone replacement has been shown to increase vertebral and femoral bone mineral density, and bone strength in older men with unequivocally low testosterone levels (16). Testosterone increases bone mass by multiple mechanisms. Testosterone's aromatization to estrogen plays an important role in regulating bone health in men. Testosterone's effects on fracture risk have not been studied.

TESTOSTERONE EFFECTS ON COGNITIVE FUNCTION

Cross-Sectional and Longitudinal Studies Correlating Sex-Hormone Levels and Cognitive Function

Several lines of evidence suggest that testosterone regulates several domains of cognition, sexually dimorphic behaviors, mood, and affect, and the neuropathology of Alzheimer's Disease (AD). Testosterone is aromatized to estrogen in the brain, and some effects of testosterone on cognition might be mediated through its conversion to estradiol. Additionally, androgen receptors are expressed in the brain (330), and androgen effects on brain organization during development (331,332) are mediated through androgen receptor. Androgens increase neurite arborization, facilitating intercellular communication (331-334). Testosterone is metabolized in neurons as well as in glial cells to DHT, which is further converted reversibly in some cell types such as type 1 astrocytes to 5α-androstane-3α,17β-diol (335), which is a potent modulator of GABA on $GABA_A$ receptors but a weak ligand for AR and ER (336). The 3β isomer of androstanediol, 5α-androstan-3β,17β-diol, is also synthesized in the brain; this steroid is a ligand for ERβ (337). Thus, testosterone treatment may potentially expose the brain to a range of biologically active metabolites, all of which may contribute to the observed responses. Testosterone also affects serotonin, dopamine, acetylcholine (333), and calcium signaling (334). Thus, testosterone could influence cognitive function and the development and

progression of AD neuropathology through multiple mechanistic pathways.

The age-related decline in serum testosterone levels has been associated with impairment in cognitive function (338). Androgens effects on cognitive function are domain-specific. For instance, observations that men outperform women in a variety of visuo-spatial skills suggest that androgens enhance visuo-spatial skills (339). In !Kung San hunter-gatherers of Southern Africa, testosterone, but not estradiol, levels correlated with better spatial ability and with worse verbal fluency (340). Women with congenital adrenal hyperplasia with high androgen levels score higher on tests of spatial cognition than their age- and gender-matched siblings (341). 46, XY rats with androgen insensitivity perform worse on tests of spatial cognition than their age-matched controls (342). Other studies have reported a complex relationship between androgen levels and spatial ability (123,343-345). Circulating levels of dihydrotestosterone, a metabolite of testosterone that is not converted to estrogen, positively correlated with verbal fluency (340). Barrett-Conner et al (122) found positive associations between total and bioavailable testosterone levels, and global cognitive functioning and mental control, but not with visuospatial skills. In the Baltimore Longitudinal Study of Aging (346), higher free testosterone index was associated with better scores on visual and verbal memory, visuospatial functioning, and visuomotor scanning. Men with low testosterone levels had lower scores on visual memory and visuospatial performance (346); however, some studies have shown no association of serum testosterone levels with domains of visual and verbal memory, and executive function in older men (347,348). In the Concord Health and Aging in Men Project, the authors found that changes in serum testosterone levels over time, rather than baseline testosterone levels, were predictive of cognitive decline (338).

The Potential Role of Testosterone in the Pathobiology of Alzheimer's Disease

A large body of preclinical and epidemiologic data shows that testosterone acts as a negative regulator of endogenous Aβ amyloid accumulation in the brain, attenuates tau phosphorylation, reduces neuro-inflammation, exerts neuronal protective effect in response to injury and disease, and promotes neuronal regeneration and connectivity. However, the randomized trials data generated largely in community dwelling middle-aged and older adults without cognitive deficits or Alzheimer's Disease neuropathology have been inconclusive for reasons that are discussed below.

Testosterone acts as a negative regulator of endogenous Aβ amyloid accumulation in the brain through multiple mechanisms. Surgical orchiectomy of male rats is associated with increased accumulation of Aβ amyloid in the brain; the accumulation of Aβ amyloid in surgically orchiectomized rats is prevented by DHT administration but not by estradiol administration (349-351). In male Brown-Norway rats, age-related decreases in testosterone and DHT are associated with increased brain levels of Aβ amyloid (350). Testosterone promotes the conversion of amyloid precursor protein (APP) to soluble APP-alpha rather than A beta amyloid. Consistent with these findings, prolonged treatment of cultured cortical neurons and neuroblastoma cell lines with testosterone resulted in increased production of soluble sAPP-α and decreased production of Aβ amyloid (349). This effect of testosterone on the processing of APP is mediated in part through its aromatization to estradiol (352). However, there is strong evidence of mediation through a direct androgen receptor (AR)-mediated pathway as well (350-353).

In 3XTg-AD mouse model of Alzheimer's Disease, orchiectomy at age 3 months is associated with

significantly increased accumulation of Aβ amyloid in hippocampus CA1, amygdala, and subinculum at age 6 months (352,353). DHT treatment of orchiectomized mice prevents the accumulation of Aβ amyloid as well as deterioration of spontaneous alternation behavior (353). DHT also reduces Tau-phosphorylation in orchiectomized triple transgenic mouse model of AD (352). Androgens upregulate the expression of neprilysin, the enzyme that catalyzes the degradation and clearance of Aβ amyloid in neuronal cells (354) and decrease Aβ amyloid accumulation (355).

Testosterone also attenuates AD-like tau pathology. In gonadectomized mice, testosterone as well as estradiol reduce tau phosphorylation (356,357). Androgens also reduce tau phosphorylation induced by acute heat shock and injury in male rats independent of estradiol (358).

Testosterone exerts neuroprotective effects in many brain regions. Androgens promote neuronal viability during neural development as well as in adult brain following mechanical injury and disease-related toxicity (359-361). Testosterone protects motor neurons in the spinal cord following axotomy (359-361). In this experimental model, testosterone treatment accelerates the rate of nerve regeneration and attenuates neuronal loss (359,360,362-364).

Testosterone exerts neuroprotective effects across the lifespan in brain areas susceptible to neurodegeneration in AD (365-368). Thus, in cultured neurons, testosterone reduces neuronal apoptosis induced by oxidative stress and Aβ amyloid (369-371).

Testosterone promotes neuronal growth, connectivity, and functioning. Testosterone increases neurite arborization, and synapse formation facilitating intercellular communication (372-375). Testosterone also has nongenomic effects, and affects serotonin, dopamine, acetylcholine and calcium signaling (376-378). Androgen receptors are expressed in the brain,

and androgen effects on organization of the brain during development are likely mediated directly through AR. Some additional effects of testosterone are mediated through its conversion to estradiol.

Testosterone also exerts protective effects against neuroinflammation. Orchiectomy as well as obesogenic diet are each associated with increased expression levels of proinflammatory cytokines TNF-alpha and IL-1beta in the cerebral cortex in middle-aged male rats (370). The castration-induced upregulation of proinflammatory cytokines TNFα and IL-1β effect is prevented by testosterone supplementation (370). Similar stimulatory effects of testosterone on the expression of proinflammatory cytokines were observed in mixed glial cell cultures *in vitro* (370). 5α Dihydrotestosterone inhibits interleukin-1α or tumor necrosis factor α-induced proinflammatory cytokine production via androgen receptor-dependent inhibition of nuclear factor κB activation (371). Low testosterone also is associated with increased macrophage infiltration in sciatic nerve in castrated male rats (371). The mechanisms of these protective effects of testosterone on neuroinflammation are incompletely understood but appear to require both androgen receptor and estrogen receptor-mediated pathways (371).

Epidemiological Data on the Association of Testosterone Levels with Cognitive Function and AD Pathology

Some but not all epidemiologic studies have found an association between low circulating testosterone levels and AD (346,379-384); the relation appears to be stronger between free testosterone levels and the risk of AD than between total testosterone and AD (346). The strength of the association between testosterone and AD is affected by apolipoprotein ε4 genotype, a genetic risk factor for AD (380); men with one or more ε4 alleles have lower testosterone levels and a higher risk of AD than men without an ε4 allele (380).

In longitudinal follow-up of male participants of the Baltimore Longitudinal Study on Aging (346), the men who were healthy at baseline and developed a clinical diagnosis of AD had significantly lower free testosterone levels than those who did not develop AD. The age-related decline in circulating free testosterone levels preceded the clinical diagnosis of AD by nearly 10 years (346).

Rosario *et al.* (384) found that low brain levels of testosterone were associated with increased risk of AD in men. In human postmortem brain tissue from neuropathologically normal men, tissue levels of testosterone but not E_2 showed an age-related decline (384). The brain tissue levels of testosterone were significantly lower in AD cases as compared with neuropathologically normal cases after controlling for age (384).

Epidemiologic investigations of the association of circulating testosterone levels with age-related changes in cognitive function are in agreement that androgens effects on cognitive function are domain-specific. Generally, men with low testosterone levels perform less well than those with normal testosterone levels on tests of verbal fluency, visuospatial abilities, verbal memory, and executive function (121,122,385-388). Some inconsistency in findings across studies is likely related to heterogeneity of study populations, lack of standardization of cognitive assessments across studies, inaccuracy and imprecision of testosterone immunoassays, and the use of variable thresholds of testosterone levels to define "low". Some studies have suggested a curvilinear relation between testosterone levels and cognitive function; both low and high testosterone levels are associated with worse function suggesting that there may be an optimal level at which cognitive performance is optimized (386).

Clinical Trials Data

No adequately powered randomized placebo-controlled trials of testosterone replacement have been conducted in men with AD (389-392). The clinical trials data on the effects of testosterone on cognition have provided conflicting results; these trials were limited by their small size, inclusion of men who were not clearly hypogonadal and who did not have cognitive impairment or AD neuropathology, and use of outcomes that were not directly related to AD phenotype. Some studies have reported improvements in verbal memory and visuospatial skill while others found no effect (389,391-393).

The Testosterone Trials, a set of 7 coordinated trials of community-dwelling older men with unequivocally low testosterone levels, measured using liquid chromatography tandem-mass spectrometry (LC-MS/MS), showed no significant effect on delayed paragraph recall – the primary outcome of the trial (26,390). Post hoc analysis of the TTrials data showed small but significant improvement in executive function (390). The TTrials had many attributes of good trial design - prospective allocation of participants, parallel groups, blinding, and high retention rates, but progression of AD was not a primary aim of the trial (26). The trial's duration of one year was not long enough to evaluate effects on clinically meaningful measures of cognitive function or AD pathology. The trial did not include any measures of AD pathology, including A beta amyloid or Tau-protein or blood or CSF markers of AD. The participants in this well conducted trial were not selected prospectively based on cognitive deficits or risk of AD. A few small testosterone supplementation studies (sample size varying from 11 to 47) of 6 weeks to 6-month duration in men with cognitive impairment of AD have reported modest improvements in verbal and spatial memory but the small sample sizes, short intervention durations, variable eligibility criteria, and inclusion of men without confirmed AD, and inclusion of men with

normal testosterone levels limit the interpretability of these data (391,392).

In a double-blind randomized placebo-controlled trial, Huang *et al* investigated the effect of testosterone administration for 3-years on multiple domains of cognitive function in a large cohort (n=280) of men 60 years and older with low or low-normal testosterone levels (394). In this trial of older, cognitively healthy men, testosterone administration was not associated with significant improvement in any domain of cognitive function (Figure 11). These findings are similar to another recent placebo-controlled clinical trial conducted in older men 65-years and older (n=493) with low testosterone levels, which showed that treatment with testosterone for 1-year was not associated with improved cognitive function or memory (Figure 11) (390). Sensitivity analysis that was limited to men with minimal cognitive impairment also did not find significant differences in measures of cognition between the testosterone and placebo groups (390).
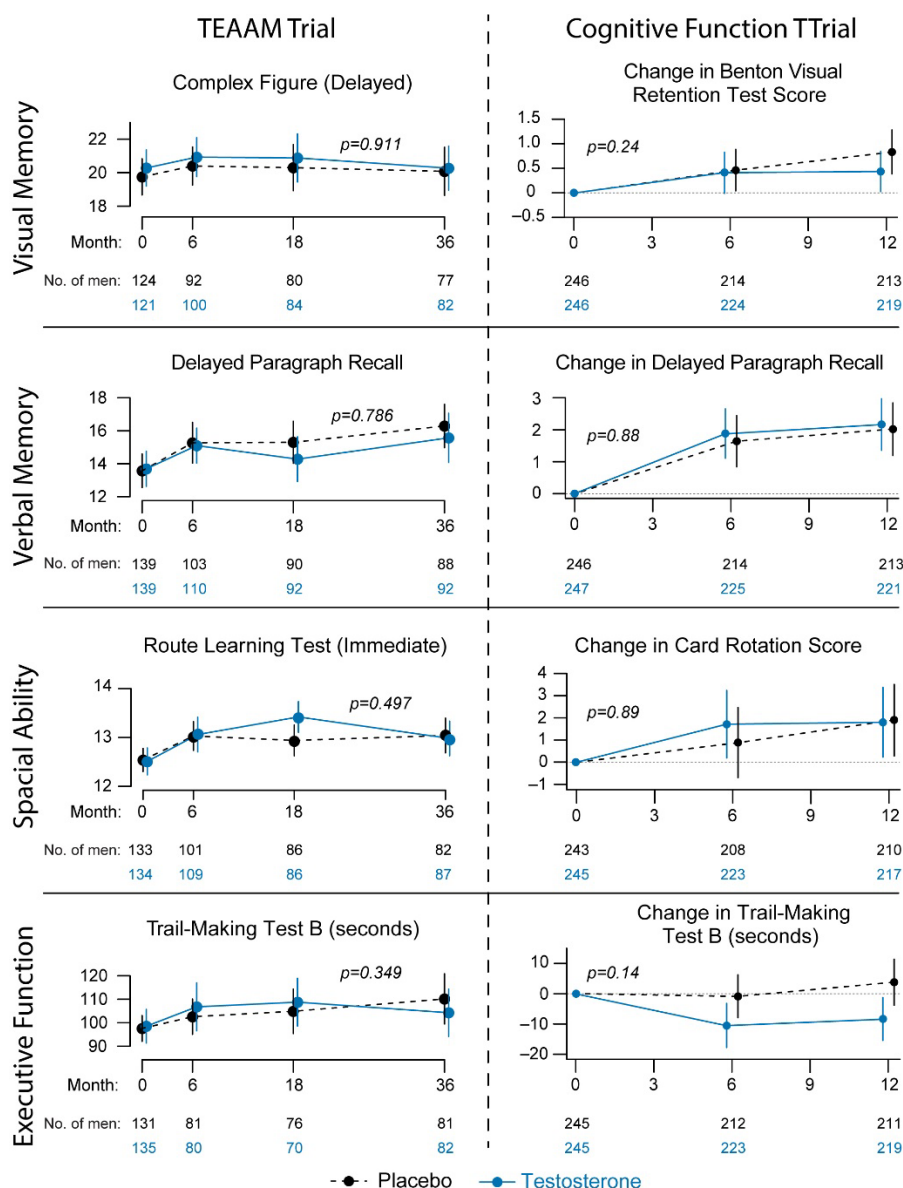

Figure 11. Effects of testosterone therapy on cognition domains in older men.

Left panels show the long-term effects of testosterone therapy in visual and verbal memory, spacial ability and executive function in the TEAAM trial (36 months of treatment). Data displayed as baseline and post-randomization cognitive function test scores by group and study visit. Error bars are 95% CIs for mean scores and p-values are for the estimated difference between treatment effects, controlling for baseline values, age, and education (figure adapted from Huang et al. J Clin Endocrinol Metab. 2018;103(4):1678-1685.) Right panels show adjusted mean change from baseline to 6 months and 12 months for men with age-associated memory impairment by treatment group in cognition domains in the Cognitive Function Trial of the TTrials (12 months duration; figure adapted from Resnick et al. JAMA. 2017;317(7):717-727).

Synopsis of the Effects of Testosterone on Cognition

In spite of the robust preclinical data that testosterone acts as a negative regulator of endogenous Aβ amyloid accumulation in the brain, attenuates tau phosphorylation, reduces neuro-inflammation, exerts neuronal protective effect in response to injury and disease, and promotes neuronal regeneration and connectivity and some epidemiologic evidence that decline in testosterone levels increases the risk of incident clinical AD, the randomized trial data on the effects of testosterone on cognition is highly equivocal. The randomized clinical trials in community dwelling older adults without cognitive deficit or AD neuropathology have not found clinically meaningful improvements in cognitive function. The inconsistency in findings cannot yet be interpreted as conclusive evidence that there is no effect. Limitations of previous studies include limited sample sizes, inclusion of men with no clear cognitive deficit or AD neuropathology, the use of a variety of neuropsychological tests that are not clinically meaningful in the context of AD or dementia; the use of differing protocols in clinical trials. The effects of testosterone therapy on clinically important outcomes in men with cognitive impairment have not been studied. The efficacy of testosterone replacement in men with cognitive impairment, such as in patients with Alzheimer's disease, needs further investigation in larger randomized controlled trials.

TESTOSTERONE EFFECTS ON MOOD, ENERGY, AND HEALTH-RELATED QUALITY OF LIFE

Circulating testosterone concentrations have not been consistently associated with major depressive disorder in men (128,129,395-398). Rather, testosterone levels appear to be associated with a late-life low grade persistent depressive disorder (dysthymia) (128,129,395-398). Intervention trials have failed to demonstrate statistically significant or clinically meaningful improvements in patients with major depressive disorder (399). Placebo-controlled trials of testosterone in men with refractory depression also have not consistently shown a beneficial effect of testosterone (399-402). A meta-analysis of randomized trials reported modest improvements in depressive symptoms in testosterone-treated men compared to placebo-treated men (403), but there is no convincing evidence that testosterone treatment can induce remission in men with major depressive disorder (404). Two small trials in men with dysthymia have reported greater improvements in depressive symptoms in testosterone-treated men than in placebo-treated men (405,406). Adequately powered long-term randomized trials are needed to determine whether testosterone replacement therapy can induce remission in older hypogonadal men with late-onset, low grade persistent disorder (dysthymia).

There is anecdotal evidence that androgens improve energy and reduced sense of fatigue (407). Testosterone administration increases hemoglobin and red cell mass, stimulates 2, 3 DPG concentrations thereby shifting the oxygen – hemoglobin dissociation curve favorably to improve greater oxygen delivery, and induces muscle capillarity (322,408,409). Additionally, testosterone stimulates mitochondrial

biogenesis and mitochondrial quality (410). All of these adaptations would be expected to improve net oxygen delivery to the muscle, improve aerobic performance, and reduce fatigability. The effects of testosterone on fatigue and vitality have been studied in some randomized trials. Endogenous levels of total and free testosterone are not significantly associated with vitality in older hypogonadal men with sexual dysfunction, diminished vitality, and/or mobility limitation (114). In the Vitality Trial of the TTrials, testosterone treatment for 1-year did not improve vitality in older men with low vitality measured using the Functional Assessment of Chronic Illness Therapy (FACIT)-scale but men receiving testosterone did report a small but statistically significant improvement in mood. These findings are consistent with other randomized controlled studies (23,171,190), showing no clear benefit on fatigue and health-related quality of life with testosterone therapy.

Supraphysiologic doses of androgenic steroids such as those abused by athletes and recreational bodybuilders have been associated with aggressive responses to provocative situations (411), increased scores on Young's manic scale, and with affective and psychotic disorders in some individuals (412); these adverse effects have not been reported with physiologic testosterone replacement.

By improving some aspects of physical and sexual function, testosterone supplementation might be expected to improve health-related quality of life. However, only a few small trials have evaluated the effects of testosterone on health-related quality of life. A systematic review of a small number of randomized trials has not revealed a significant improvement in composite health-related quality of life scores, but testosterone therapy improves scores on the physical component of MOS SF-36 (16,159).

Risks of Testosterone Administration in Older Men

Short-term testosterone administration in healthy, young, androgen-deficient men with classical hypogonadism is associated with a low frequency of relatively mild adverse effects such as acne, oiliness of skin, and breast tenderness. However, the long-term risks of testosterone supplementation in older men are largely unknown. There are several unique considerations in older men that may increase their risks of testosterone administration. Serum total and free testosterone concentrations are higher in older men than young men at any dose of testosterone therapy, due to decreased testosterone clearance in older men (61). Older men exhibit greater increments in hemoglobin and hematocrit in response to testosterone administration than young men (321), adjusting for testosterone dose. Altered responsiveness of older men to testosterone administration might make them susceptible to a higher frequency of adverse events, such as erythrocytosis, or to unique adverse events not observed in young hypogonadal men. The baseline prevalence of disorders such as prostate cancer, benign prostatic hypertrophy, and cardiovascular disease that might be exacerbated by testosterone administration is high in older men; therefore, small changes in risk in either direction could have enormous public health impact. Furthermore, the clustering of co-morbid conditions in the frail elderly might render these men more susceptible to the adverse effects of testosterone therapy than healthy young hypogonadal men.

The contraindications for testosterone administration include history of prostate or breast cancer (16). Benign prostatic hypertrophy by itself is not a contraindication, unless it is associated with severe symptoms, as indicated by IPSS symptom score of greater than 21. Testosterone should not be given without prior evaluation and treatment to men with baseline hematocrit greater than 50%, severe untreated sleep apnea, or congestive heart failure with Class III or IV symptoms (16). Testosterone suppresses spermatogenesis and should not be prescribed to men who are considering having a child in the near future.

The risks of testosterone administration include acne, oiliness of skin, erythrocytosis, induction or exacerbation of sleep apnea, leg edema, transient breast tenderness or enlargement, and reversible suppression of baseline spermatogenesis (16) (Table 2). Abnormalities of liver enzymes, hepatic neoplasms, and peliosis hepatis that have been reported previously with orally administered, 17-alpha alkylated androgens, have not been observed with replacement doses of transdermal or injectable testosterone formulations. The two major areas of concern and uncertainty are the effects of long-term testosterone administration on prostate cancer and major adverse cardiovascular events.

Table 2. Potential Adverse Effects of Testosterone Replacement in Older Men
Adverse Events for Which There is Evidence of Association with Testosterone Administration
1.　　Erythrocytosis
2.　　Acne and oily skin
3.　　Detection of subclinical prostate cancer
4.　　Growth of metastatic prostate cancer
5.　　Reduced sperm production and fertility
Potential Adverse Events for Which There is Weak Evidence of Association with Testosterone Administration
1.　　Gynecomastia
2.　　Male pattern balding (familial)
3.　　Growth of breast cancer
4.　　Induction of worsening of obstructive sleep apnea
Formulation Specific Adverse Effects
1.　　Oral Tablets (not recommended) 　　　- Effects on liver enzymes and HDL cholesterol (methyltestosterone)
2.　　Pellet Implants 　　　-. Infection, extrusion of pellet
3.　　Intramuscular Injections 　　　- Fluctuations in mood or libido 　　　- Pain at injection site 　　　- Coughing episodes immediately after injection
4.　　Transdermal Patches 　　　- Skin reaction at the patch application site
5.　　Transdermal Gel 　　　- Potential risk of transference to partner 　　　- Skin irritation and odor at application site 　　　- Stickiness, slow drying, dripping
6.　　Buccal Testosterone Tablets 　　　- Alterations in taste 　　　- Irritation of gums

Adapted with permission from the Endocrine Society Guideline for Testosterone Therapy in Men with Hypogonadism in: Bhasin et al J Clin Endocrinol Metab 2018;103(5):1715-1744.

TESTOSTERONE EFFECTS ON THE RISK OF ATHEROSCLEROTIC HEART DISEASE

The long-term consequences of testosterone supplementation on the risk of heart disease remain unknown and have been the subject of debate (145,413-417). Some known effects of testosterone such as increase in hematocrit, suppression of plasma HDL cholesterol, and salt and water retention, might be expected to increase cardiovascular risk. Some other effects such as testosterone's vasodilator effect on coronary arteries resulting in increased coronary blood flow, reduction of whole body and abdominal fat mass, and improved brachial reactivity might be perceived as beneficial. Testosterone's effects on coagulation are complex; testosterone administration is associated with stimulation of both anti-coagulant and pro-coagulant proteins.

Androgen Effects on Plasma Lipids

Cross-sectional studies of middle-aged men found a positive relationship between serum testosterone levels and plasma HDL-cholesterol concentrations (415,418-421). Lower testosterone levels in men are associated with higher levels of dense LDL particles (418), triglycerides (421,422) and prothrombotic factors (423).

The effects of androgen supplementation on plasma lipids depend on the dose, the route of administration (oral or parenteral), the type of androgen (aromatizable or not), and the subject population (whether young or old, and hypogonadal or not). Supraphysiological doses of testosterone and non-aromatizable androgens frequently employed by bodybuilders undoubtedly decrease plasma HDL-cholesterol levels (424-427). However, administration of replacement doses of testosterone in older men has been associated with only a modest decrease or no change in plasma HDL-cholesterol

(16,22,23,25,184,186-189,428-430), and without a significant effect on cholesterol efflux capacity from macrophages (431), suggesting preserved HDL function.

Androgens and Other Cardiovascular Risk Factors

Cross-sectional studies have found a positive association between circulating testosterone concentrations and tissue plasminogen activator activity (432), and a negative relationship between testosterone and plasminogen activator inhibitor-1 activity, fibrinogen, and some other prothrombotic factors (432), suggesting an antithrombotic effect of testosterone. However, testosterone increases hematocrit (433), as well as neutrophil, monocyte and platelet counts (434). In men, higher neutrophil counts – even within the normal range - are associated with cardiovascular disease (435). Similarly, higher monocyte counts within the normal range have been suggested as a risk-factor for coronary artery plaque formation and cardiovascular mortality (436). Additionally, testosterone administration increases thromboxane A2 receptor density on human platelets, increasing platelet aggregability *ex vivo* (437,438). Observational studies have not found a consistent relationship between testosterone treatment and the risk of venous thromboembolism (439-443), although one study reported a small increase in VTE risk in the first few months after starting testosterone treatment (439).

Cross-sectional studies have reported conflicting findings on the association of endogenous testosterone levels and inflammatory markers (444-449). Intervention trials of testosterone generally have not found a significant effect of testosterone on inflammatory markers (430,450). Even supraphysiological doses of testosterone have been found not to affect C-reactive protein (451). Similarly,

a prospective cohort study did not find meaningful changes in inflammatory markers in men with prostate cancer receiving androgen deprivation therapy (452).

Androgens and Coronary Artery Disease

Whether variation of testosterone within the normal range is associated with risk of coronary artery disease remains controversial. Of the 30 cross-sectional studies reviewed by Alexandersen (145), 18 reported lower testosterone levels in men with coronary heart disease, 11 found similar testosterone levels in controls and men with coronary artery disease and 1 found higher levels of DHEAS. Prospective studies have failed to reveal an association of total testosterone levels and coronary artery disease (146-150,453-455). The common carotid artery intimal media thickness, a marker of generalized atherosclerosis, is negatively associated with circulating testosterone levels (150).

One interventional study (456), reported that testosterone undecanoate given orally improved angina pectoris in men with coronary heart disease. Testosterone infusion acutely improves coronary blood flow in a canine model and in men with coronary artery disease (457-463). Short-term administration of testosterone induces a beneficial effect on exercise-induced myocardial ischemia in men with coronary artery disease (462). This effect may be related to a direct vasodilator effect of testosterone on the coronary arteries resulting in increased coronary blood flow. Testosterone replacement has been shown to increase the time to 1-mm ST-segment depression (460). However, in another study, there were no differences between the placebo or testosterone groups in peak heart rate, systolic blood pressure, maximal rate pressure product, perfusion imaging scores, or the onset of ST-segment depression (462). Yue et al (463) reported that testosterone induces

endothelium-independent relaxation of rabbit coronary arteries via potassium conductance. Testosterone is a potent vasodilator; it induces nitric oxide synthesis in human aortic endothelial cells *in vitro* (464). Testosterone has been shown to be an inhibitor of L-type Ca^{2+} channel. In human cells transfected with α_{1C} subunit of the human cardiovascular L-type Ca^{2+} channel, testosterone inhibits these calcium channels with a potency that is similar to that of dihydropyridine calcium channel blockers (465).

Effects of Testosterone Supplementation on Atherosclerosis Progression

In some animal models, orchiectomy accelerates and testosterone administration retards atherogenesis progression (466). The protective effect of testosterone on aortic atherogenesis in this preclinical model is mediated through its conversion to estradiol by the CYP19A1 in the blood vessel wall (466).

Two large placebo-controlled trials have evaluated the effects of testosterone treatment on atherogenesis progression in middle-aged and older men. The Testosterone's Effects on Atherosclerosis Progression in Aging Men (TEAAM) Trial determined the effects of testosterone therapy on progression of subclinical atherosclerosis in the common carotid artery using sonographic measurement of common carotid artery intima-media thickness (CCA-IMT) and the coronary artery calcium scores measured using MDCT. The participants in the TEAAM Trial were 308 men, 60 years and older, with total testosterone between 100 and 400 ng/dL or free testosterone below 50 pg/mL (23). Men were randomized to receive either 75 mg of transdermal testosterone gel or placebo gel daily and received for 3 years. Neither the progression of CCA-IMT nor coronary artery calcium scores differed between the men randomized to the testosterone and placebo groups (Figure 12) (23).
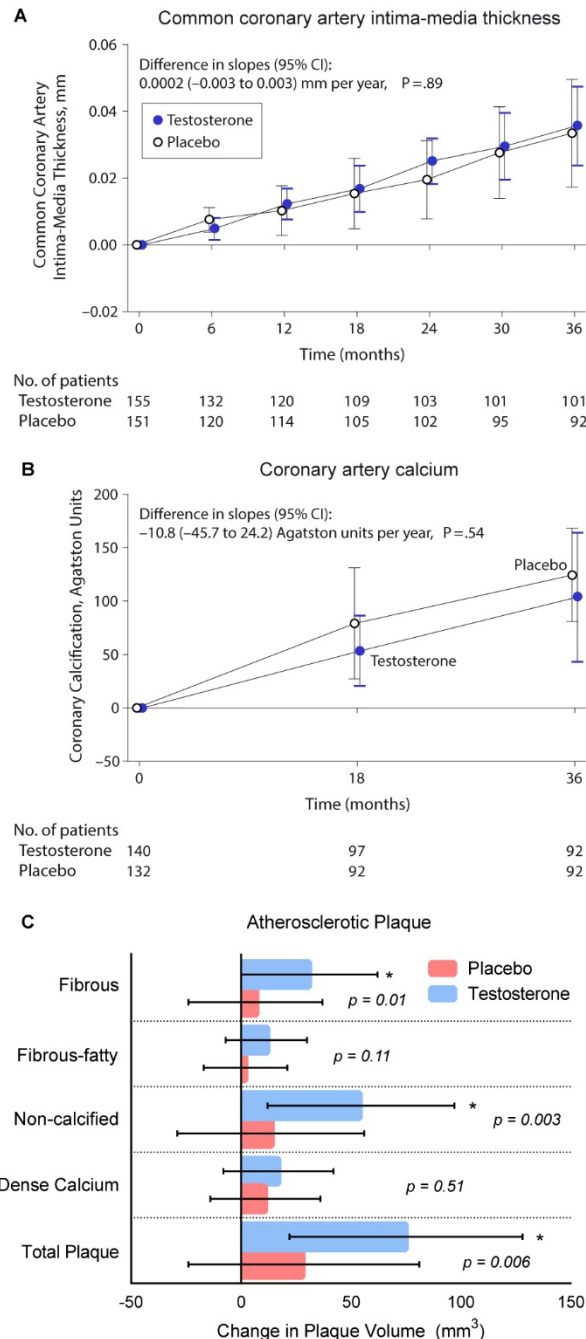
Figure 12. Effects of testosterone administration on atherosclerosis progression. Panels A and B show data from the TEAAM trial (Basaria et al. JAMA. 2015;314(6):570-81; figure reproduced with permission from JAMA) Panel C shows data from the Cardiovascular Trial of the TTrials (data from Budoff et al. JAMA. 2017;317(7):708-716; figure adapted from Gagliano-Jucá & Basaria, Asian J Androl. 2018;20(2):131-137).

In the cardiovascular trial of the TTrials, 138 men with serum total testosterone below 275 ng/dL received either testosterone gel or placebo gel for one year and were evaluated by coronary computed tomographic angiography for progression of non-calcified and calcified coronary artery plaque volume, as well as

coronary artery calcium score (467). Consistent with the findings of the TEAAM Trial, the changes in coronary artery calcium scores did not differ between the testosterone and placebo groups over one year of intervention. However, the increase in non-calcified plaque volume (primary endpoint) was significantly greater in men assigned to the testosterone arm than in those assigned to placebo arm (Figure 12) (467); there were baseline differences in non-calcified plaque volume between the two groups. The clinical implications of these findings to cardiovascular risk remain to be established.

Testosterone and Cardiac Arrhythmias

Testosterone has important effects in cardiac electrophysiology (468); it increases potassium currents derived from the human ether-a-go-go related gene (hERG) (469), and inhibits the depolarizing delayed calcium current (I_{CaL}) (470), with its effects on I_{CaL} being more meaningful than on hERG (471). These effects lead to shortening of ventricular cardiomyocyte repolarization time, which can be seen in the electrocardiogram as shortening of the heart-rate corrected QT interval (QTc). Indeed, cross-sectional studies have observed a negative association between serum testosterone levels and QTc duration (472). Additionally, in randomized trials of testosterone replacement to men with low testosterone levels, testosterone treatment shortened QTc duration in community-dwelling older men (473) and in men with chronic heart failure (474). Similarly, in a prospective cohort study, androgen deprivation therapy in men with prostate cancer was associated with QTc prolongation compared with men with prostate cancer not receiving the therapy (475). As QTc prolongation is associated with an increased risk of ventricular tachyarrhythmias (torsades de pointes) and sudden cardiac death (476-478), it is not surprising that androgen deprivation therapy is associated with a higher risk of arrhythmia, cardiac conduction disturbances and sudden death (479,480). A small case series study and analysis of the

European pharmacovigilance database concluded that "*conditions or drugs leading to male hypogonadism were associated with torsades de pointes*", and "*correction of hypogonadism with testosterone replacement therapy can treat or prevent torsades de pointes*" (481).

Cross-sectional studies have also linked low androgen levels in men to an increased risk of atrial fibrillation (482-484), and normalization of testosterone levels with testosterone replacement is associated with a decreased incidence of atrial fibrillation compared with untreated hypogonadal men (485). These findings need corroboration in randomized trials.

The Effects of Testosterone on Major Adverse Cardiovascular Events (MACE)

To-date, no randomized trials have been large enough or of sufficiently long duration to determine the effects of testosterone treatment on MACE (416). The frequency of MACE reported in randomized testosterone trials has been low—even lower than that expected for the age and comorbid conditions of the participants (18,486,487). A randomized trial of testosterone in older men (The TOM Trial) with mobility limitation was stopped early due to a higher frequency of cardiovascular-related events in men assigned to testosterone than in those assigned to placebo (22), heightening concern about the cardiovascular safety of testosterone in frail older men. In contrast to many other testosterone trials in older men, which recruited relatively healthy older men, the participants in the TOM trial had a high prevalence of chronic conditions, such as heart disease, diabetes mellitus, obesity, hypertension, and hyperlipidaemia (22). Men, 75 years of age or older, and men with high on-treatment testosterone levels seemed to be at the greatest risk of cardiovascular-related events. In secondary analyses, these events were found to be associated with changes in serum free testosterone and estradiol levels (488). The dose of testosterone used in the TOM trial was higher than

that used in some previous trials, but not dissimilar from or lower than that used in some other trials. The cardiovascular events were small in number and of variable clinical significance. The TOM trial was not designed for cardiovascular events; therefore, the cardiovascular events were not a pre-specified endpoint, and were not collected in a standardized manner, nor adjudicated prospectively. Additionally, many of the cardiovascular events were not MACE.

The higher cardiovascular adverse event incidence in testosterone-treated older men observed in the TOM trial was not reproduced in two larger trials of longer duration published more recently; in the TEAAM trial, the incidence of major adverse cardiac events throughout the 3 years of intervention was similar between groups (23). Similarly, in the TTrials, the number of MACE (myocardial infarction, stroke or death related to cardiovascular disease) during the one year of treatment was similar in the two groups, with seven men in each group experiencing an event (26). The number of MACE in the TEAAM and Ttrials were too few to permit strong inferences on the effects of testosterone treatment on MACE.

The Hormonal Regulators of Muscle and Metabolism in Aging (HORMA) trial reported a significantly greater increase in blood pressure in men treated with testosterone than in those treated with placebo (489). Testosterone administration causes salt and water retention (490), which can induce edema and worsen pre-existing heart failure.

Several meta-analyses of randomized testosterone trials have been published (413,486,487,491,492); however, these meta-analyses are limited by the small size of most trials, heterogeneity of study populations, poor quality of adverse-event reporting, and short treatment duration in many trials. None of the testosterone trials to date was sufficiently powered to adequately assess safety outcomes. The rigor of adverse-event reporting varied greatly among studies.

The MACE was not ascertained rigorously nor adjudicated in most trials except in the TTrials.

The meta-analyses of randomized testosterone trials (487,491-494) and retrospective analyses of electronic medical records data (495-498) have also yielded inconsistent findings. These meta-analyses and pharmacovigilance studies have suffered from many limitations that are inherent in retrospective analysis of electronic medical records data. These studies included heterogeneous populations, and differed in the duration of intervention and study design. They used variable definitions and ascertainment of cardiovascular outcomes. The cardiovascular events were not prespecified, not collected prospectively and were not adjudicated. Treatment indications, treatment regimens, on-treatment testosterone levels and exposure differed among studies. These studies also suffered from a potential for residual confounding in that the patients assigned to testosterone therapy differed from comparators in baseline cardiovascular risk factors. Because of these inherent limitations and inconsistency of findings, these epidemiologic studies do not permit strong inferences about the relation between testosterone therapy and mortality and cardiovascular outcomes.

Synopsis of the Effects of Testosterone on Cardiovascular Risk

The long-term effects of testosterone replacement therapy on MACE remain unknown. The FDA conducted an extensive review and concluded "*the studies...have significant limitations that weaken their evidentiary value for confirming a causal relationship between testosterone and adverse cardiovascular outcomes*". Nevertheless, the FDA directed the pharmaceutical companies to add in the drug label information about a possible increased risk of cardiovascular events with testosterone use. An independent review conducted by the European Medicines Agency also found no consistent evidence

of an increased risk of coronary heart disease associated with testosterone treatment of hypogonadal men. Long-term randomized trials of the effects of testosterone replacement on MACE are needed and are particularly important because even small changes in incidence rates could have significant public health impact.

A large randomized, placebo-controlled trial to study the effects of testosterone replacement therapy on the incidence of major adverse cardiovascular events in men 45 to 80 years of age with low testosterone levels and one or more symptoms of testosterone deficiency, who are at increased risk for cardiovascular events is currently underway (The TRAVERSE Trial, NCT03518034). The intervention duration is up to 5 years in this trial of over 6,000 men. The efficacy outcomes include adjudicated clinical fractures, remission of low-grade persistent depressive disorder (dysthymia), progression from pre-diabetes to diabetes, correction of anemia, and overall sexual activity, sexual desire, and erectile function. This randomized, placebo-controlled trial offers an historical opportunity to advance our understanding of the cardiovascular safety and long-term efficacy of testosterone replacement in middle-aged and older hypogonadal men.

TESTOSTERONE, DIABETES, AND METABOLIC SYNDROME

Spontaneous (156) and experimentally induced (222) androgen deficiency is associated with increased fat mass, and testosterone replacement decreased fat mass in older men with low testosterone levels (16). In epidemiologic studies, low testosterone levels are associated with higher levels of abdominal adiposity (499,500). Testosterone administration promotes the mobilization of triglycerides from the abdominal adipose tissue in middle-aged men (501). Surgical castration in rats impairs insulin sensitivity; physiologic testosterone replacement reverses this metabolic derangement (502). However, high doses of testosterone impair insulin sensitivity in castrated rats (502), suggesting a biphasic relationship in which both low and high testosterone levels impair insulin resistance. Androgens increase insulin-independent glucose uptake (503) and modulate LPL activity in a region-specific manner (504).

Testosterone levels are lower in men with type 2 diabetes mellitus compared with controls (505-510). Low total testosterone levels have been associated with lower insulin sensitivity (505,511) and increased risk of type 2 diabetes mellitus and metabolic syndrome in community dwelling men both cross-sectionally and longitudinally (508-510,512-520). However, the association of free testosterone and type 2 diabetes mellitus has been inconsistent; some studies have reported a weak relationship (509,510,512) while others have failed to find any relationship (508,514). Circulating sex hormone binding globulin (SHBG) and some SHBG polymorphisms also have been associated negatively with the risk of type 2 diabetes (508-510,512-516,521-524). For instance, individuals with the rs6257and rs179994 variant alleles of the SHBG single nucleotide polymorphism (SNP) have lower plasma SHBG levels and a higher risk of type 2 diabetes (521-524). Similarly, individuals with the rs6259 variant have higher SHBG levels and lower type 2 diabetes risk (524). We performed longitudinal analyses of men participating in the Massachusetts Male Aging Study (525), a population-based study of men aged 40-70 years (Figure 13) to evaluate whether SHBG is an independent predictor of T2DM (526). After adjustment for age, body mass index, hypertension, smoking, alcohol intake and physical activity, the hazard ratio for incident type 2 diabetes was 2.0 for each one SD decrease in SHBG and 1.29 for each one SD decrease in total testosterone (525). Free testosterone was not significantly associated with type 2 diabetes. The strong association of T2DM risk with SHBG persisted even after additional adjustment for free testosterone. The association of SHBG polymorphisms with type 2 diabetes suggests a potential role of SHBG in the pathogenesis of type 2

diabetes. In a Mendelian randomization analysis of the UK Biobank data, genetically determined free testosterone levels were associated with the risk of type 2 diabetes mellitus in a sexually dimorphic manner after adjusting for SHBG levels; men with low genetically determined free testosterone levels had increased risk of type 2 diabetes while women with low genetically determined free testosterone levels had reduced risk of type 2 diabetes (527).

Interventional studies have yielded inconsistent results. Acute and severe androgen deficiency induced by administration of a GnRH agonist or antagonist worsens measures of insulin sensitivity; acute withdrawal of testosterone therapy in men with

idiopathic hypogonadotropic hypogonadism (528) and administration of androgen deprivation therapy in men with prostate cancer is associated with the worsening of insulin sensitivity (452,529,530). Men with prostate cancer who are receiving androgen deprivation therapy are at increased risk of developing type 2 diabetes and metabolic syndrome (479,529,531). In the TIMES2 study (532), men with type 2 diabetes mellitus and/or metabolic syndrome were randomized to either 2% testosterone gel or placebo gel for 6 months. Randomization to testosterone arm was associated with greater improvements in sexual function and plasma lipid levels than placebo. However, the changes in
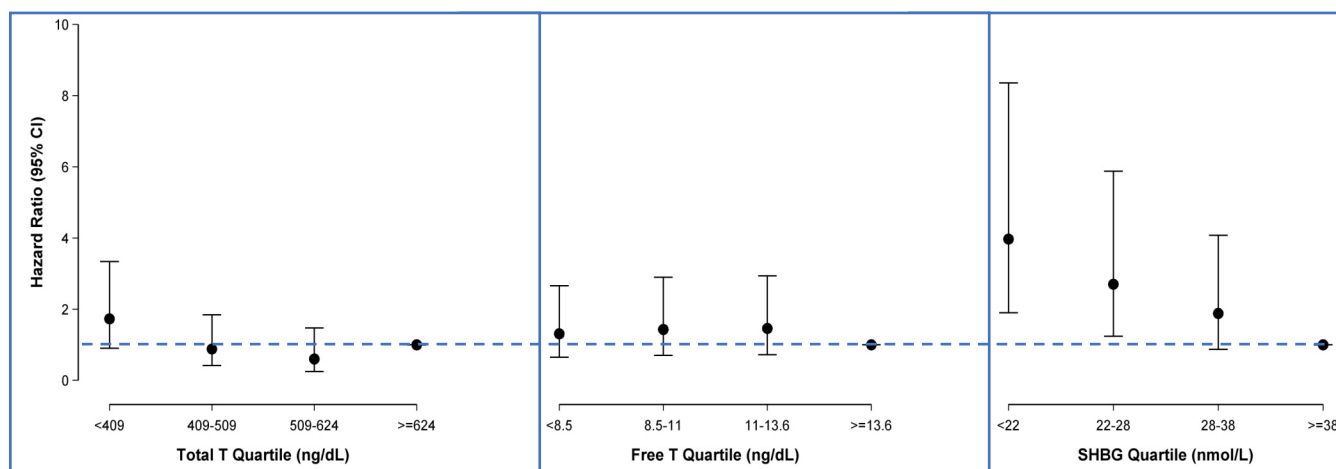

Figure 13. Circulating Concentrations of SHBG, but not total or free testosterone, were associated prospectively with risk of incident diabetes in the Massachusetts Male Aging Study (MMAS). In a prospective analysis of data from the Massachusetts male Aging Study, total testosterone (left panel) and free testosterone (middle panel) were not associated significantly with risk of incident diabetes. Only SHBG concentrations were associated with incident diabetes in longitudinal analysis. Reproduced with permission from Lakshman et al J Gerontol A Biol Sci Med Sci. 2010;65(5):503-9.

HbA$_{1c}$ levels did not differ between groups. Homeostasis model assessment of insulin resistance (HOMA-IR), a marker of insulin resistance, improved modestly in men who were assigned to testosterone

compared with placebo (532). Dhindsa et al reported improvement of insulin sensitivity with testosterone replacement for 24 weeks in hypogonadotropic hypogonadal men with type 2 diabetes (511). Overall,

studies have failed to show improvements in diabetes outcomes or consistent changes in measures of insulin sensitivity (18,430,451,532-536) even though interventional trials have found a consistent reduction in whole body fat as well as abdominal fat (18,123,343). Indeed, in the TEAAM trial, 3 years of testosterone supplementation decreased fat mass in community-dwelling older men with low or low-normal serum testosterone concentrations, but did not improve insulin sensitivity (Figure 14) (537). The T4DM trial, one of the largest testosterone trials

conducted to-date, evaluated the effects of 2 years of testosterone treatment in men aged 50 to 74 years with impaired glucose tolerance or newly diagnosed type 2 diabetes and a serum testosterone concentration below 404 ng/dL enrolled in a lifestyle program. Compared to placebo, testosterone treatment in conjunction with a life style program was associated with a lower proportion of participants with type 2 diabetes (538). It is important to note, however, that the men enrolled in the T4DM trial were not hypogonadal.
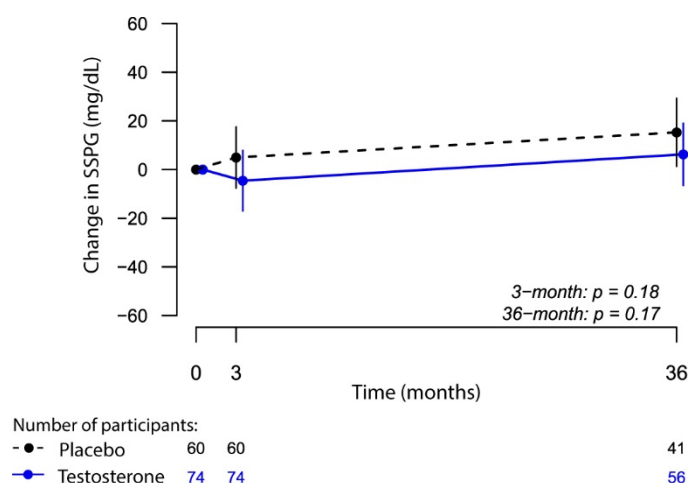

Figure 14. Long-term effects of testosterone therapy on insulin sensitivity in older men. Change in insulin sensitivity over time measured by the octreotide insulin suppression test and estimated as the mean concentration of glucose at equilibrium (SSPG). Figure adapted from Huang et al. J Clin Endocrinol Metab. 2018;103(4):1678-1685.

TESTOSTERONE AND PROSTATE RISK

There is no evidence that testosterone causes prostate cancer (539). A retrospective analysis of the Registry of Hypogonadism in Men (RHYME) (540) and several meta-analyses of randomized controlled trials (486,541,542) did not find an increased risk of prostate cancer in men receiving testosterone. Also, there is no consistent relationship between endogenous serum testosterone levels and the risk of prostate cancer (16,18,123,343,542-545). A meta-analyses of prospective cohort studies did not find a significant association between endogenous total testosterone levels and prostate cancer (542). Conversely, an

analysis of 20 prospective studies found that men in the lowest tenth of free testosterone concentration had a lower risk of prostate cancer (OR=0.77, 95%CI= 0.69 to 0.86; p<0.001) compared with men with higher concentrations (545). Similarly, in the male participants in the UK Biobank followed for a mean of 6.9 years, higher genetically determined free testosterone was associated with a higher risk of prostate cancer, while total testosterone was not associated with prostate cancer risk (Figure 15) (546). The men with Klinefelter Syndrome have lower risk of prostate cancer than the general population. Taken together, these data suggest that life-long exposure to

testosterone treatment in hypogonadal men could potentially increase the risk of prostate cancer.
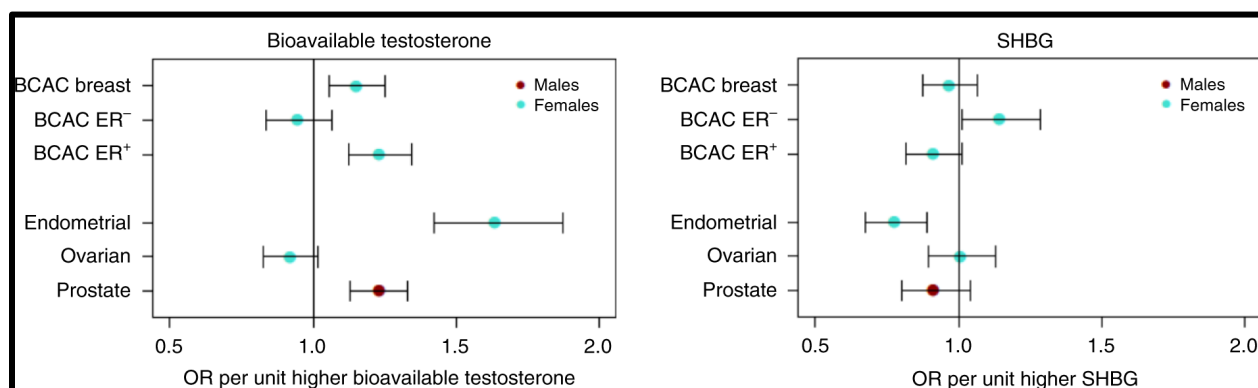

Figure15. Mendelian Randomization: Genetically Determined Bio-Testosterone Associated with Increased Prostate Cancer Risk. Legend: UK Biobank Study: Genetic determinants of bioavailable testosterone were positively associated with risk of prostate cancer in men and ER+ breast cancer and endometrial cancer in women. Reproduced with permission from: Ruth KS, et al. Nat Med. 2020;26(2):252-258.

Prostate cancer is an androgen–dependent tumor, and testosterone treatment is known to promote the growth of metastatic prostate cancer (544,547). Testosterone administration has been historically contraindicated in men with history of prostate cancer (16,543). The prevalence of subclinical, microscopic foci of prostate cancer in older men is high (548-555). There is concern that testosterone administration might make these subclinical foci of cancer grow and become clinically overt. In addition, older men with low testosterone levels may have prostate cancer (556,557). Morgentaler et al (556,557) reported a high prevalence of biopsy-detectable prostate cancer in men with low total or free testosterone levels despite normal PSA levels and normal digital rectal examinations. However, this study did not have a control group, and we do not know whether sextant biopsies of age-matched controls with normal testosterone levels would yield a similarly high incidence of biopsy-detectable cancer. Therefore, this study should not be interpreted to conclude that there is a higher prevalence of prostate cancer in older men with low testosterone levels, or that low testosterone levels are an indication for performing prostate biopsy.

Effects of Testosterone Therapy on Prostate Events

None of the testosterone trials in middle-aged or older men had sufficient power or intervention duration to detect meaningful differences in the incidence of prostate cancer between testosterone and placebo-treated men. Testosterone treatment of hypogonadal men increases PSA levels (16,558), which may lead to urological referral for prostate biopsy. A systematic review of randomized testosterone trials in middle-aged and older men found (413) that men treated with testosterone in clinical trials were at significantly higher risk for undergoing prostate biopsy than placebo-treated men (413). Because of the high prevalence of subclinical prostate cancer in older men, the higher number of prostate biopsies in testosterone-treated men could lead to increased detection rates of subclinical prostate cancer in comparison with placebo-treated men. Thus, testosterone therapy of middle-aged and older men is associated with a higher risk of prostate biopsy and a bias towards detection of a higher number of prostate events (18,413).

Administration of exogenous testosterone or suppression of circulating levels of testosterone by administration of a GnRH antagonist is not associated with proportionate changes in intra-prostatic testosterone or DHT concentrations. For instance, in a randomized controlled trial, Marks et al (559) measured intraprostatic testosterone and DHT levels in older men treated with placebo or testosterone. Surprisingly, intraprostatic DHT concentrations were not significantly higher in testosterone-treated men than in placebo-treated men (559). Similarly, the expression levels of androgen-dependent genes in the prostate were not significantly altered by testosterone administration (559). In separate studies, lowering of circulating testosterone levels by administration of a GnRH antagonist was not associated with changes in intraprostatic androgen concentrations (560,561).

Effects of Testosterone Replacement on Serum PSA Levels

Serum PSA levels are lower in androgen–deficient men and are restored to normal following testosterone replacement (16,558,562-570). Lowering of serum testosterone concentrations by withdrawal of androgen therapy in young, hypogonadal men is associated with a decrease in serum PSA levels. Similarly, treatment of men with benign prostatic hyperplasia with a 5-alpha reductase inhibitor, finasteride, is associated with a significant lowering of serum and prostatic PSA levels (570,571). However, serum PSA levels do not increase progressively in healthy hypogonadal men with replacement doses of testosterone. The increase in PSA levels during testosterone replacement might trigger evaluation and biopsy in some patients (16,543).

More intensive PSA screening and follow-up of men receiving testosterone replacement might lead to an increased number of prostate biopsies and the detection of subclinical prostate cancers that would have otherwise remained undetected (16,543). Serum PSA levels tend to fluctuate when measured repeatedly in the same individual over time (572-574).

There is considerable test-retest variability in PSA measurements (572-574). Some of this variability is due to the inherent assay variability, and a significant portion of this variability is due to unknown factors. Fluctuations are larger in men with high mean PSA levels. Variability can be even greater if measurements are performed in different laboratories that use dissimilar assay methodology (572-574).

An important issue is what increment in PSA level should warrant a prostate biopsy in older men receiving testosterone replacement. To address this issue, we conducted a systematic review of published studies of testosterone replacement in hypogonadal men (543). This review indicated that the weighted effect size of the change in PSA after testosterone replacement in young, hypogonadal men is 0.68 standard deviation units (95% confidence interval 0.55 to 0.82). This means that the effect of testosterone replacement therapy is to increase PSA levels by an average 0.68 standard deviations over baseline. Because the average standard deviation was 0.47 in this systematic analysis, the standard deviation score of 0.68 translates into an average increase in serum PSA levels of about 0.30 ng/ml in young hypogonadal men (543). The average change in serum PSA levels after testosterone replacement in studies of older men was 0.43 ng/mL (543). The data from the Proscar Long-Term Efficacy and Safety Study (PLESS) demonstrated that the 90% confidence interval for the change in PSA values measured 3 to 6 months apart is 1.4 ng/mL (570). Therefore, a change in PSA of >1.4 ng/ml between any two values measured 3 to 6 month apart in the same patient is unusual (16,543). In the TTrials, 2.4% of men receiving testosterone had increases above 1.4 ng/mL at 3 months, and 4.7% at 12 months (26).

Carter et al, based on the analysis of PSA data from the Baltimore Longitudinal Study of Aging, reported that PSA velocity, defined as the annual rate of change of PSA, is different in men who develop prostate cancer than in those who do not (575-577). Thus, PSA velocity greater than 0.7 ng/ml/year was unusual in

men without prostate cancer whose baseline PSA was between 4 and 10 ng/ml (575-577). However, most men being considered for testosterone replacement will have baseline PSA less than 4 ng/ml. In a subsequent analysis, the same group reported that the PSA velocity in men with baseline PSA between 2 and 4 ng/ml was 0.2 ng/ml/year (577). Because test-to-retest variability in PSA measurement is far greater than this threshold, it is likely that the use of this threshold of 0.2 ng/ml/year to select men for prostate biopsy would lead to many unnecessary biopsies.

In eugonadal, young men, administration of supraphysiological doses of testosterone does not further increase serum PSA levels (166,169,578). These data are consistent with dose response studies in young men that demonstrate that maximal serum concentrations of PSA are achieved at testosterone levels that are at the lower end of the normal male range; higher testosterone concentrations are not associated with higher PSA levels (166,169).

In summary, these data suggest that the administration of replacement doses of testosterone to androgen-deficient men can be expected to produce a modest increment in serum PSA levels. Increments in PSA levels after testosterone supplementation in androgen-deficient men are generally less than 0.5 ng/mL and increments in excess of 1.4 ng/mL over a 3–6-month period are unusual. Nevertheless, administration of testosterone to men with baseline PSA levels between 2.6 and 4.0 ng/mL will cause PSA levels to exceed 4.0 ng/mL in some men. Increments in PSA levels above 4 ng/mL will trigger a urological consultation and many of these men will be asked to undergo prostate biopsies. However, considering the controversy over prostate cancer screening and monitoring, the decision to monitor PSA levels during testosterone treatment and the decision to refer a patient for consideration of prostate biopsy should be made only after informing him of the risks and benefits of prostate cancer screening and monitoring and engaging the patient in a shared decision-making process.

Monitoring PSA Levels in Older Men Receiving Testosterone Replacement (Tables 3 and 4)

Older men considering testosterone supplementation should undergo evaluation of risk factors for prostate cancer; the Endocrine Society guideline suggest a baseline PSA measurement and a digital prostate examination (16). Prostate cancer screening has some risks; therefore, initiation of prostate monitoring should be a shared decision, made only after a discussion of the risks and benefits of prostate cancer monitoring. Men with history of prostate cancer, should not be given androgen supplementation and those with palpable abnormalities of the prostate or PSA levels greater than 3 ng/ml should undergo urological evaluation. After initiation of testosterone replacement therapy, PSA levels should be repeated at 3 months and annually thereafter (16). Although measurements of free PSA and PSA density have been proposed to enhance the specificity of PSA measurement, long term data, especially from studies of testosterone replacement in older men, are lacking. Considering the interassay variability and the longitudinal change in PSA previously discussed, an Endocrine Society Expert Panel recently suggested that men receiving testosterone replacement should be referred to urological consultation if: 1) PSA increases more than 1.4 ng/mL in the first 12 months of treatment; 2) a PSA above 4 ng/mL is confirmed; or 3) a prostatic abnormality is detected on digital rectal examination (16). After 12 months of treatment, prostate monitoring should follow standard guidelines for prostate cancer screening taking into account the age and race of the patient (16).

Table 3. Recommendations for Monitoring of Men Receiving Testosterone Therapy
A. Explain the potential benefits and risks of monitoring for prostate cancer and engage the patient in shared decision making regarding the prostate monitoring plan.
B. Evaluate the patient at 3–12 months after treatment initiation and then annually to assess whether symptoms have responded to treatment and whether the patient is suffering from any adverse effects
C. Monitor testosterone concentrations 3–6 months after initiation of therapy: --Therapy should aim to raise testosterone into the mid-normal range. --Injectable testosterone enanthate or cypionate: measure testosterone midway between injections. If midinterval T is >600 ng/dL (24.5 nmol/L) or <350 ng/dL (14.1 nmol/L), adjust dose or frequency. --Transdermal gels: assess testosterone 2–8 h following the gel application, after the patient has been on treatment for at least 1 week; adjust dose to achieve testosterone in the mid-normal range. --Transdermal patches: assess testosterone 3–12 h after application; adjust dose to achieve concentration in the mid-normal range. --Buccal T bioadhesive tablet: assess concentrations immediately before or after application of fresh system. --Testosterone pellets: measure concentrations at the end of the dosing interval. Adjust the number of pellets and/or the dosing interval to maintain serum T concentrations in the mid-normal range. --Oral T undecanoate: monitor serum T concentrations 3–5h after ingestion with a fat-containing meal. --Injectable testosterone undecanoate: measure serum T levels at the end of the dosing interval just prior to the next injection and aim to achieve nadir levels in low-mid range.
D. Check hematocrit at baseline, 3–6 months after starting treatment, and then annually. If hematocrit is >54%, stop therapy until hematocrit decreases to a safe level; evaluate the patient for hypoxia and sleep apnea; reinitiate therapy with a reduced dose.
E. Measure BMD of lumbar spine and/or femoral neck after 1–2 year of testosterone therapy in hypogonadal men with osteoporosis, consistent with regional standard of care.
F. For men 55–69 years of age and for men 40–69 years of age who are at increased risk for prostate cancer who choose prostate monitoring, perform digital rectal examination and check PSA level before initiating treatment; check PSA and perform digital rectal examination 3–12 months after initiating testosterone treatment, and then in accordance with guidelines for prostate cancer screening depending on the age and race of the patient.
G. Obtain urological consultation if there is: • An increase in serum PSA concentration.1.4 ng/mL within 12 months of initiating testosterone treatment • A confirmed PSA > 4 ng/mL at any time • Detection of a prostatic abnormality on digital rectal examination • Substantial worsening of lower urinary tract symptoms

Adapted with permission from the Endocrine Society Guideline for Testosterone Therapy in Men with Hypogonadism in: Bhasin et al J Clin Endocrinol Metab 2018;103(5):1715-1744.

Table 4. Indications for Urological Consultation in Men Receiving Testosterone Replacement
1) An increase in serum or plasma PSA concentration >1.4 ng/mL within any 12-month period after initiating testosterone treatment
2) A PSA >4.0 ng/mL
3) Detection of a prostatic abnormality on digital rectal examination
4) An AUA/IPSS prostate symptom score of >19

Adapted with permission from the Endocrine Society Guideline for Testosterone Therapy in Men with Hypogonadism in: Bhasin et al J Clin Endocrinol Metab 2018;103(5):1715-1744.

Testosterone and Benign Prostatic Hypertrophy

Testosterone replacement can be administered safely to men with benign prostatic hypertrophy who have mild to moderate symptom scores. The severity of symptoms associated with benign prostatic hypertrophy can be assessed by using either the International Prostate Symptom Score (IPSS) or the American Urological Association (AUA) Symptom questionnaires. Androgen deficiency is associated with decreased prostate volume and androgen replacement increases prostate volume compared to age–matched controls (559,562,566,567). Meta-analyses of testosterone trials have not found statistically significant difference in lower urinary tract symptoms scores in hypogonadal men receiving testosterone replacement compared to placebo (Figure 16) (256,579). However, in patients with pre–existing, severe symptoms of benign prostatic hypertrophy, even small increases in prostate volume during testosterone administration may exacerbate obstructive symptoms. In these men, testosterone should either not be administered or administered with careful monitoring of obstructive symptoms.
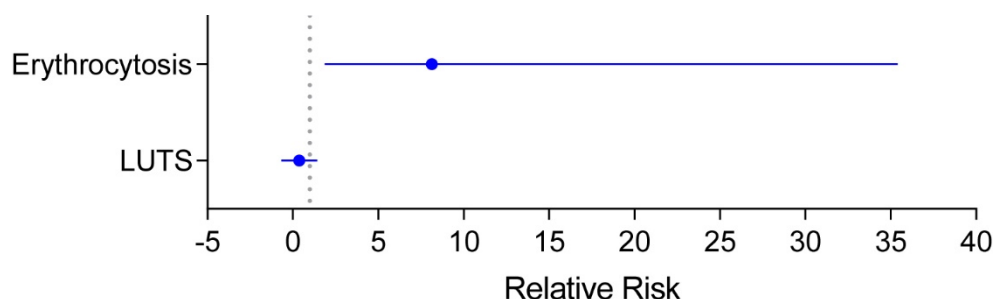

Figure 16. Adverse events associated with testosterone therapy in randomized trials. The relative risk and 95% CI for development of erythrocytosis (RR= 8.14; 95%CI= 1.87 to 35.40) and lower urinary tract symptoms (LUTS; RR= 0.38; 95%CI= -0.67 to 1.43) in randomized testosterone trials derived from meta-analyses published by Ponce et al., 2018 are shown. The figure was adapted with permission from Ponce et al. J Clin Endocrinol Metab. 2018;103(5):1745-54.

ERYTHROCYTOSIS

Testosterone replacement is associated with increased red cell mass and hemoglobin levels (Figure 16) (256,329,580-585). Therefore, testosterone replacement should not be administered to men with baseline hematocrit of 52% or greater without appropriate evaluation and treatment of erythrocytosis (16) (Table 3). Administration of testosterone to androgen–deficient young men is typically associated with a small increase in hemoglobin levels. Clinically significant erythrocytosis is uncommon in young hypogonadal men during testosterone replacement therapy, but can occur in men with sleep apnea, significant smoking history, or chronic obstructive lung disease. Testosterone administration in older men is associated with greater increments in hemoglobin than observed in young, hypogonadal men (321). The magnitude of hemoglobin increase during testosterone therapy appears related to the testosterone dose, the increase in testosterone concentrations during testosterone therapy, and age (321). Testosterone replacement by means of a transdermal system has been reported to produce a lesser increase in hemoglobin levels than that associated with intramuscular testosterone enanthate and cypionate presumably because of the substantially higher testosterone dose and average circulating testosterone levels achieved with testosterone esters (586).

Testosterone increases hemoglobin and hematocrit by multiple mechanisms (322,408,409,587). Testosterone administration stimulates iron-dependent erythropoiesis by suppressing hepcidin transcription and increasing iron availability for erythropoiesis (322,408,409,587). Additionally, testosterone stimulates erythropoiesis by a direct effect on bone marrow hematopoietic progenitors and increasing the numbers of myeloid progenitors.

Testosterone also stimulates erythropoietin and alters the set-point of the relationship between erythropoietin and hemoglobin (322). Testosterone supplementation can correct anemia in older men with unexplained anemia of aging and anemia of inflammation (322,329,409). Suppression of testosterone secretion in men receiving androgen deprivation therapy reduces hematocrit and hemoglobin levels by slowing erythropoiesis independently of changes in erythropoietin levels (588).

Monitoring Hematocrit During Testosterone Replacement Therapy (Table 3)

Hematocrit levels should be measured at baseline and 3 months after institution of testosterone replacement or after increase in dosage, and every 12 months thereafter. It is not clear what absolute hematocrit level or magnitude of change in hematocrit warrants discontinuation of testosterone administration. Plasma viscosity increases disproportionately as hematocrit rises above 50%. Hematocrit levels above 54% may be associated with increased risk of neuro-occlusive events. Therefore, testosterone dose should be withheld if hematocrit rises above 54%; once hematocrit falls to a safe level, testosterone therapy may be re-initiated at a reduced dose or with a different formulation (16).

SLEEP APNEA

Circulating testosterone concentrations are related to sleep rhythm and are generally higher during sleep than during waking hours (589-592). Testosterone secretory peaks coincide with the onset of rapid-eye movement sleep. Aging is associated with decreased sleep efficiency, reduced numbers of REM sleep episodes, and altered REM sleep latency, which may contribute to lower circulating testosterone concentrations (590-594). The degree of sleep-

disordered breathing increases with age and is associated with reduced overnight plasma bioavailable testosterone. Thus, changes in sleep efficiency and architecture are associated with alterations in testosterone levels in older men (590-594). Sleep apnea and disordered sleep are often associated with low testosterone levels (595), particularly in patients with more severe cases of OSA (i.e. severe hypoxemia) (596). Some potential mechanisms by which OSA may decrease endogenous testosterone levels include disruption of pulsatile luteinizing hormone secretion from restricted sleep and/or recurrent nocturnal hypoxia (597,598), which is further exacerbated by obesity. OSA treatment with continuous positive airway pressure has been demonstrated to increase serum testosterone levels (599).

Testosterone can induce or exacerbate sleep apnea in some individuals, particularly those with obesity or chronic obstructive lung disease (589-594,600). This appears to be due to direct effects of testosterone on laryngeal muscles. Testosterone administration depresses hypercapnic ventilator drive and induces apnea in primate infants (594). Short-term administration of high doses of testosterone shortens sleep duration and worsens sleep apnea in older men (601). The frequency of sleep apnea in randomized testosterone trials in older men has been very low (16,486) and no randomized trial has reported an increased incidence of OSA or OSA worsening in men randomized to the testosterone arm compared to the placebo arm.

Testosterone should not be given to men with severe untreated OSA without evaluation and treatment of sleep apnea. Several screening instruments can be used to detect sleep apnea. A history of loud snoring, and daytime somnolence, in an obese individual with hypertension increases the likelihood of having sleep apnea; such patients should be referred for a sleep study.

BREAST ENLARGEMENT AND TENDERNESS

Testosterone administration can induce breast tenderness; however, gynecomastia is an uncommon complication of testosterone replacement therapy. Even with administration of supraphysiological doses of testosterone enanthate, less than 4% of men in a contraceptive trial developed detectable breast enlargement (580). Breast cancer is listed as a contraindication for testosterone replacement therapy primarily because of concern that increased estrogen levels during testosterone treatment might exacerbate breast cancer growth. There are, however, few case reports of breast cancer occurring as a complication of testosterone treatment. Men with Klinefelter's syndrome have a higher risk of breast cancer than the general population (602).

An Individualized, Patient-Centric Approach to Shared Decision Making in the Evaluation and Treatment of Older Men with Low Testosterone Levels

Recent large randomized clinical trials, especially the TTrials, have substantially expanded our understanding of the efficacy and short-term safety of testosterone in older men with low testosterone levels. However, none of the trials has been long enough or large enough to determine the effects of testosterone treatment on major adverse cardiovascular events and prostate cancer risk. Furthermore, the long-term efficacy of testosterone treatment in improving hard outcomes – physical disability, fractures, falls, progression to dementia, progression from prediabetes to diabetes, remission of late-life low grade persistent depressive disorder (dysthymia) - remains to be established. Adherence with testosterone treatment is poor and in one survey, nearly 50% of men prescribed testosterone, discontinued treatment within 3 months. Population level screening of all older men for androgen deficiency is not justified (16) because of the lack of

agreement on a case definition, the paucity of data on the performance characteristics of the screening instruments (e.g., the ADAM questionnaire (603), the Aging Male Symptoms questionnaire (604), and the MMAS questionnaire (605)) and the lack of clarity on the public health impact of the androgen deficiency syndrome in the general population.

Recognizing the lack of evidence of the long-term safety and efficacy of testosterone therapy in older men with symptomatic androgen deficiency, the expert panel of the Endocrine Society recommended against testosterone therapy of all men 65 years or older with low testosterone levels (16). Instead, the panel suggested that *"in men >65 years who have symptoms or conditions suggestive of testosterone deficiency (such as low libido or unexplained anemia) and consistently and unequivocally low morning testosterone, clinicians offer testosterone therapy on an individualized basis after explicit discussion of the potential risks and benefits"* (16).

The decision to offer testosterone treatment to older men with low testosterone levels should be guided by an individualized assessment of potential benefits and risks (Figure 17) (606). Determine whether the patient has clear evidence of testosterone deficiency recognizing the imprecision and inaccuracy of many available immunoassays and the substantial overlap in the symptoms of hypogonadism and aging *per se*. Perform a careful general health evaluation to identify conditions, such as prostate cancer, erythrocytosis, heart failure, or a hypercoagulable state that could increase the risk of harm. Weigh the burden of patient's symptoms and conditions associated with testosterone deficiency against the potential benefits and the uncertainty of long-term harm. Evaluate prostate cancer risk recognizing that prostate cancer screening and monitoring has some risks. Weigh the bother and distress associated with symptoms of testosterone deficiency and patient's values and risk tolerance against the uncertainty of benefits and long-term risks, and the burden, cost, and risks of treatment and monitoring. The participation of the patient who is well informed of the potential benefits and risks in the shared decision to initiate testosterone treatment can enable a more thoughtful treatment plan and increased adherence with the treatment and monitoring (606).
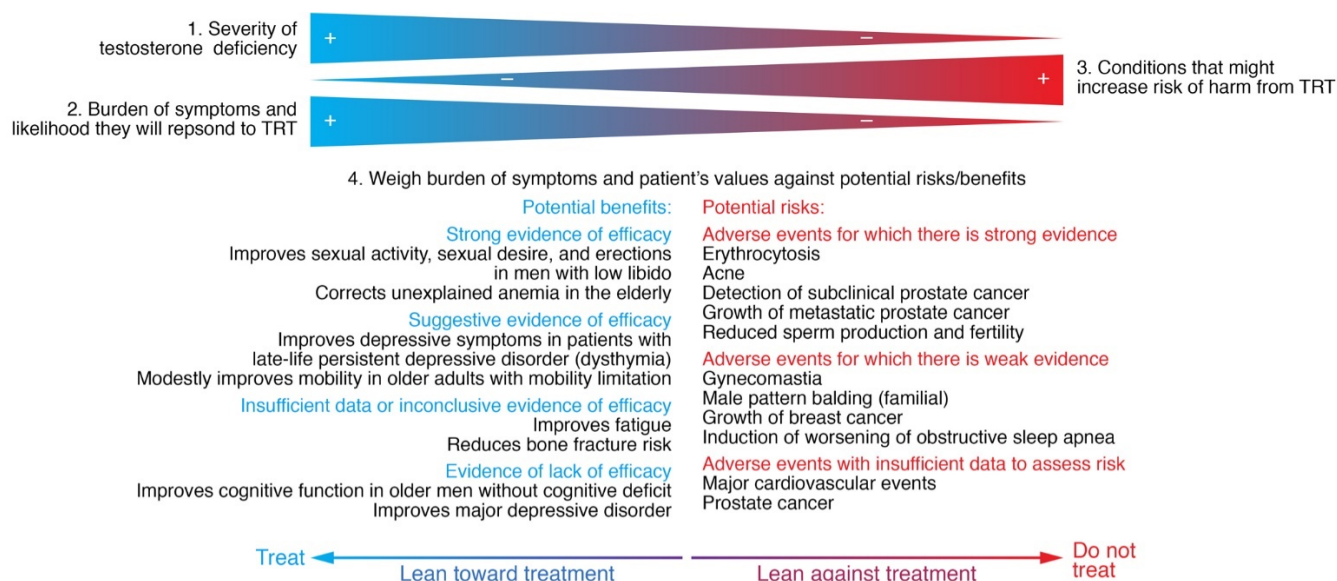

Figure 17. An evidence-based, individualized approach to testosterone therapy in older men with testosterone deficiency. The decision to offer testosterone treatment to older men with low testosterone

levels should be guided by an individualized assessment of potential benefits and risks. Testosterone deficiency needs to be evaluated using reliable assays for the measurement of total and free testosterone levels. Patients should also be evaluated for conditions that are likely to respond to testosterone replacement therapy (TRT) as well as conditions that could be adversely impacted, such as prostate cancer, erythrocytosis, heart failure, or a hypercoagulable state. It is important to consider each patient's burden of symptoms, individual preferences, and risk tolerance against the uncertainty of long-term benefits and risks, the burden and risks of monitoring, and the cost. Reproduced with permission from Bhasin S. 2021. J Clin Invest. 2021;131(4):e146607.

Testosterone therapy can be instituted using any of the available approved formulations based on considerations of pharmacokinetics, patient convenience and preference, cost, and formulation-specific adverse effects (16). The men receiving testosterone therapy should be monitored using a standardized monitoring plan to facilitate early detection of adverse events and to minimize the risk of unnecessary prostate biopsies (Table 2), as recommended by the Endocrine Society expert panel (Table 3) (16).

CHANGES IN THE SPERMATOGENIC COMPARTMENT OF THE TESTIS

Women are more fertile below the age of 40, and fecundity decrease after age 35 and fertility ceases at the inception of menopause, around age 50. Increasing age in women confers greater risk for infertility, spontaneous abortion, and genetic and chromosomal defects among offspring. In contrast, there is no critical age at which sperm production or function, and fertility cease in men (607-614). Although serum testosterone concentrations decrease below the normal range in a significant minority of older men, men over the age of 60 years commonly

father children; the oldest father on record was 94-years old (607,609). Even though many older men are fertile, the overall fertility and fecundity decline with aging. The interpretability of data on the effects of aging on male fertility is limited by the small size of the studies and the low overall event rates.

Paternal age is associated with an increased risk of germ line mutations in FGFR2, FGFR3, and RET genes and inherited autosomal dominant diseases, such as Apert's syndrome, achondroplasia, and Costello Syndrome, respectively, in the offspring of older men (614-623). These monogenic disorders have been referred to as paternal age effect (PAE) disorders. Approximately one third of babies with diseases due to new autosomal dominant mutations are fathered by men aged 40 years or older (624).

Some other disorders such as schizophrenia, autism, and bipolar disorder have also been linked to paternal age (Figure 18) (615,616,622,623). The rate of de novo mutations increases with paternal age (622), which may contribute to the increase risk of neurodevelopmental diseases such as schizophrenia and autism (622).
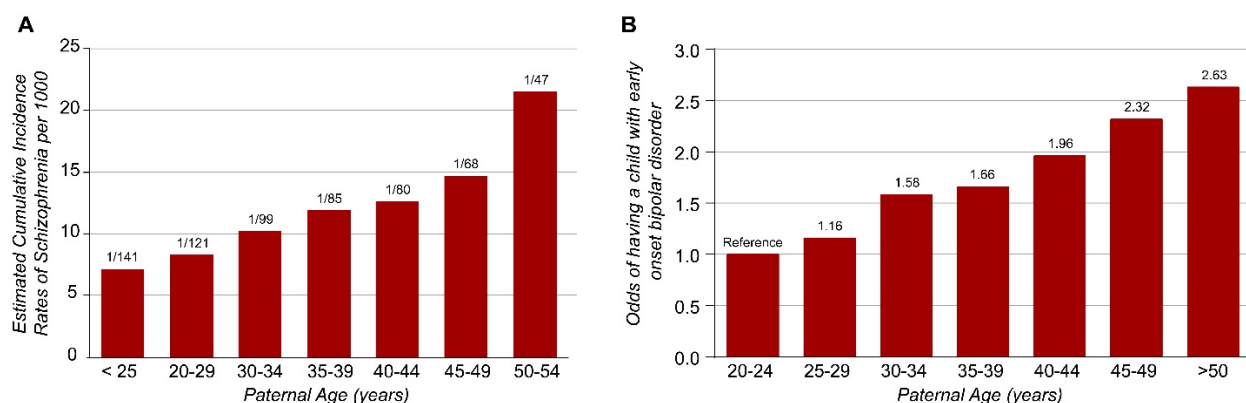
Figure 18. Impact of paternal age on incidence of schizophrenia and early-onset bipolar disorder. Increasing paternal age at conception increases the relative risk of having an offspring with schizophrenia (panel A; figure adapted from Malaspina et al. Arch Gen Psychiatry. 2001 Apr;58(4):361-7.) and the odds ratio of having a child with early-onset bipolar disorder (compared to fathers aged 20 to 24 years; panel B; data derived from Frans et al. Arch Gen Psychiatry. 2008 Sep;65(9):1034-40)

The accumulation of these de novo germ line mutations with increasing paternal age has been explained by the "selfish spermatogonial selection" hypothesis (618,619). According to this hypothesis, the somatic mutations in male germ cells that enhance the proliferation of germ cells could lead to within-testis expansion of mutant clonal lines (620,621), thus favoring the propagation of germ cells carrying these pathogenic mutations, and increasing the risk of mutations in the offspring of older fathers (620,621). Interestingly, the risk of autism has also been associated with the age of the father as well as the grandparent (623). These concerns have prompted the American Society of Reproductive Medicine to state in their guidelines that semen donors should be younger than 40 years of age so that potential hazards related to aging are diminished (610).

Some cardiac defects have also been attributed to aberrant genetic input from older men. For instance, a case-control study of 4,110 individuals with congenital heart defects born between 1952 and 1973 in British Columbia, found a general pattern of increasing risk with increasing paternal age among cases relative to controls for ventricular septal defects, atrial septal defects, and patent ductus arteriosus (617,624). The risk of schizophrenia has also been reported to increase with paternal age (618) and possible loci affecting this risk have been identified (625). In addition, a modest proportion of preeclampsia, normally associated with increased maternal risk factors including age, might be attributable to an increase in paternal age although no gene loci have been identified (626). These observations need further corroboration.

Although there is a positive association between paternal age and incidence of aneuploidy, it has been difficult to dissociate the effect of paternal age from the confounding influence of the advanced maternal age. After accounting for various confounders, there does not appear to be a major independent effect of increased paternal age on the incidence of autosomal aneuploidies (608,609,615,616,627,628). The existence of a paternal age effect on Down syndrome is controversial. Earlier studies from the 1960s and

1970s found no correlation between Down syndrome and paternal age (e.g.,(629)). However, a study in New York from 1983 to 1997 found a significant greater numbers of mothers and fathers 35 years of age and older, respectively, among parents of patients with Down's syndrome (630). Among the cases of Down syndrome evaluated, paternal age had a significant effect only when the mothers were 35 years of age or older, and was the highest when both the Mother and Father were older than 40 years in which case the risk of Down Syndrome was 6 times that observed among couples younger than 35 years of age (630).

Changes in Fertility of Older Men

A review of studies examining fertility at different ages demonstrated significant age-related differences in fertility rates in men; men older than 50 have lower pregnancy rates, increased time to pregnancy, and subfecundity compared to younger men (608,609,615,631,632). Some changes in fertility rates might be related to age-related decrease in sexual activity. A literature review found no significant change in sperm concentration with aging when comparing men under the age of 30 to those greater than 50 years (613). However, in general, semen volume, sperm motility, and the number of morphologically normal sperm decrease with advancing age (Table 5; (45,608-614,622,627,628,631,633)). A number of these studies, however, did not control for important confounding variables. Of the 21 studies in which sperm densities were compared among men of different age groups (613), only four studies adjusted for the duration of abstinence, well known to affect sperm concentration. In addition, there is significant heterogeneity in the populations studied; most of the studies examined data from semen of sperm donors while others examined men from infertility clinics. Sperm donors might represent a healthier group of men than the general population; conversely men in infertility clinics might be more likely to have abnormalities of sperm number or function. Even studies that have controlled for abstinence as well as alcohol and tobacco use have shown an age-related decrease in semen volume. In one study of men whose partners had bilateral tubal obstruction or absence of both tubes and who were treated by conventional IVF, the odds ratio of failure to conceive was higher for men 40 years of age or older (634).

Table 5: Changes in Semen Quality and Fertility in Men with Age		
Parameter	**Age comparison**	**Change**
Semen volume	30 versus ≥50 years	3-22% decrease
Sperm concentration	Varying	None
Abnormal sperm morphology	≤30 versus ≥ 50 years	4-18% increase
Time to pregnancy	<30-35 versus >30-50 years	5-20% increase
Pregnancy rates	<30 versus > 50 years	23-38% decrease
Subfecundity	Varying	11-250% increase

From a Literature Review by Kidd et al., 2001 (501)

CHANGES IN THE GERM CELL COMPARTMENT

In a comparison of younger men (21-25 years) with older men (>50) referred for andrological evaluation, the ejaculate volume, progressive sperm motility, and sperm morphology were lower in older men than younger men after adjustment for duration of sexual abstinence (635). The older men also had a higher frequency of sperm tail defects, suggesting epididymal dysfunction (636). In addition, the fructose content was significantly lower in older men suggesting a defect in the seminal vesicle contribution to semen. There were no significant differences in sperm concentration and testicular size between the young and older men in this study.

Necropsies on adult men of different ages have revealed that the testicular volume is lower only in men in the 8th decade of life (637). A recent study examined testicular germ cells obtained by orchiectomy from 36 older men with advanced prostate cancer and by testicular biopsy from 21 younger men with obstructive azoospermia, as controls (638). The ratios of primary spermatocytes, round spermatids, and elongated spermatids to Sertoli cells were significantly decreased in the testes of older men, but the ratio of spermatogonia to Sertoli cell number remained unchanged (638,639). Older men are characterized by lower rates of germ cell apoptosis and cell proliferation compared with younger men, suggesting that germ cell proliferation and apoptosis diminish with aging (639).

Other studies evaluating the fidelity of the germ cell compartment are cross-sectional and depend on analyses of sperm number and semen quality; large-scale chromosomal analyses in healthy community dwelling men are scarce as most data are derived from fertility clinics. A review of studies examining semen quality at different ages demonstrated significant age-related decrease in semen volume and sperm morphology. The change in sperm morphology has been hypothesized to be due to an increase in aneuploidy with age. Härkönen et al (628) found that sperm morphology was directly associated with the number of chromosomes in sperm and that men with higher aneuploidy rates for chromosomes 13, 18, 21, X and Y had lower sperm motility and sperm concentrations. Despite the changes in sperm morphology and motility from older men, *in vitro* fertilizing capacity of the sperm is well preserved (45,634).

There are several difficulties in interpreting these data on age-related changes in sperm density and function. The normal range for sperm concentration in men is wide where sperm concentration above 15 million/ml (total sperm per ejaculate > 39 million) is considered normal. Thus, even though average sperm concentrations decline with aging, they are still are in the normal range (45,632,638). Furthermore, normal sperm counts do not always correlate with normal sperm function.

Studies in flies demonstrate more germ cells during larval than adult stages suggesting age-related quiescence of the germ line (640). Significant age-related decreases in germ cells and spermatogenesis also have been reported in rodents and primates (641-645). The Brown Norway rat has been studied as a model of aging of the human male reproductive system because in this rodent model, serum testosterone levels decrease with aging, as they do in humans (642-644). Along with changes in hypothalamic-pituitary hormones, alterations in sperm counts, sperm maturation, Sertoli cell number, and progeny outcomes have been observed in this rodent model (Table 6) (626,642-651). Analysis of ribosomal DNA from germ cells of the male brown Norway rat

has revealed hypermethylation of ribosomal DNA (645,652). Alterations in ribosomes have been theorized to promote aging of cells by multiplying errors in protein synthesis which initially might elude gross morphological analysis but eventually might lead to germ cell degeneration (652). Further assessment of spermatogonial stem cell populations is needed. In many animal models of life span extension, there is a trade-off between longer life and fecundity, although there are some exceptions (653).

Table 6. Changes in the Reproductive Axis in the Brown-Norway Rat		
Parameter	Change	Reference
GnRH	↓	530, 531
FSH	↑	530, 531
LH	→	530, 531
Testosterone	↓	530-532, 534
Germ Cells	↓	535
Sertoli Cells	↓	531, 537
Spermatogenesis	↓	531, 537
Seminiferous Tubules	↓	531, 537
Seminiferous Tubule Function	altered	534, 537
Epididymal function	↓	538
Sperm morphology	altered	538
Sperm motility	↓	538
Sperm Count	↓	532

CHANGES IN SUPPORTIVE CELLS AND ACCESSORY GLANDS

Since Sertoli and Leydig cells are crucial to spermatogenesis, changes in these cells could affect sperm number and function. Age-related changes in the supporting structures for sperm maturation have been described in the Brown Norway rat. These changes include reductions in the numbers of Leydig and Sertoli cells (643-645). Changes in the supporting cells and structures for sperm maturation have been invoked to explain the age-related decrease in sperm number and fecundity in rats. In stallions, the numbers of Sertoli cells decreases with aging but individual Sertoli cells display a remarkable capacity to accommodate greater numbers of developing germ cells (654).

In men, Sertoli cell number has been reported to be lower in men aged 50 to 85 years than in men aged 20 to 48 years (655). The apoptotic rate of primary spermatocytes in aged men was also significantly elevated compared with that of younger men, resulting in a decrease of the number of primary spermatocytes per Sertoli cell (639), leading the authors to suggest that there might be a failure of the Sertoli cells to support spermatogenesis in older men.

Sertoli cells produce inhibin, which regulates gonadotropin expression from the pituitary. Inhibin B has been identified as the physiologically important

form of inhibin in men and as a valuable serum marker of Sertoli cell function and spermatogenesis. Higher gonadotropins and lower inhibin levels in older men suggest a decline in Sertoli cell function ([655](#)); however changes in circulating inhibin B levels with advancing age have been inconsistent ([70,655-657](#)). Overall, these data suggest a possible decline in Sertoli cell number and function in older.

Aging is accompanied by a progressive, albeit variable, decline of Leydig cell function with a decrease of mean serum free (or bioavailable) testosterone levels in the population between age 25 and 75 years ([658](#)). Total Leydig cell volume and the absolute number of Leydig cells decline with advancing age, although total testis weight does not change substantially with age ([658-662](#)). In one study, age accounted for more than a third of the variation in Leydig cell number, and explained more than half the variation in daily sperm production ([661](#)). This might in part be explained by a fusion of Leydig cells resulting in fewer but multinucleated Leydig cells with age ([662](#)). The functionality of the multinucleated cells is not known.

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