**Androgens and cardiovascular disease In men**

**Bu B. Yeap,** Medical School, University of Western Australia, Perth, Western Australia, Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Perth, Western Australia. bu.yeap@uwa.edu.au

**Girish Dwivedi,** Medical School, University of Western Australia, Perth, Western Australia, Harry Perkins Institute of Medical Research, University of Western Australia, Perth, Western Australia, Department of Cardiology, Fiona Stanley Hospital, Perth, Western Australia.

**Updated December 13, 2022**

**ABSTRACT**

Testosterone is the principal male sex hormone or androgen, which regulates sexual characteristics and body composition. Testosterone is converted to bioactive metabolites dihydrotestosterone and estradiol. Circulating testosterone peaks in early adulthood and declines gradually across middle and older age: older men exhibit lower testosterone and dihydrotestosterone concentrations compared to younger men. In older men, lower testosterone concentrations are associated with higher incidence of cardiovascular events. Lower testosterone and dihydrotestosterone concentrations have also been associated with higher cardiovascular mortality in older men. However, causation is unproven as a randomized placebo-controlled trial of testosterone treatment sufficiently powered to examine outcomes of cardiovascular events or mortality has yet to be reported. Potential mechanisms by which testosterone could exert beneficial actions in the vasculature include reduction in cholesterol accumulation and modulation of inflammation. Smaller randomized trials of testosterone therapy have shown improvements in surrogate endpoints related to cardiovascular risk. However, other trials of testosterone have not shown improvements in carotid atherosclerosis, as assessed by carotid intima-media thickness. One study reported an increase in coronary atheroma assessed using coronary computed tomography angiography in older men receiving testosterone therapy over 12 months. Although one randomized trial of testosterone therapy in older men with mobility limitations reported an excess of adverse events in the treatment arm, larger recent trials in middle-aged to older men did not find any excess of cardiovascular adverse events with testosterone treatment. Meta-analyses of testosterone trials generally have not shown an increase in cardiovascular adverse events. Retrospective case-control studies of health insurance databases have major methodological limitations. The results from such studies are inconsistent, associating testosterone prescriptions with either increased or decreased risk of cardiovascular events, and with lower mortality. While androgen deprivation in men with prostate cancer results in adverse metabolic effects, abuse of high dosages of androgenic steroids is associated with harm. Thus, while some epidemiological studies associate higher circulating concentrations (but within the normal range) of endogenous androgens with lower risk of cardiovascular events and mortality, the effects of exogenous androgens in the form of testosterone therapy seeking to maintain physiological circulating androgen concentrations on the cardiovascular system remain uncertain. This evidence gap has to be accommodated in the current clinical management of hypogonadal men and should be addressed by further randomized interventional studies to clarify whether testosterone treatment has beneficial, neutral or adverse effects on the cardiovascular system.

**Introduction**

**Androgen Physiology**

THE HYPOTHALAMIC-PITUITARY AXIS

Testosterone (T) is the principal male sex hormone or androgen that regulates sexual maturation and secondary sexual characteristics and body composition in adult men (1). T undergoes conversion into two major bioactive metabolites, dihydrotestosterone (DHT) a more potent ligand for the androgen receptor, and estradiol (E2), a ligand for estrogen receptors (2). T is produced primarily by the testis, under stimulation of luteinizing hormone (LH) from the pituitary gland, itself under regulation of gonadotrophin releasing hormone (GnRH) from the hypothalamus. The hypothalamic-pituitary-testicular (HPT) axis is under negative feedback regulation via T and E2, acting on the central components of the HPT axis (1,3-5).

CONSEQUENCES OF ANDROGEN DEFICIENCY

Androgens play diverse roles in the body, and androgen deficiency in men results in multiple symptoms and signs extending from loss of libido, lethargy, fatigue, poor concentration to gynecomastia, accumulation of fat, loss of muscle mass, and osteopenia or osteoporosis (6,7). Actions of T are amplified by local conversion to DHT in tissues such as the prostate and skin by the enzyme 5α-reductase (8). Of note, some of actions of T, such as in bone and adipose tissue, are mediated via conversion to E2 by the enzyme aromatase (9-11).

MEASUREMENT OF CIRCULATING ANDROGEN CONCENTRATIONS

Immunoassays have been the standard method for measurement of circulating sex hormones: however, these can exhibit non-specificity and method-dependent bias, particularly at lower hormone concentrations (12,13). Mass spectrometry is regarded as the gold standard for assay of T concentrations (14). Although mass spectrometry is preferred, it is not widely available and validated immunoassays can be informative (6,7). In the circulation, T (and DHT and E2) are bound with high affinity to sex hormone-binding globulin (SHBG), T is also bound with lower affinity to albumin with a small fraction unbound or free (15). However, whether unbound or “free” T represents a more biologically active form of the hormone *in vivo* is controversial (16). A major difficulty is that measurement of free T using equilibrium dialysis is technically demanding and thus rarely performed. Instead, free T is typically calculated, and this result may vary according to the equations used (15-18). Furthermore, established reference ranges for free T are lacking (7,19). Thus, analysis of actions of T in the male body and cardiovascular system involves consideration of not only its bioactive metabolites, but also the accuracy of assays used and the limitations of calculated free T as a biomarker.

**Androgens and Male Ageing**

DECLINE IN CIRCULATING ANDROGENS WITH MALE AGING

Circulating T peaks in early adulthood and declines gradually across middle and older age, thus older men exhibit lower T and DHT concentrations compared to younger men (20-22). Observational studies have shown longitudinal declines in T and DHT in ageing men, with parallel increases in LH and SHBG (23-25). This phenomenon suggests that in some men, there is progressive impairment of testicular endocrine function with ageing (25). However, whether older men with symptoms consistent with androgen deficiency and lower circulating concentrations of T compared to younger men, have an androgen deficiency state remains unclear (26,27). The bioactive metabolites of T, DHT and E2 measured with mass spectrometry have been associated with longer leucocyte telomere length, a measure of slower biological ageing, in middle-aged and older men (28,29). However, a study in mostly middle-aged men showed no association between T measured with immunoassay and leucocyte telomere length (30). Thus, there is considerable interest in the question of whether lower T concentrations might contribute to various manifestations of ill health in ageing men.

CONTROVERSIES OVER FUNCTIONAL HYPOGONADISM

Men with disorders of the hypothalamus, pituitary, or testes resulting in hypogonadism have symptoms and signs of androgen deficiency, and low circulating T concentrations (6). These men are classified as having pathological (or classic or organic) hypogonadism, and T treatment is routinely offered to improve symptoms and restore body composition (6,7). However, low circulating T concentrations are found in many conditions where the HPT axis is intact, including ageing, obesity, and systemic illnesses (7,31). In this context, low T concentrations result from reduction or suppression of HPT axis function, rather than an intrinsic disorder of the HPT axis, with the label of “functional hypogonadism” applied (7,31). Obesity is closely associated with reduced circulating T and SHBG, and loss of excess weight restores endogenous production of T (32-35). Thus, low T might be a biomarker for the presence of systemic illnesses, rather than a contributing or causal factor. Whether or not, and if so which, men with functional hypogonadism should receive T treatment, remains the subject of debate (7,19,20).

**Androgens and Risks of Cardiovascular Disease**

AGE AND OBESITY AS COMMON RISK FACTORS

Advancing age is an established risk factor for chronic diseases including cardiovascular disease (CVD) and mortality (36,37). Age is a component of all major cardiovascular risk calculators (38). Similarly, obesity, with its close associations with insulin resistance and diabetes risk, is a robust cardiovascular risk factor, and - unlike age - is a potentially modifiable one, whether via bariatric surgery or incretin-based medical therapy (41-44). This is illustrated by observational studies which show a reduction in cardiovascular events and mortality following bariatric surgery in patients who are obese (mean age 48 years) (41,42). Thus, advancing age and obesity are both associated with low circulating T concentrations, and are also risk factors for CVD.

CONFLUENCE OF AGE, OBESITY, LOW TESTOTERONE AND CARDIOVASCULAR DISEASE

Given that age and obesity are associated with both low circulating T concentrations and increased risk of CVD, understanding the relationship between these factors becomes vitally important (45). Demographic change will result in increasing numbers of older men in communities worldwide, who will have lower circulating T concentrations and who are at risk of ill health including from CVD (36). If low T contributes to CVD risk, either directly or via its association with obesity, then this represents a potential pathway for intervention to preserve health in ageing men. Conversely, if T is a biomarker for CVD, it may still have a role in risk stratification and identification of men at risk who may benefit from non-hormonal interventions directed at conventional risk factors for CVD.

**Epidemiological studies**

**Associations of Androgens with Cardiovascular Events**

PROSPECTIVE COHORT STUDIES: IMMUNOASSAY RESULTS

Prospective cohort studies report the association of endogenous sex hormones with incidence of cardiovascular events (Table 1). Analyses invariably adjust for age, and typically also include adjustment for body mass index (or waist circumference) and other conventional cardiovascular risk factors. From studies reporting sex hormone results based on immunoassays, some longitudinal analyses have shown no association of total T concentrations with incidence of myocardial infarction (MI) or ischemic heart disease (IHD) events (46,49,53,63,64). In an analysis of 3,443 men aged ≥70 years from the Western Australian Health In Men Study (HIMS), low total T concentrations were associated with an increased incidence of stroke (50). This finding was confirmed by later studies (57,59). In another analysis from HIMS, higher LH was associated with incident IHD (52). One smaller study reported a U-shaped association of total T with incidence of cardiovascular events (54). There were conflicting associations of total E2 with stroke (47,48), and there was no association of total E2 with the incidence of MI (49). In one study, men with lower total T had a higher risk of heart failure, but this was not confirmed in another study (65,68).

|  |
| --- |
| **Table 1. Cohort Studies Examining Associations Between Sex Hormones with Cardiovascular Events in Middle-Aged and Older Men** |
| **Study author and year** | **Size (n of men)** | **Follow-up (yr)** | **Age (yr)** | **Summary of results** |
| Smith GD, 2005 (46) | 2,512 | 16.5 | 45-59 | No association of total T with IHD events or deaths. |
| Arnlov J, 2006 (47) | 2,084 | 10 | 56 | Higher total E2 at baseline associated with lower incidence of CVD events, total T was not associated. |
| Abbott RD, 2007 (48)  | 2,197 | ≤7 | 71-93 | Baseline total E2 in top quintile (≥125 pmol/L) associated with higher risk of stroke, total T was not associated. |
| Vikan T, 2009 (49)  | 1,568 | ≤13 | 59.6 | No association of total T or E2 with incident MI, or with CVD or IHD mortality. |
| Yeap BB, 2009 (50) | 3,443 | 3.5 | ≥70 | Total and free T in the lowest quartiles (<11.7 nmol/liter and <222 pmol/liter) predicted increased incidence of stroke or TIA. |
| \* Ohlsson C, 2011 (51)  | 2,416 | 5 | 69-81 | Men with total Ta in highest quartile (≥19 mol/L) had lower risk of CVD events. E2 was not associated. |
| Hyde Z, 2011 (52)  | 3,637 | 5.1 | 70-88 | Higher LH was associated with incident IHD. |
| Haring R, 2013 (53)  | 254 | 5, 10 | 75.5 | No associations of baseline total T or total E2 with incident CVD events. |
| Soisson V, 2013 (54) | 495; 146 | 4 | >65 | Total T in lowest and highest quintiles associated with CHD or stroke. |
| \* Shores MM, 2014 (55)  | 1,032 | 9 | 76 | DHTb <1.7 or >2.6 nmol/L associated with cardiovascular events. Total T was not associated. |
| \* Shores MM, 2014 (56) | 1,032 | 10 | 76 | Non-linear association of DHTb with stroke with lowest risk in men with DHT 1.7-2.6 nmol/L. Total Tb was not associated with stroke. DHT <0.86 nmol/L associated with CVD mortality.  |
| \* Yeap BB, 2014 (57) | 3,690 | 6.6 | 70-89 | Higher total Tc (>12.6 nmol/L) or DHT (>1.34 nmol/L) associated with lower incidence of stroke. Tc, DHT and E2 were not associated with MI. |
| \* Srinath R, 2015 (58) | 1,558 | 12.8 | 63.1 | Td was not associated with incidence of CHD events, or cardiac-related mortality. |
| Holmegard HN, 2016 (59) | 4,602 | 20 | 57 | Total T in lowest decile (0-10th percentile) associated with stroke. |
| \* Chan YX, 2016 (60) | 1,804 | 14.9 | 50.3 | Total Tc, DHT and E2 were not associated with CVD events. |
| \* Srinath R, 2016 (61) | 1,558 | 14.1 | 63.1 | Td was not associated with stroke. |
| Wang A, 2019 (62) | 5,553 | 6 | 63.5 | Neither total T nor free T were associated with CVD events. |
| \* Gyawali P, 2019 (63) | 1,492 | 4.9 | 54.2 | Higher total Tc associated with lower risk of incident CVD events, but not with CVD mortality. E2 not associated. |
| Hatami H, 2020 (64) | 816 | 12 | 46.1 | Total T was not associated with risk of CVD events. |
| Zhao D, 2020 (65) | 4,107 | 19.2 | 63.2 | Lower total T associated with increased risk of incident heart failure. |
| \* Collett T-H, 2020 (66) | 552 | 7.4 | 72.4 | Total Td not associated with CVD events. |
| \* Boden WE, 2020 (67) | 2,118 | 3 | ≥40 | 643 men with total Tb <10.4 nmol/L had higher risk of combined endpoint of CHD death, MI or stroke, compared with 1,475 men with total T ≥10.4 nmol/L.  |
| Shafer S, 2021 (68) | 3,865 | 13.8 | 48.2 | Lower total T not associated with incident heart failure. |
| Yeap BB, 2022 (69) | 210,700 | 9 | 58 | Total T not associated with incident MI, stroke, heart failure or MACE. Calculated free T not associated with incident MI, stroke or heart failure, but associated with incidence of MACE. |

IHD=ischemic heart disease, CVD=cardiovascular disease, MI=myocardial infarction, TIA=transient ischemic attack, CHD=coronary heart disease, MACE=major cardiovascular adverse event. \* denotes studies where total T, DHT and/or E2 were measured using mass spectrometry. aT and E2 assayed using gas chromatography-mass spectrometry (GC-MS), bT and DHT assayed using liquid chromatography-tandem mass spectrometry (LC-MS), cT, DHT and E2 assayed by LC-MS, dT assayed using LC-MS

Recently the association of testosterone with CVD events was examined in the largest prospective cohort study to date, the United Kingdom (UK) Biobank (69). In this study of 210,700 men aged 40-69 years at baseline, with 9 years follow-up, 8,790 had an incident CVD event. Total T was not associated with risk of incident MI, ischemic stroke, hemorrhagic stroke, heart failure, nor major cardiovascular adverse events (MACE) defined as the composite endpoint of non-fatal MI, non-fatal ischemic stroke, and CVD death. The large size of the UK Biobank, and accumulation of outcome events over the period of follow-up, provided power to examine these associations in a robust fashion. UK Biobank used an immunoassay for measurement of serum T which may underestimate results compared to mass spectrometry (70), and UK Biobank men were generally healthier than the UK male population as a whole (71). Therefore, while these results are convincing, their generalizability to different populations in other regions needs to be established.

PROSPECTIVE COHORT STUDIES: MASS SPECTROMETRY RESULTS

Prospective cohort studies where sex hormones were measured using mass spectrometry are shown (Table 1, marked with \*). In the Osteoporotic Fractures in Men Study in Sweden (MrOS), a large prospective cohort study of 2,416 men aged 69-81 years, men with higher total T had a lower incidence of CVD events (51). In HIMS, a later analysis of 3,690 men aged 70-89 years with sex hormones measured using mass spectrometry confirmed the association of low total T with higher incidence of stroke (57). In these analyses, total E2 was not associated with these outcomes (51,57). Analyses from the Cardiovascular Health Study (CHS) of 1,032 men aged 76 years suggested a U-shaped association of DHT with CVD events and stroke risk (55,56). Of note, an analysis from the Busselton Health Study (BHS) of 1,804 predominantly middle-aged men found no association of sex hormones with incidence of CVD events (60). Analyses from the Atherosclerosis Risk in Communities Study of 1,558 men aged 63 years found no association of sex hormones with CVD events (58,61), similar to the findings from a small subset of the MrOS USA study (66). By contrast, in the Men Androgen Inflammation Lifestyle Environment and Stress (MAILES) cohort of 1,492 men followed for 4.9 years, higher total T was associated with a lower risk of CVD events (63). Furthermore, in a post-hoc analysis of 2,118 men with metabolic syndrome participating in a trial of niacin or placebo plus simvastatin, men who had a baseline total T <10.4 nmol/L had higher risk of the combined endpoint of coronary heart disease death, MI or stroke, compared with men with higher total T concentrations (65).

SUMMARY: ENDOGENOUS SEX HORMONES VS. CARDIOVASCULAR DISEASE

Taken together, these epidemiological studies suggest that there may be an association of lower endogenous T concentrations with increased risk of CVD events in middle-aged and older men. However, the studies provide a mix of positive, equivocal and negative results. Major cohort studies using mass spectrometry for assay of sex hormones associated higher total T concentrations with lower risk of CVD events in older (51,57) and middle-aged to older men (63,67). There may be a predilection for lower testosterone, measured by mass spectrometry, to be associated with stroke risk (57). In one study lower DHT was associated with CVD events including stroke, with a non-linear association (55,56). Inconsistent results in other studies may have been due to smaller cohort sizes and fewer outcome events reducing the power available to detect underlying associations. However, in the largest ever prospective cohort study, the UK Biobank, there was no association of testosterone with a range of CVD events (69). Overall, epidemiological studies would suggest a possible protective effect of endogenous androgens against CVD events in the older population of men, rather than in relatively healthy middle-aged men.

**Associations of Androgens with Cardiovascular Mortality**

PROSPECTIVE COHORT STUDIES: IMMUNOASSAY RESULTS

Several of the studies in Table 1 reported CVD-related mortality in addition to events. Other studies where the outcome was based on CVD-related mortality are summarized in Table 2. Studies almost invariably adjusted for age, and typically adjusted for BMI and other cardiovascular risk factors. In two studies using immunoassay for assay of sex hormones, T was not associated with CVD or ischemic heart disease (IHD) mortality (46,49). However, several other studies using immunoassay for sex hormones did find associations of lower endogenous total T concentrations with increased risk of CVD-related death (72,74,77,81). Lower calculated free T was also associated with increased CVD-related mortality in some studies (76,78,81), but was associated with lower IHD mortality in one study (73). Lower total E2 was associated with CVD mortality in one study (76). In an analysis from the UK Biobank of 149,436 men followed for 11.3 years, there was no association of either total or calculated free T with risk of CVD mortality (84).

|  |
| --- |
| **Table 2. Cohort Studies Examining Associations Between Sex Hormones and CVD-Related Mortality in Middle-Aged and Older Men** |
| **Study author and year**  | **Size (n of men)** | **Follow-up (yr)** | **Age (yr)** | **Summary of results** |
| Khaw K-T, 2007 (72) | 825 and 1489  | ≤10  | 40-79  | Total T inversely related to mortality from all causes, CVD and cancer. |
| Araujo AB, 2007 (73) | 1,686 | 15.3 | 40-70 | Lower free T associated with lower IHD mortality. Equivocal association of lower DHT with IHD mortality. |
| Laughlin GA, 2008 (74) | 794 | 11.8 | 50-91 | Total T in the lowest quartile (<8.4 nmol/L) predicted increased mortality from all causes and from CVD and respiratory causes. |
| \* Tivesten A, 2009 (75) | 3,014 | 4.5 | 75 | Total Ta and E2 levels in the lowest quartiles predicted all-cause and non-CVD mortality. T and E2 were not associated with CVD mortality. |
| Menke A, 2010 (76)  | 1,114 | 18 | ≥20 | Lower free T associated with overall and CVD mortality in first 9 years of follow-up. Lower total E2 associated with CVD mortality. (Difference between 90th and 10th percentiles for free T and total E2) |
| Haring R, 2010 (77)  | 1,954 | 7.2 | 20-79 | Total T <8.7 nmol/L associated with increased all-cause, CVD and cancer mortality. |
| Hyde Z, 2012 (78) | 3,637 | 5.1 | 70-88 | Lower free T (100 vs 280 pmol/L) predicted all-cause and CVD mortality. |
| \* Yeap BB, 2014 (79)  | 3,690 | 7.1 | 70-89 | Optimal total Tb (9.8-15.8 nmol/L) predicted lower all-cause mortality. Higher DHT (>1.3 nmol/L) predicted lower IHD mortality. E2 was not associated with mortality. |
| \* Pye SR, 2014 (80)  | 2,599 | 4.3 | 40-79 | Presence of sexual symptoms and total Tc <8 nmol/L associated with all-cause and CVD mortality, total or free T not associated. |
| Holmboe SA, 2015 (81) | 5,323 | 18.5 | 30-70 | Higher T or free T (highest vs lowest quartile) associated with lower CVD mortality. |
| \* Hsu B, 2016 (82) | 1,705, 1,367 and 958 | 0, 2 and 5  | ≥70  | Decrease in total Td over time associated with all-cause but not CVD mortality. Decrease in total E2d was associated with all-cause and CVD mortality. |
| \* Chasland L, 2017 (83) | 1,649 | 20 | 49.8 | Higher physical activity and total Tb, DHT and E2 were not associated with CVD events. Men with higher physical activity and DHT had the lowest risk of CVD death. Men with lower physical activity and higher E2 had greater risk of CVD death. |
| Yeap BB, 2021 (84) | 149,436 | 11.3 | 58.0 | Men with lower total T had higher all-cause and cancer-related mortality, no association with CVD deaths.  |

IHD=ischemic heart disease, CVD=cardiovascular disease, CHD=coronary heart disease. \* denotes studies where total T, DHT and/or E2 were measured by mass spectrometry; free T was calculated. aT and E2 measured using gas chromatography-mass spectrometry (GC-MS). bT, DHT and E2 measured using liquid chromatography-tandem mass spectrometry (LC-MS), cT measured using GC-MS, dT and E2 measured using LC-MS.

PROSPECTIVE COHORT STUDIES: MASS SPECTROMETRY RESULTS

Prospective cohort studies using mass spectrometry for assay of sex hormones are of interest. In an analysis from MrOS in Sweden, lower endogenous total T and E2 concentrations were associated with all-cause and non-CVD mortality, but not with CVD mortality (75). Interestingly, in an analysis from HIMS of 3,690 men aged 70-89 years at baseline, optimal endogenous total T concentrations were associated with survival, and higher DHT predicted lower IHD mortality (79). The CHS study reported consistent findings with lower DHT concentrations being associated with CVD mortality (56). An analysis from the European Male Ageing Study found that the combination of sexual symptoms and lower total T was associated with all-cause and CVD mortality, rather than total T or free T on their own (80). In an analysis from the Concord Health and Ageing in Men Project (CHAMP), longitudinal decreases in total T, DHT or E2 were associated with all-cause mortality, but only the longitudinal decrease in total E2 was predictive of CVD mortality (82). Finally, in an analysis from BHS in which physical activity and sex hormones concentrations were analyzed, men with higher levels of physical activity and higher DHT concentrations had the lowest risk of CVD death (83).

SUMMARY: ENDOGENOUS SEX HORMONES VS. CARDIOVASCULAR MORTALITY

Several cohort studies have reported an association between lower endogenous T concentrations and increased mortality related to CVD, after adjusting for age and other cardiovascular risk factors. Of large studies using mass spectrometry for assay of sex steroids, MrOS in Sweden found an association of lower total T and E2 with all-cause rather than CVD mortality, while HIMS found an optimal total T to be associated with survival (75,79). HIMS found higher DHT was associated with lower IHD mortality (79), consistent with results from CHS (56), and in BHS the combination of higher DHT and higher levels of physical activity was associated with lower risk of death from CVD (83). Declining E2 may also have a role, being associated with CVD mortality in CHAMP (82). However, in relatively healthy middle-aged men (UK Biobank), there was no evidence of an association between total T and CVD mortality risk (84).

Therefore, allowing for some heterogeneity in cohort characteristics and results, lower endogenous T concentrations, measured using mass spectrometry, may be predictors of CVD related deaths in older men, as might lower or declining concentrations of its bioactive metabolites DHT and E2. However, this may not be the case in generally healthy middle-aged men. Whether lower concentrations of endogenous sex hormones are biomarkers or possibly contributing factors to these outcomes remains unclear from these observational studies, as proof of causality ultimately requires interventional studies and randomized controlled trials (RCTs).

**Mechanistic Studies**

**Potential Mechanisms**

Knowledge of potential mechanisms by which androgens might exert protective effects against atherosclerosis and reduce the risk of cardiovascular events would bridge the findings from epidemiological studies and clinical investigation. There are a substantial number of such studies with diverse models and results, a comprehensive discussion of each being beyond the scope of this chapter (for reviews, see (85,86)). Selected studies are discussed briefly in this context.

**Cholesterol Accumulation in Animal Models**

Experimental studies in castrated male rabbits fed a high cholesterol diet reported effects of testosterone treatment to reduce accumulation of cholesterol in the aortic wall and to reduce atheromatous plaque area and aortic intimal thickness (87-90). Similar results have also been reported in miniature pigs (91). Castration of low-density lipoprotein receptor (LDLR)-deficient male mice results in increased fatty steak lesion formation in the aorta compared to non-castrated controls, which is attenuated with testosterone supplementation (92). At least part of this effect may be mediated via conversion of T to E2. Of note, T and DHT increased calcification of plaque in apolipoprotein E (ApoE)-null mice, even as T had a neutral effect on plaque volume and DHT decreased plaque volume (93). In testicular feminized mice with a non-functional androgen receptor (AR) and low circulating T concentrations, T supplementation to physiological levels reduced fatty streak formation (94). Similarly, AR knockout mice (ARKO) showed increased aortic atherosclerosis, and atherosclerotic lesion area that was reduced with T treatment (95). In wild-type mice, T treatment reduced the presence of necrotic cores within plaque compared with placebo. Therefore, these animal studies suggest an effect of T treatment in reducing cholesterol accumulation and the development of atheromatous plaque, while increasing calcification. However, the actions of sex hormones are complex, being mediated partly via aromatization of T to E2, and occurring at least to an extent via AR-independent mechanisms.

**Neointimal Formation and Vascular Smooth Muscle Proliferation**

NEOINTIMAL RESPONSES TO INJURY

In a male rabbit aorta model of neointimal plaque formation induced by endothelial denudation, T treatment *in vitro* inhibited plaque development (96). In a male porcine model of coronary neointimal plaque formation following moderate angioplasty-induced arterial injury, castrated males exhibited greater intimal area compared to intact males and castrated males treated with T (97). T inhibited proliferation and increased expression of the cell-cycle regulator p27kip1 during neointimal formation. However, despite castration of wild-type mice resulting in increased neointimal formation following wire injury, selective deletion of AR from endothelial cells or smooth muscle cells did not affect lesion size (98). Therefore, effects of T on neointimal formation may be indirect, or mediated by AR-independent mechanisms.

VASCULAR SMOOTH MUSCLE

Vascular smooth muscle cells contribute to progression of atherosclerotic lesions and formation of the fibrous cap (99). T was shown to regulate expression of proliferation-associated genes in skeletal myocytes and in myofibers in different muscles (100,101). Its role in smooth muscle cells in the vasculature is not well defined. In one study, T exerted a pro-proliferative effect on vascular smooth muscle cells *in vitro*, with increased DNA synthesis assessed using a thymidine incorporation assay (102). In that study the effect of T was blocked by the AR antagonist flutamide. In another study, T induced apoptosis in cultured vascular smooth muscle cells, in an AR-dependent manner (103). In one study, deletion of the AR in vascular smooth muscle cells did not change atherosclerotic plaque size in LDLR knockout mice (104). However, another study demonstrated that T, acting via the AR in vascular smooth muscle cells, might be involved in promoting vascular calcification (105). Therefore, T seems to exert indirect effects on neointimal proliferation in response to injury and may play a secondary role in the development and calcification of atheromatous plaque via complex actions in vascular smooth muscle.

**Inflammation**

INFLAMMATION AND ATHEROTHROMBOSIS

A mechanistic link likely exists between inflammation and atherothrombosis (for reviews, see (106,107)). Statin therapy lowered both LDL cholesterol and C-reactive protein (CRP) concentrations, reducing the risk of cardiovascular events in a primary prevention setting in adults with LDL <3.4 mmol/L and high-sensitivity CRP ≥2.0 mg/L (108). Recently, anti-inflammatory intervention utilizing canakinumab, a monoclonal antibody targeting interleukin-1β, in a secondary prevention setting in adults with high-sensitivity CRP ≥2.0 mg/L demonstrated a modest reduction in major cardiovascular events (109). However, a major trial using low-dose methotrexate showed no benefit in adults with previous MI or multivessel coronary artery disease and either type 2 diabetes or metabolic syndrome (110). By contrast, colchicine has shown promise in major RCTs as an anti-inflammatory agent to reduce cardiovascular risk in both acute and chronic secondary prevention settings (111,112). These results underscore the relationship between inflammation and atherosclerosis.

TESTOSTERONE EFFECTS ON IMMUNE CELLS

Of note, *in vitro* studies have shown effects of T to reduce production of inflammatory cytokines from monocytes, macrophages and endothelial cells (113-115). Deletion of monocyte-macrophage AR in LDLR knockout mice resulted in reduced atherosclerosis compared to LDLR knockout mice, suggesting a role for AR-mediated actions in inflammatory cells (104). In an elegant study, pre-pubertal castration of male ApoE knockout mice increased atherosclerotic lesion area, which was abolished by an anti-CD3 antibody targeting T cells, linking hormonal and immunologic regulation of atherosclerosis (116). In that study, both castration and depletion of AR in epithelial cells resulted in increased thymus weight, and mice with depletion of AR in epithelial cells showed increased atherosclerosis and increased infiltration of T cells in the vascular adventitia (116). These findings support a mechanism by which deficiency of androgen action modulates immune/inflammatory responses to promote atherosclerosis.

TESTOSTERONE EFFECTS ON CYTOKINES

Older men treated with gonadotrophin-releasing hormone agonists to suppress HPT axis function showed increased concentrations of circulating tumor necrosis factor-α and interleukin-6 (117). In a randomized cross-over trial of 27 men ranging in age from 36-78 years, T treatment given via intramuscular injections over one month reduced circulating concentrations of tumor necrosis factor-α and interleukin-1β, and increased concentrations of the anti-inflammatory cytokine interleukin-10 (118). In another study of 20 men with type 2 diabetes, there was an inverse correlation of baseline T and interleukin-6, but T treatment over three months, while reducing waist circumference, did not alter tumor necrosis factor-α, interleukin-6, or C-reactive protein (CRP) concentrations (119). In the Testosterone Trials (T-Trials), T treatment via transdermal gel over 12 months in men aged ≥65 years did not change concentrations of high-sensitivity CRP or interleukin-6 (120). In a study of men treated with DHT or with recombinant human chorionic gonadotrophin over three months, neither intervention affected markers of endothelial cell activation or inflammation (121). By contrast, in a trial of men with metabolic syndrome, men in the T treatment arm showed a reduction in high sensitivity CRP after 12 months of treatment (122). In a trial of 76 men with newly diagnosed type 2 diabetes, T treatment over 9 months reduced markers of endothelial cell activation and inflammation, namely circulating concentrations of intracellular adhesion molecule type 1, p-selectin, and CRP (123). Therefore, the results of clinical studies are not wholly consistent. In summary, although the concept that T might exert anti-inflammatory actions protective against atherosclerosis is plausible, more evidence is needed using a direct measure of atherosclerosis.

**Clinical trials with surrogate endpoints**

**Testosterone Effects on Angina and Vascular Function**

EFFECTS OF EXOGENOUS TESTOSTERONE ON ANGINA

Mechanistic studies in cell and animal models provide a plausible rationale for the epidemiological findings associating lower endogenous T concentrations with higher risk of CVD. However, clinical studies are necessary to clarify whether administration of T modulates clinical manifestations of CVD *in vivo*. Case series from the 1940s reported a beneficial effect of T therapy using intramuscular testosterone propionate to decrease the frequency and severity of angina attacks in an era where nitrate therapy was the mainstay of therapy (124-126). These early reports in men (and a small number of women) describe gradual improvements in symptoms over periods ranging from weeks to months. Conversely, a study in the 1960s found that administration of oral conjugated estrogen (that would suppress the HPT axis and serum androgen concentrations) to men resulted in adverse cardiovascular effects (127). In any case, as T is the native hormone which is metabolized *in vivo* to DHT and E2 (2), it is the preferred treatment for hypogonadal men (128), and represents the logical candidate for interventional studies.

More recent RCTs have revisited the issue of T treatment in men with CAD (Table 3A). In studies lasting from eight weeks to 12 months, T supplementation in men with CAD increased post-exercise ST segment depression (129), time to ischemia on exercise testing (130,132) and in a study in older men with diabetes, reduced the frequency of angina and silent myocardial ischemia during ECG Holter monitoring (133). These findings suggest either a protective effect of T on the myocardium, or an improvement in exercise capacity. A cross-over study found increased perfusion of myocardium supplied by unobstructed arteries, consistent with a vasodilatory action (131). Therefore, while existing data are limited, contemporary RCTs support historical observations suggesting a potentially beneficial effect of T supplementation in men with CAD.

|  |
| --- |
| **Table 3. Selected Randomized Controlled Trials (RCTs) of Testosterone Supplementation in Middle-Aged and Older Men Reporting Outcomes Related to Angina (A), Artery Health (B), and Cardiovascular Adverse Events (C)** |
| **Study author and year**  | **Population (men)** | **Formulation of T**  | **N** **active** | **N placebo** | **Duration** | **Result** |
| A |  |  |  |  |  |  |
| Jaffe MD, 1977 (129) | Men with ST segment depression on exercise (mean age 58 years) | T cypionate 200 mg weekly | 25 | 25 | 8 weeks | Decreased postexercise ST segment depression in T-treated but not placebo group |
| English KM, 2000 (130) | Men with coronary artery disease (mean age 62 years) | Transdermal patch 5 mg | 22 | 24 | 12 weeks | Increased time to 1-mm ST- segment depression during treadmill exercise |
| Webb CM, 2008 (131) | Men with angiographically proven coronary artery disease, 40-75 years | Oral T undecanoate 80 mg bd | 22 | 8 weeks, cross-over | No difference in angina or endothelial function, decreased arterial stiffness, increased perfusion of myocardium |
| Mathur A, 2009 (132) | Men with chronic stable angina (men age 65 years) | Depot intramuscular T undecanoate | 7 | 6 | 12 months | Increased time to ischemia, non-significant trend for decreased CIMT |
| Cornoldi A, 2009 (133) | Men with proven coronary artery disease and type 2 diabetes (mean age 74 years) | Oral T undecanoate 40 mg tds | 45 | 44 | 12 weeks | Reduced number of angina attacks and silent ischemic episodes on ECG Holter monitoring |
| B |  |  |  |  |  |  |
| Aversa A, 2010 (122) | Men with metabolic syndrome, T ≤11 nmol/L or free T ≤250 pmol/L (mean age 57 years) | Depot intramuscular T undecanoate 1,000 mg every 12 weeks | 40 | 10 | 12 months\* | Decreased high sensitivity CRP, improvement in CIMT |
| Basaria S, 2015 (164) | ≥60 years, T 3.5-13.9 nmol/L or free T <173 pmol/L | Transdermal T gel 75 mg daily | 156 | 152 | 3 years | No difference in rates of change in CIMT or coronary artery calcium |
| Budoff MJ, 2017 (168) | Men aged ≥65 years with T <9.5 nmol/L | Transdermal T gel 50 mg daily | 73 | 65 | 12 months | Greater increase in non-calcified coronary plaque volume |
| Hildreth KL, 2018 (141) | Mean age 66 years, T 6.9-12.1 nmol/L | Transdermal gel, titrated to 13.9-19.1 or 20.8-34.7 nmol/L | 41, 43 | 40 | 12 months | No effect of T on endothelial function or on CIMT |
| C |  |  |  |  |  |  |
| Basaria S, 2010 (172) | ≥65 years, T 3.5-12.1 nmol/L or free T <173 pmol/L, mobility limitation | Transdermal gel 100 mg daily | 106 | 103 | 6 months | Trial stopped prematurely due to excess cardiovascular events in T arm |
| Srinivas-Shankar U, 2010 (173) | ≥65 years, T ≤12 nmol/L or free T ≤250 pmol/L, frail or intermediate frail | Transdermal gel 50 mg daily | 138 | 136 | 6 months | T improved muscle strength and physical function, no signal for cardiovascular adverse events |
| Snyder P, 2016 (174) | ≥65 years, T <9.5 nmol/L, sexual dysfunction (A), diminished vitality (B) and/or mobility limitations (C) | Transdermal gel 50 mg daily | 395(A 230,B 236,C 193) | 395 (A 229,B 238,C 197) | 12 months | Modest benefit of T on sexual function, no signal for cardiovascular adverse events |
| Wittert GA, 2021 (175) | 50-74 years, waist ≥95 cm, T ≤14 nmol/L, and impaired glucose tolerance or newly diagnosed type 2 diabetes  | Depot intramuscular testosterone undecanoate 1000 mg every 3 months | 504 | 503 | 24 months | All men received background lifestyle intervention (Weight Watchers). T reduced risk of type 2 diabetes at 2 years by 40%. |

T=testosterone, CIMT=carotid intima media thickness, CRP=C-reactive protein. \* men in placebo group switched to T after 12 months, extension study to 24 months no longer randomized.

EFFECTS OF EXOGENOUS TESTOSTERONE ON VASCULAR FUNCTION

Brachial artery endothelial function is an established measure of cardiovascular health examining both endothelial and vascular smooth muscle function, which mirrors responses in the coronary arteries (134). Assessment of arterial stiffness provides complementary insights into vascular health (135,136). In uncontrolled open-label studies in men with low baseline T concentrations, T supplementation improved both endothelial function and arterial stiffness (137,138). A study in hypogonadal older men found an improvement in arterial stiffness with transdermal T therapy (139). In a RCT of 55 obese men with type 2 diabetes, one year’s treatment with T undecanoate given as a depot intramuscular injection every 10 weeks improved endothelial function compared to placebo (140). However, other studies in middle-aged and older men did not show any effect of transdermal T treatment on endothelial function (141,142). There is a pathway by which T treatment is expected to improve endothelial function as *in vitro* studies demonstrate stimulation of nitric oxide synthesis in human aortic endothelial cells exposed to T (143). T might exert beneficial effects in the vasculature via actions to improve endothelial function and arterial stiffness, but additional studies are needed before a definitive conclusion can be made.

**Testosterone and Atherosclerosis**

CIRCULATING CHOLESTEROL CONCENTRATION

Clinical studies of T have shown consistent results for T treatment to reduce circulating concentrations of total cholesterol to a modest degree (144-146). A trend for T to lower LDL cholesterol has been noted (144). T treatment appears to lower high density lipoprotein (HDL) cholesterol again to a small degree (147). HDL cholesterol is involved in reverse cholesterol transport thus exerting anti-atherogenic activity, such that HDL function is an independent predictor of cardiovascular events (148,149). However, T may modulate HDL concentrations without a corresponding effect on HDL function (150). Of note, in observational studies, endogenous T concentrations correlated with circulating HDL and were inversely associated with total cholesterol (151,152). Thus, the prognostic significance of T-induced changes in lipid profiles, and the effect of T treatment on HDL-mediated anti-atherogenic action in men at risk for CVD remains unclear.

CAROTID ATHEROSCLEROSIS

Carotid intima-media thickness (CIMT) and the presence of carotid plaque are measures of preclinical carotid atherosclerosis, which can be assessed non-invasively using ultrasound (153). While low endogenous T concentrations are associated with increased CIMT in observational studies (154,155), it is less clear whether low endogenous T (or E2) predicts progression of CIMT (156-158). One study implicated low-grade inflammation in this process, finding an association of low non-SHBG-bound T with CIMT in older men with CRP ≥2 mg/L, but not in those with CRP <2 mg/L (159). Two cohort studies observed cross-sectional associations of low T concentrations with greater carotid plaque area (158,160). However, no association was found between baseline sex hormone concentrations and change in plaque area during follow-up, possibly due to increased use of anti-hypertensive and lipid-lowering therapy (158). One study reported an association of higher T concentrations with reduced CIMT and lower prevalence of carotid plaque in a cohort of community-dwelling men, but not in a cohort of men with angiographically proven CAD (161). In men with proven CAD, higher DHT was associated with less carotid plaque. Of note, E2 was associated with increased CIMT in community-dwelling men, but with less carotid plaque in men with CAD (161). One study found that higher E2 was associated with the presence of lipid core in carotid plaques in men, with no association of T concentrations (162). In a study of men who underwent carotid endarterectomy, the ratio of circulating T/E2 was inversely associated with plaque calcifications, macrophage staining and plaque neutrophil content, as well as plaque IL-6 protein (163). These findings associate sex hormone concentrations with CIMT and carotid plaque in men.

Several interventional studies reporting CIMT as an outcome are summarized (Table 3B). Of note, in a RCT of intramuscular T undecanoate 1,000 mg given every 12 weeks to men with metabolic syndrome, there was improvement in CIMT after 12 months of treatment (122). However, a larger RCT, Testosterone Effects on Atherosclerosis Progression in Aging Men (TEAAM), conducted over three years showed no effect of transdermal T treatment on rates of progression of CIMT (164). A smaller study, which tested both exercise training and transdermal T treatment over a period of 12 months, found no effect of either intervention on CIMT (141). Thus, while the RCT data are limited, the effects of T treatment on preclinical carotid atherosclerosis may be beneficial or neutral, but are unlikely to be adverse. See table 3, part B.

CORONARY ATHEROSCLEROSIS

Coronary computed tomography angiography (CCTA) has emerged as a non-invasive method for imaging coronary atherosclerosis (165-167). Normal findings on CCTA are associated with a low risk of cardiovascular events, while the presence and extent of CAD demonstrated on CCTA are risk predictors for future cardiovascular events in large epidemiological studies (165-167). Therefore, there is considerable interest in the Cardiovascular sub-study of the T-Trials which reported CCTA outcomes for 73 men treated with transdermal T and 65 men receiving placebo over a 12-month period (168). In this study, men in the T-treated group experienced a greater increase in non-calcified and total coronary artery plaque volume, compared to men in the placebo group (168). However, the groups were unbalanced with men in the T-treated group having considerably lower non-calcified and total plaque volumes at baseline and at the end of the study compared with placebo-treated men. The fact that the two groups of men differed substantively in a key baseline characteristic makes the result of the study challenging to interpret (169). There was no difference in the rate of change of coronary calcium score between groups, albeit men in the T-treated group had lower coronary calcium scores at baseline and at the end of the study compared with men in the placebo group (168). The results for coronary calcium scores are concordant with the TEAAM trial that also showed no difference in coronary calcium scores with T treatment (164). Men in the T-Trials Cardiovascular sub-study overall had a high burden of plaque at baseline with 32% of T-treated men and 38% of placebo recipients having baseline Agatston scores ≥300 (168).

Of note, in the T-Trials Cardiovascular sub-study, data on plaque volume were not analyzed relative to vessel lumen to address the issue of whether vascular remodeling was occurring (170). Since then, CCTA technology has progressed to allow more detailed and sophisticated analysis of plaque characteristics associated with higher risk of coronary events, which were not applied in that study (171). While these findings are important and noteworthy, a larger RCT with balanced groups using current CCTA methodology would be needed to clarify the effect of T on coronary plaque characteristics. The findings are a timely reminder that until definitive RCTs are available, the effects of T on the cardiovascular system remain uncertain and may be beneficial, neutral or adverse.

**trials reporting adverse events**

**Testosterone RCTs and Cardiovascular Adverse Events**

Selected T RCTs, which have influenced this field, are summarized (Table 3, part C). These studies were underpowered for cardiovascular events and the possibility of type 1 and type 2 errors should be considered. A key RCT randomized 209 men aged 65 years and older, with mobility limitations and low or low-normal baseline T or free T, to transdermal T gel vs placebo for six months (172). Of note the starting dose of transdermal T (100 mg daily) was greater than the usual recommended starting dose (50 mg daily). The study was discontinued after an excess of adverse events was noted in the T arm (172). A contemporaneous RCT in a comparable population of 274 older men who were frail or intermediate frail, using a 50 mg daily dose of transdermal T over 6 months, was successfully completed showing improved muscle strength and physical function, with no signal for cardiovascular adverse events (173). The Testosterone Trials (T-Trials) has reported results from the main study and component sub-studies (174,176). These have been extensively reviewed (169,176). In T-Trials, 790 men aged 65 years and older, with symptoms of sexual dysfunction, diminished vitality or mobility limitations and baseline T <9.5 nmol/L (<275 ng/dL), were randomized to transdermal T gel at a starting dose of 50 mg daily vs placebo for 12 months (174). In T-Trials, T treatment improved sexual function to a moderate degree, while the primary outcomes for physical function and vitality were not met (174). T treatment improved anemia and volumetric bone density, with a neutral effect on cognition (176). The effects on coronary artery plaque volume have been discussed (see Section 4.2.3). T-Trials had a low rate of major cardiovascular adverse events (7 in each of the T and placebo arms).

Recently, a larger Australian RCT, Testosterone for the Prevention of Type 2 Diabetes Mellitus (T4DM) has been reported (175). T4DM was a randomized, double-blind, placebo-controlled, 2-year, phase 3b trial done at six Australian tertiary care centers, which randomized 1,007 men to depot intramuscular testosterone undecanoate injections given every three months for two years, vs placebo, on a background of a lifestyle intervention (Weight Watchers) given to all participants. Inclusion criteria were age 50-74 years, waist circumference ≥95 cm and baseline T ≤14.0 nmol/L (≤403.8 ng/dL), and the presence of either impaired glucose tolerance or newly diagnosed type 2 diabetes based on oral glucose tolerance testing (175). In T4DM, testosterone treatment reduced the risk of type 2 diabetes at two years by 40% beyond the effect of the lifestyle intervention (relative risk 0·59, 95% confidence interval 0·43 to 0·80; p=0·0007). T4DM is the largest T RCT reported to date, and the incidence of cardiovascular adverse effects were similar in both T and placebo arms. In T4DM, 17 men in the placebo group and 12 in the T group had a major cardiovascular adverse event (13 men in the placebo arm and 7 in the testosterone arm had an ischemic heart disease event, 3 and 4 had cerebrovascular disease events respectively, and one in each group died from a cardiovascular-related cause) (175). Therefore, in keeping with T-Trials, the rates of major cardiovascular adverse events in T4DM were low, and comparable in testosterone and placebo-treated men.

At this point, it is worth commenting on the outcome of cardiorespiratory fitness. Low cardiorespiratory fitness, assessed as maximal oxygen consumption during exercise testing (VO2peak), is a strong independent predictor of all-cause and CVD mortality in apparently healthy men and in men with established CVD (177-179). Cardiorespiratory fitness has been recommended as a vital sign for use in clinical assessment (180). An earlier study over 12 months did not show an effect of T treatment on fitness (141), nor did a more recent study with a 12-week intervention (181). That study used a 2x2 factorial design, while exercise training resulted in improved VO2 peak within the 12-week period of intervention, T treatment did not, and there was no evidence within this relatively short timeframe of additive benefit (181). In the TEAAM study conducted over 3 years, placebo-treated men showed a decline in VO2peak over time, but the decline was attenuated in T-treated men (182). In TEAAM, there was no signal for cardiovascular adverse events with T (164). Part of the beneficial effect of T treatment on VO2peak might be mediated via its effect to raise hemoglobin concentrations (147,169,176), or its action on skeletal muscle (183,184). The net effect might be to preserve (or at least attenuate the loss of) cardiorespiratory fitness in ageing men, that could translate into a reduction in cardiovascular risk. However, in keeping with the results of T4DM, an extended duration of T intervention may be required to realize these benefits.

**Meta-analyses of Cardiovascular Adverse Events in Testosterone RCTs**

To date, no T RCT large and long enough to be powered for the outcome of cardiovascular events has been reported. However, meta-analyses of reported T RCTs have been performed to determine whether T is associated with a difference in the rate of cardiovascular adverse events (Table 4). Earlier meta-analyses done in 2007 and 2010 had found no significant difference in risk for cardiovascular adverse events (147,185). One analysis done in 2013 claimed an association of T treatment with increased risk of cardiovascular-related adverse events (186). However, subsequent meta-analyses done from 2013 to 2020 have not supported this finding (187-193). Instead, these have found no association of T treatment with risk of cardiovascular adverse events (Table 4).

|  |
| --- |
| **Table 4. Meta-Analyses of Cardiovascular Adverse Events in Randomized Controlled Trials (RCTs) of T Supplementation in Men** |
| **Study characteristics** | **Results** |
| Study author and year  | N of RCTs | N active | N placebo | Adverse signal | No adverse signal |
| Haddad RM, 2007 (185) | 30 | 808 | 834 |  | No significant difference in odds ratio for any cardiovascular adverse event or MI. |
| Fernandez-Balsells MM, 2010 (147) | 51 | 2,716 |  | No significant difference for all-cause mortality, coronary bypass surgery or MI. |
| Xu L, 2013 (186) | 27 | 2,994 | T associated with increased risk cardiovascular-related event (OR 1.54, 95% CI=1.09-2.18)\*. |  |
| Ruige JB, 2013 (187) | 10 (>100 participants) | 1,289 | 848 |  | No significant difference in cardiovascular adverse events. |
| Corona G, 2014 (188) | 75 | 3,016 | 2,448 |  | No association of T supplementation with cardiovascular risk. For MACE OR=1.01 (95% CI 0.57-1.77). |
| Borst SE, 2015 (189) | 35 | 3,703 |  | No significant risk for cardiovascular-related adverse events. |
| Alexander GC, 2017 (190) | 39, cut-off for T 10.4-16.7 nmol/L | 3,230 | 2,221 |  | No significant increase in risk of MI OR=0.87 (95% CI 0.39-1.93)\*, stroke or mortality. |
| Elliott J, 2017 (191) | 87, cut-off for T 12 nmol/L or cFT 225 pmol/L | 1,462-2088 | 1,372-1,851 |  | Improved QoL, libido, depression and erectile function. No increase in risk of adverse events. |
| Corona G, 2018 (192) | 93 | 4,653 | 3,826 |  | No clear effect of T on incidence of CVD events. For MACE OR=0.97 (95% CI 0.64-1.46). |
| Diem SJ, 2020 (193) | 38 | N/A | N/A |  | Small improvement in sexual function and quality of life. Pooled risk for adverse cardiovascular outcomes did not differ between groups (OR=1.22, 95% CI 0.66-2.23).\* |
| Hudson J, 2022 (194) | 35 RCTs: 17 included in IPD meta-analysis  | 1,750 (IPD) | 1,681 (IPD) |  | No significant difference between groups. For cardiovascular or cerebrovascular events OR=1·07 (95% CI 0·81–1·42). |

MI=myocardial infarction, MACE=major adverse cardiovascular events, OR=odds ratio, 95% CI=confidence interval. QoL=quality of life, IPD=individual participant data. Unless otherwise specified, meta-analyses were conducted using random effects models. \*fixed effects model

It is worth commenting on more recent meta-analyses that have included the results of the T-Trials. In the meta-analysis by Alexander et al. (2017), 39 RCTs were included. The meta-analysis found no significant increase in risk of MI (data from 30 RCTs utilized), stroke (9 RCTs) or mortality (20 RCTs) (190). However, caveats were noted with respect to the quality of the available evidence. In a network meta-analysis, Elliott et al. (2017) included RCTs that enrolled men with baseline T ≤12 nmol/L (≤346 ng/dl) or free T ≤225 pmol/L, including 87 RCTs overall (191). T treatment was associated with improved quality of life and libido, improvement in depression and in erectile function. There was no increase in risk of adverse events such as cardiovascular death, MI or stroke (191). Corona et al. (2018) studied 93 RCTs and found no clear effect of T on incidence of CVD events, with an odds ratio of 0.97 (95% confidence interval 0.64-1.46) for major cardiovascular adverse events (192).

Diem et al. (2020) examined 38 RCTs of at least six months duration, noting that few exceeded a 1-year duration, there was a lack of power to assess important harms, and limited data for men aged 18-50 years (193). They concluded that in older men with lower testosterone concentrations, in the absence of organic hypogonadism, T treatment resulted in small improvements in in sexual functioning and quality of life, with long-term safety and efficacy still uncertain. Recently Hudson et al. (2022) analyzed RCTs involving men with T ≤12 nmol/L and minimum duration of three months, identifying 35 studies and obtaining individual participant data from 17 of these with 3,431 participants (including T-Trials but not T4DM) (194). There was no significant difference in cardiovascular adverse events in testosterone vs placebo-treated men. The authors concluded that their results provide some reassurance about the short- to medium-term safety of T treatment for male hypogonadism (194). Clearly, the results of the TRAVERSE study, a testosterone cardiovascular safety trial, will be of considerable interest (195). For now, bearing in mind the limitations of meta-analyses of RCTs using reported adverse events as the endpoint, the weight of the currently available evidence from these sources indicates that T treatment is not associated with risk of cardiovascular adverse events.

**Retrospective case-control studies**

**Retrospective Studies of Testosterone Prescriptions**

Pending an adequately powered T RCT to clarify its effect on the risk of cardiovascular events, retrospective case-control studies have sought to fill this gap (Table 5). These studies are typically based on health insurance databases recording prescriptions for T and subsequent outcomes in men prescribed or not prescribed T. Limitations of these studies include lack of clinical data such as indications for prescribing, the absence of randomization and the possibility of recall and misclassification bias (196). Initial studies in male veterans and in men with type 2 diabetes associated T prescriptions with lower mortality (197,198). By contrast, two studies associated T prescriptions with increased risk of major cardiovascular events (199,200). Both have been subject of criticisms: the first over confusing statistical methodology and data inaccuracies (resulting in publication of an erratum), the second over the lack of an appropriate comparison group (209,210). Subsequent studies have associated T prescriptions with no increase in risk of MI (201), and with lower risk of death, MI and stroke (202,203). An interesting distinction was made in the studies by Sharma et al. (2015) and Andersen et al. (2016) in that men who were prescribed T who then had “normal” T concentrations, did better than men who had persistently low T concentrations or who did not receive T (202,203). However, it is important to note that these studies did not systematically assess testosterone concentrations at multiple times or multiple days. A single measurement of testosterone on a particular day, may not be an accurate reflection of testosterone concentrations achieved over sustained periods of time with different testosterone formulations (211).

|  |
| --- |
| **Table 5. Retrospective Case-Control Studies of Men Prescribed T that Examined Associations of T Prescriptions with Cardiovascular Events and Mortality in Middle-Aged and Older Men** |
| **Study characteristics** | **Results** |
| **Study author and year**  | **Size (n of men)** | **Follow-up (yr)** | **Age (yr)** | **Favors no T** | **Favors T** |
| Shores MM, 2012 (197) | 1,031 | 3.4 | 62.1 |  | Male veterans with total T ≤8.7 nmol/L, T prescribed in 398. T supplementation associated with lower mortality. |
| Muraleedharan V, 2013 (198) | 581 | 5.8 | 59.5 |  | Men with Type 2 diabetes, 238 with total T ≤10.4 nmol/L. T supplementation associated with lower mortality. |
| Vigen R, 2013 (199) | 8,709 | 2.3 | 63.4 | Male veterans who had coronary angiography and total T ≤10.4 nmol/L. T prescription associated with increased risk of death, MI or stroke. |  |
| Finkle WD, 2014 (200)  | 55,593 | 90 days | 54.4 | Men prescribed T. Higher rate of non-fatal MI in 90 days following prescription compared to preceding 1 year. |  |
| Baillargeon J, 2014 (201) | 6,355; 19,065 | 4.1; 3.3 | ≥66 |  | 6,355 men prescribed T vs 19,065 matched non-users. T prescription not associated with increased risk of MI. For men with worse prognostic scores, T associated with reduced risk of MI. |
| Sharma R, 2015 (202) | 83,010 | 6.2; 4.6; 4.7 | 66 |  | Male veterans with low T. TRT resulting in normalization of circulating T (n=43,931) was associated with lower risk of death, MI and stroke, compared to TRT without normalization of T (n=25,701) or no TRT (n=13,378).  |
| Anderson J, 2016 (203) | 4,736 | ≥3 | 61.2 |  | Men with T <7.4 nmol/L. T therapy achieving normal T (n=2,241) was associated with reduced risk of MACE compared to persistent low T (n=801). T therapy achieving either normal T or high T (n=1,694) associated with lower all-cause mortality compared to persistent low T. |
| Wallis CJD, 2016 (204) | 10,311: 28,029 | 5.3 | ≥66 |  | Men treated with T. T treatment associated with lower mortality HR=0.88 (95% CI=0.84-0.93) and prostate cancer risk HR=0.86 (95% CI=0.75-0.99). Shorter exposure (2 months) associated with increased risk of cardiovascular events and mortality, longer exposure (35 months) with reduced risk. |
| Cheetham TC, 2017 (205) | 8,808: 35,527 | 3.2 | 58.4 |  | Men ≥40 years, diagnosis or T <10.4 nmol/L. T associated with reduced risk of outcome of MACE, unstable angina, coronary revascularization, TIA. HR=0.67 (95% CI=0.62-0.73). |
| Loo SY, 2019 (206) | 15,401 | 4.7 | ≥45 | Men with low T and no evidence of HPT axis disease. T associated with increased risk of composite outcome of stroke/TIA/MI (HR=1.21, 95% CI 1.00-1.46), with risk highest in first 6 months to 2 years of T use (HR 1.35, 95% CI, 1.01-1.79). Risk of all-cause mortality lower with current T use (HR=0.64, 95% CI 0.52-0.78) and higher with past T use (HR=1.72, 95% CI 1.21-2.45), compared with non-use. |  |
| Oni OA, 2019 (207) | 1,470 | 3.2-4.0 | ≥50 |  | Male veterans with low total T and history of MI. All-cause mortality lower in men treated with T who normalized total T (N=755), vs men treated with T who did not normalize total T (N=542, HR=0.76, 95% CI 0.64-0.90), or men not treated with T (N=173, HR=0.76, 95% CI 0.60-0.98). No significant difference in the risk of recurrent MI between groups. |
| Shores MM, 2021 (208) | 204,857 | 4.3 | 60.9 |  | Male veterans with low T. Current transdermal T use not associated with risk for incident MI/ischemic stroke/venous thromboembolism (HR=0.89, 95% CI 0.76-1.05) in men without prevalent CVD, and in those with prevalent CVD was associated with lower risk (HR=0.80; 95% CI, 0.70-0.91). Current intramuscular T use not associated with risk for composite endpoint in men without or with prevalent CVD (HR=0.91, 95% CI 0.80-1.04; HR=0.98, 95% CI 0.89-1.09, respectively). |

MI=myocardial infarction, TRT=testosterone replacement therapy, MACE=major cardiovascular adverse event comprising death, non-fatal MI and non-fatal stroke, TIA=transient ischemic attack.

A study by Wallis et al. (2016) found that T treatment was associated with lower mortality overall, but men who had T for a relatively shorter duration of exposure had increased risk, while men with longer duration of exposure had reduced risk (204). In a study by Cheetham et al. (2017) of men aged ≥40 years diagnosed with low T or with T <10.4 nmol/L, T treatment was associated with a reduced risk of major cardiovascular adverse events (205). Loo et al. (2019) reported contrary findings: analyzing a cohort of men with no evidence of HPT axis disease via the UK Clinical Practice Research Datalink, they associated use of testosterone with higher risk of a composite outcome of stroke, transient ischemic attack or MI, with the risk highest in the first six to 24 months of T use (206). In that study all-cause mortality risk was lower with current T use, and higher with past T use. Those findings are not supported by two more recent analyses, involving men with prior heart disease or with multiple comorbidities (207,208). In a study of male veterans with low T concentrations and a history of MI, men receiving T treatment who had a subsequent normal testosterone concentration had a lower risk of death from any cause compared to men receiving T treatment who had a subsequent low T concentration (207). Those men also had a lower risk of death compared with men who did not receive T treatment. Finally, in a cohort of 204,857 male veterans with a mean age of 60.9 years and 4.7 chronic medical conditions who were followed for 4.3 years, current transdermal T use was not associated with risk for the composite outcome of incident MI, ischemic stroke or venous thromboembolism in men without prevalent CVD (208). On the other hand, it was associated with lower risk in men with prevalent CVD. In that study, current intramuscular T use not associated with risk for the composite endpoint in men without or with prevalent CVD (208).

In an earlier retrospective cohort study that compared the use of T gel with T injections, T injections were associated with greater risk of cardiovascular adverse events (212). Bearing in mind the limitations of non-randomized studies and the possibility of bias, and the absence of a control group not receiving T, an additional factor is that more than 90% of the T injections were T cypionate, enanthate or propionate (212), which are short acting formulations typically requiring fortnightly administration with marked fluctuations in blood concentrations. The analysis would not apply to long-acting depot injections of T undecanoate typically administered every 12 weeks, which provide more stable pharmacokinetics (128). It is worth noting in this context that a population-based case-control study (involving 19,215 patients with confirmed venous thromboembolism and 909,530 age-matched controls) found an increased risk of venous thromboembolism within the first six months of T treatment but not thereafter (213). By contrast a systematic review and meta-analysis including six RCTs (2,236 participants) and five observational studies (1,249,640 participants) found no evidence of an association between T treatment and venous thromboembolism (214). However, the authors of that study noted that the available RCT data might have had inadequate power to detect an increased risk.

In summary, earlier findings associating T prescriptions with adverse cardiovascular outcomes were echoed in a more recent study. However, most studies do not show such adverse signals, and associated T use with lower risk of adverse cardiovascular events or mortality, including several recent large studies. There is a suggestion that T treatment which achieves normal T concentrations may relate to lower risk of cardiovascular events and mortality. Bearing in mind the limitations of these retrospective, observational and non-randomized studies, which cannot prove causality, the available data provide some reassurance but are far from definitive.

**Abuse of Androgenic Steroids**

Androgenic steroids can serve as appearance and performance-enhancing drugs and are abused by some competitive athletes, recreational sportspersons and body builders (1,215,216). The use/abuse of androgenic steroids occurs in contravention of medical advice and applicable sporting regulations, typically without medical supervision using unapproved formulations often in excessive doses (216). The use/abuse can be interspersed with periods of non-use. Adverse effects include suppression of the endogenous HPT axis, reduced spermatogenesis and impaired fertility, decreased testicular volume, hair loss and gynecomastia and are well-recognized (1,215). There is also an appreciation that long-term abuse results in cardiovascular toxicity in the form of myocardial dysfunction and accelerated coronary atherosclerosis (217). However, this study could be confounded as men who abuse androgenic steroids may also consume many other substances and the androgen preparations might have harmful contaminants. Abuse using pharmacological dosing of various products is very distinct from medically supervised T therapy aiming to achieve physiological circulating concentrations of T (128). Nevertheless, this is a reminder that excessive exposure to androgens carries the risk of harm (1).

**Discussion**

**Lessons from the Available Evidence**

Epidemiological data are consistent with a protective role for endogenous androgens against CVD. In some studies of middle-aged and older men, lower circulating concentrations of endogenous T are associated with higher incidence of cardiovascular events, particularly stroke. Lower circulating T and DHT concentrations have also been associated with higher cardiovascular mortality (discussed in sections 2.1-2.2). Potential mechanisms by which T could exert beneficial actions in the vasculature have been explored in experimental models. These include reduced cholesterol accumulation and modulation of inflammation (sections 3.1-3.4). Clinical studies have reported favorable effects of T treatment on angina symptoms and exercise tolerance, but its effect on subclinical atherosclerosis remains uncertain (sections 4.1-4.2). The T-Trials, T4DM and meta-analyses of existing T RCTs in general do not show any signal for cardiovascular adverse events (sections 5.1-5.2). Retrospective case-control studies have reported contrasting results but in general, men receiving T prescriptions appear to have lower risk of major cardiovascular events and lower mortality compared to men who did not receive T, particularly if T treatment was associated with subsequent normal concentrations of circulating T (section 6.1). It is important to bear in mind that there are contrasting findings, and beneficial associations of T with cardiovascular outcomes may be less evident in healthier middle-aged men. Therefore, epidemiological evidence and mechanistic data could be used to argue for an anti-atherogenic or a protective effect of T on the cardiovascular system, as could the majority of retrospective case-control studies. However, this remains to be proven in the context of prospective RCTs of T intervention.

**Gaps in the Current Evidence Base**

RCT data are lacking as to whether treatment of middle-aged and older men with T would reduce the risk of cardiovascular events. The T-Trials which used transdermal T gel over a 12-month intervention offer important evidence as to benefits of T treatment for sexual function, anemia and bone density in older men without apparent diseases of the HPT axis, who had lower circulating T concentrations compared with younger men and symptoms suggestive of (but not diagnostic for) androgen deficiency (174,176). T4DM demonstrated the benefit of T treatment to reduce the risk of type 2 diabetes in men at high risk, beyond the effects of a lifestyle intervention (175). T4DM also showed a beneficial effect of T treatment on sexual function, and on bone microarchitecture and density (175,218). The T-Trials and T4DM are also noteworthy for the absence of any adverse cardiovascular safety signal for T treatment in these populations of men (174,175). However, the findings of the T-Trials Cardiovascular sub-study regarding an increase in total coronary atheroma plaque volume, in men with substantial baseline atheromatous disease, require clarification (168).

Major evidence gaps pertain to the effects of T on the cardiovascular system, as to whether T acts to slow development or progression of coronary or carotid atheromatous plaque in middle-aged and older men, in the differing contexts of either primary or secondary prevention for CVD. If the action of T is to reduce cholesterol accumulation, and to reduce inflammation and neointimal response to injury (sections 3.1-3.4) then these actions may have more impact to prevent or reduce progression of early atherosclerosis, rather than to reverse established disease. The related questions are whether T intervention in a primary prevention setting will reduce growth of coronary or carotid atheromatous plaque, or whether in a secondary prevention setting T intervention would influence the incidence of cardiovascular events. Another important question relates whether transdermal vs depot intramuscular (T undecanoate) formulations of T have similar or differing effects on the cardiovascular system.

Neither T-Trials nor T4DM had any cardiovascular endpoints and will not answer the question as to whether T exerts beneficial, neutral or adverse effects on the cardiovascular system. The US multicenter RCT “A study to evaluate the effect of testosterone replacement therapy (TRT) on the incidence of major adverse cardiovascular events (MACE) and efficacy measures in hypogonadal men (TRAVERSE)” commenced recruitment in 2018 of men aged 45-80 years with T <10.4 nmol/L (<300 ng/dl) with evidence of CVD or at increased risk for CVD (195). TRAVERSE was designed as a cardiovascular safety study with the endpoint of myocardial infarction, stroke or death due to cardiovascular causes, aimed to enroll 6,000 men randomized to transdermal T gel or placebo, and is planned to complete in 2022. TRAVERSE will also examine outcomes of prostate cancer, sexual function, bone fractures, depression, anemia and diabetes (195). TRAVERSE will address the issue of the cardiovascular safety of T treatment in what would largely be a secondary prevention setting. This leaves unanswered the question of whether T intervention in a primary prevention setting would reduce development or progression of coronary or carotid atheroma.

**Application to Clinical Practice**

Current clinical practice recommendations prioritize the identification of men with classical or pathological hypogonadism who are androgen deficient due to diseases of the hypothalamus, pituitary or testes (6,7). In such men, T treatment consistently resolves symptoms and signs of androgen deficiency (6,7,19). In men with classical or pathological hypogonadism the benefits of T treatment likely outweigh possible cardiovascular risks. In any case, individualized assessment and management of cardiovascular risk factors and disease should be part of routine clinical care. Of note, the US regulatory agency required labelling to warn of a possible increased risk of cardiovascular events with T, but the European regulatory agency concluded there was no consistent evidence of increased risk of coronary heart disease with T therapy (19). Until more evidence is available, it may be prudent to adopt a degree of caution in older men who are frail or who have known CVD, and to optimize management of cardiovascular risk factors and disease before starting T treatment. Treatment should aim for physiological replacement of T using approved formulations and avoid excessive doses (128).

It is beyond the scope of this chapter to discuss controversies regarding the management of men with low endogenous T concentrations due to obesity or presence of systemic illnesses where the HPT axis is intact, but its activity may be suppressed (19,31). However, it is worth noting that in men where a clear indication for T treatment is lacking, the risks and benefits of an intervention need to be considered with special care. Further research is needed to determine whether and how T treatment might impact on the risk of CVD in men.

**Conclusions**

Some epidemiological studies have associated higher circulating concentrations (but within the normal range) of endogenous androgens with lower risk of cardiovascular events and mortality. In men with pathological hypogonadism, the benefits of T treatment likely outweigh putative risks of cardiovascular adverse events. However, the effects of exogenous androgens in the form of T therapy seeking to maintain physiological circulating androgen concentrations on the cardiovascular system remain uncertain. Additional information will be forthcoming once the results of the TRAVERSE trial are known. Current clinical care of hypogonadal men should recognize this evidence gap and allow for individualized assessment and management of pre-existing cardiovascular risk factors and disease in men requiring T therapy. Well-designed and adequately powered RCTs are needed to clarify whether T treatment has beneficial, neutral or adverse effects on the cardiovascular system in the general population of middle-aged and older men.

**REFERENCES**

1. Handelsman DJ. Androgen physiology, pharmacology, use and misuse. In [www.endotext.org](http://www.endotext.org), version of October 5, 2020, published by MDTEXT.COM, Inc., South Dartmouth, MA 02748.
2. Lakshman KM, Kaplan B, Travison TG, et al. The effects of injected testosterone dose and age on the conversion of testosterone to estradiol and dihydrotestosterone in young and older men. J Clin Endocrinol Metab 2010; 95: 3955-3964.
3. Finkelstein JS, Whitcomb RW, O’Dea LStL, Longcope C, Schoenfeld DA, Crowley WF. Sex steroid control of gonadotrophin secretion in the human male. I. Effects of testosterone administration in normal and gonadotrophin-releasing hormone-deficient men. J Clin Endocrinol Metab 1991; 73: 609-620.
4. Finkelstein JS, O’Dea LStL, Whitcomb RW, Crowley WF. Sex steroid control of gonadotropin secretion in the human male. II. Effects of estradiol administration in normal and gonadotropin-releasing hormone-deficient men. J Clin Endocrinol Metab 1991; 73: 621-628.
5. Bagatell CJ, Dahl KD, Bremner WJ. The direct pituitary effect of testosterone to inhibit gonadotropin secretion in men is partially mediated by aromatization to estradiol. J Androl 1994; 15: 15-21.
6. Yeap BB, Grossmann M, McLachlan RI, et al. Endocrine Society of Australia position statement on male hypogonadism (part 1): assessment and indications for testosterone therapy. Med J Aust 2016; 205: 173-178.
7. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone therapy in men with hypogonadism: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018; 103: 1715-1744.
8. Ryl A, Rotter I, Grzywacz A, et al. Molecular analysis of the SRD5A1 and SRD5A2 genes in patients with benign prostatic hyperplasia with regard to metabolic parameters and selected hormone levels. Int J Environ Res Public Health 2017; 14: 1318.
9. Mellstrom D, Vandenput L, Mallmin H, et al. Older men with low serum estradiol and high serum SHBG have an increased risk of fractures. J Bone Mineral Res 2008; 23: 1552-1560.
10. LeBlanc ES, Nielson CM, Marshall LM, et al. The effects of serum testosterone, estradiol, and sex hormone binding globulin levels on fracture risk in older men. J Clin Endocrinol Metab 2009; 94: 3337-3346.
11. Finkelstein JS, Lee H, Burnett-Bowie S-A, et al. Gonadal steroids and body composition, strength, and sexual function in men. N Engl J Med 2013; 369: 1011-1022.
12. Sikaris K, McLachlan RI, Kazlauskas R, et al. Reproductive hormone reference intervals for healthy fertile young men: evaluation of automated platform assays. J Clin Endocrinol Metab. 2005; 90:5928-5936.
13. Jasuja GK, Travison TG, Davda M, et al. Age trends in estradiol and estrone levels measured using liquid chromatography tandem mass spectrometry in community-dwelling men of the Framingham Heart Study. J Gerontol Med Sci 2013; 68:733-740.
14. Handelsman DJ, Wartofsky L. Requirement for mass spectrometry sex steroid assays in the Journal of Clinical Endocrinology and Metabolism. J Clin Endocrinol Metab. 2013; 98:3971-3973. Subsequent comment in Wierman ME, et al. J Clin Endocrinol Metab. 2014; 99:4375.
15. Goldman AL, Bhasin S, Wu FCW, et al. A reappraisal of testosterone’s binding in circulation: physiological and clinical implications. Endocr Rev 2017; 38: 302-324.
16. Handelsman DJ. Free testosterone: pumping up the tires or ending the free ride? Endocr Rev 2017; 38: 297-301.
17. Ly LP, Sartorius G, Hull L, et al. Accuracy of calculated free testosterone formulae in men. Clin Endocrinol 2010; 73: 382-388.
18. Fiers T, Wu F, Moghetti P, et al. Reassessing free-testosterone calculation by liquid chromatography-tandem mass spectrometry direct equilibrium dialysis. J Clin Endocrinol Metab 2018; 103: 2167-2174.
19. Yeap BB, Wu FCW. Clinical practice update on testosterone therapy for male hypogonadism: contrasting perspectives to optimise care. Clin Endocrinol 2019; 90: 56-65.
20. Bhasin S, Valderrabano RJ, Gagliano-Juca T. Age-related changes in the male reproductive system. In [www.endotext.org](http://www.endotext.org), version of February 10, 2022, published by MDTEXT.COM, Inc., South Dartmouth, MA 02748.
21. Handelsman DJ, Yeap BB, Flicker L, et al. Age-specific population centiles for androgen status in men. Eur J Endocrinol 2015; 173: 809-817.
22. Yeap BB, Alfonso H, Chubb SAP, et al. Reference ranges and determinants of testosterone, dihydrotestosterone and estradiol levels measured using liquid chromatography-tandem mass spectrometry in a population-based cohort of older men. J Clin Endocrinol Metab. 2012; 97:4030-4039.
23. Hsu B, Cumming RG, Hirani V, et al. Temporal trend in androgen status and androgen-sensitive outcomes in older men. J Clin Endocrinol Metab 2016; 101: 1836-1846.
24. Ahern T, Swiecicka A, Eendebak RJAH, et al. Natural history, risk factors and clinical features of primary hypogonadism in ageing men: longitudinal data from the European Male Ageing Study. Clin Endocrinol 2016; 85: 891-901.
25. Yeap BB, Manning L, Chubb SAP, et al. Progressive impairment of testicular endocrine function in ageing men: testosterone and dihydrotestosterone decrease, and luteinising hormone increases, in men transitioning from the 8th to 9th decades of life. Clin Endocrinol 2018; 88: 88-95.
26. Wu FCW, Tajar A, Beynon JM, et al. Identification of late-onset hypogonadism in middle-aged and elderly men. N Engl J Med 2010 363: 123-135.
27. Tajar A, Huhtaniemi IT, O’Neill TW, et al. Characteristics of androgen deficiency in late-onset hypogonadism: results from the European Male Aging Study (EMAS). J Clin Endocrinol Metab 2012; 97: 1508-1516.
28. Yeap BB, Knuiman MW, Divitini ML, et al. Epidemiological and Mendelian randomisation studies of dihydrotestosterone and estradiol, and leucocyte telomere length in men. J Clin Endocrinol Metab 2016; 101: 1299-1306.
29. Yeap BB, Hui J, Knuiman MW, et al. Cross-sectional associations of sex hormones with leucocyte telomere length, a marker of biological age, in a community-based cohort of older men. Clin Endocrinol 2019; 90: 562-569.
30. Coburn SB, Graubard BI, Trabert B, McGlynn KA, Cook MB. Associations between circulating sex steroid hormones and leucocyte telomere length in men in the National Health and Nutrition examination Survey. Andrology 2018; 6: 542-546.
31. Wittert GA, Yeap BB, Grossmann M. Hypothalamo-pituitary-testicular axis function in systemic diseases and effects of medications. In: Oxford textbook of Endocrinology and Diabetes, 3rd Edition, Section 10, Chapter 6, pages 1597-1604. Oxford University Press, Oxford, United Kingdom, 2022.
32. Yeap BB, Marriott RJ, Antonio L, et al. Sociodemographic, lifestyle and medical influences on serum testosterone and sex hormone-binding globulin in men from UK Biobank. Clin Endocrinol 2021; 94: 290-302.
33. Grossmann M. Low testosterone in men with type 2 diabetes: significance and treatment. J Clin Endocrinol Metab 2011; 96: 2341-2353.
34. Ng MTF, Dupuis P, Grossmann M. Lowered testosterone in male obesity: mechanisms, morbidity and management. Asian J Androl 2014; 16: 223-231.
35. Grossmann M. Hypogonadism and male obesity: Focus on unresolved questions. Clin Endocrinol 2018; 89: 11-21.
36. Global Burden of Disease Study 2013 Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: a systematic analysis for the Global Burden of disease Study 2013. Lancet 2015; 386: 743-800.
37. Roth GA, Nyugen G, Forouzanfar MH, et al. Estimates of global and regional premature cardiovascular mortality in 2015. Circulation 2015; 132: 1270-1282.
38. Allan GM, Nouri F, Korownyk C, et al. Agreement among cardiovascular disease risk calculators. Circulation 2013; 127: 1948-1956.
39. Haslam DW, James WP. Obesity. Lancet 2005; 366: 1197-1209.
40. Fan J, Song Y, Chen Y, et al. Combined effect of obesity and cardio-metabolic abnormality on the risk of cardiovascular disease: a meta-analysis of prospective cohort studies. Int J Cardiol 2013; 168: 4761-4768.
41. Kwok CS, Pradhan A, Khan MA, et al. Bariatric surgery and its impact on cardiovascular disease and mortality: a systematic review and meta-analysis. Int J Cardiol 2014; 173: 20-28.
42. Zhou X, Yu J, Li L, et al. Effects of bariatric surgery on mortality, cardiovascular events, and cancer outcomes in obese patients: a systematic review and meta-analysis. Obes Surg 2016; 26: 2590-2611.
43. Wilding JPH, Batterham RL, Calanna S, et al. Once-weekly semaglutide in adults with overweight or obesity. N Engl J Med 2021; 384: 989-1002.
44. Jastreboff AM, Aronne LJ, Ahmad NN, et al. Tirzepatide once weekly for the treatment of obesity. N Engl J Med 2022; 387: 205-216.
45. Yeap BB, Araujo AB, Wittert GA. Do low testosterone levels contribute to ill-health during male ageing? Crit Rev Clin Lab Sci. 2012; 49:168-182.
46. Smith GD, Ben-Shlomo Y, Beswick A, et al. Cortisol, testosterone, and coronary heart disease. Prospective evidence from the Caerphilly Study. Circulation 2005; 112: 332-340.
47. Arnlov J, Pencina MJ, Amin S, et al. Endogenous sex hormones and cardiovascular disease incidence in men. Ann Intern Med 2006; 145: 176-184.
48. Abbott RD, Launer LJ, Rodriguez BL, et al. Serum estradiol and risk of stroke in elderly men. Neurology 2007; 68: 563-568.
49. Vikan T, Schirmer H, Njolstad I, Svartberg J. Endogenous sex hormones and the prospective association with cardiovascular disease and mortality in men: the Tromso study. Eur J Endocrinol 2009; 161: 435-442.
50. Yeap BB, Hyde Z, Almeida OP, et al. Lower testosterone levels predict incident stroke and transient ischemic attack in older men. J Clin Endocrinol Metab 2009; 94: 2353-2359.
51. Ohlsson C, Barrett-Connor E, Bhasin S, et al. High serum testosterone is associated with reduced risk of cardiovascular events in elderly men. J Am Coll Cardiol 2011; 58: 1674-1681.
52. Hyde Z, Norman PE, Flicker L, et al. Elevated luteinizing hormone predicts ischaemic heart disease events in older men. The Health In Men Study. Eur J Endocrinol 2011; 164: 569-577.
53. Haring R, Teng Z, Xanthakis V, et al. Associations of sex steroids, gonadotrophins, and their trajectories with clinical cardiovascular disease and all-cause mortality in elderly men from the Framingham Heart Study. Clin Endocrinol 2013; 78: 629-634.
54. Soisson V, Brailly-Tabard S, Helmer C, et al. A J-shaped association between plasma testosterone and risk of ischemic arterial event in elderly men: The French 3C Cohort Study. Maturitas 2013; 75: 282-288.
55. Shores MM, Biggs ML, Arnold AM, et al. Testosterone, dihydrotestosterone, and incident cardiovascular disease and mortality in the cardiovascular health study. J Clin Endocrinol Metab 2014; 99: 2061-2068.
56. Shores MM, Arnold AM, Biggs ML, et al. Testosterone and dihydrotestosterone and incident ischaemic stroke in men in the Cardiovascular Health Study. Clin Endocrinol 2014; 81: 746-753.
57. Yeap BB, Alfonso H, Chubb SAP, et al. In older men, higher plasma testosterone or dihydrotestosterone is an independent predictor for reduced incidence of stroke but not myocardial infarction. J Clin Endocrinol Metab 2014; 99: 4565-4573.
58. Srinath R, Golden SH, Carson KA, Dobs A. Endogenous testosterone and its relationship to preclinical and clinical measures of cardiovascular disease in the Atherosclerosis Risk in Communities Study. J Clin Endocrinol Metab 2015; 100: 1602-1608.
59. Holmegard HN, Nordestgaard BG, Jensen GB, et al. Sex hormones and ischemic stroke: a prospective cohort study and meta-analyses. J Clin Endocrinol Metab 2016; 101: 69-78.
60. Chan YX, Knuiman MW, Hung J, et al. Neutral associations of testosterone, dihydrotestosterone and estradiol with fatal and non-fatal cardiovascular events, and mortality in men aged 17-97 years. Clin Endocrinol 2016; 85: 575-582.
61. Srinath R, Gottesman RF, Golden SH, et al. Association between endogenous testosterone and cerebrovascular disease in the ARIC Study (Atherosclerosis Risk in Communities). Stroke 2016; 47: 2682-2688.
62. Wang A, Arver S, Boman K, et al. Testosterone, sex hormone-binding globulin and risk of cardiovascular events: a report from the Outcome Reduction with an Initial Glargine Intervention trial. Eur J Prev Cardiol. 2019; 26:847-854.
63. Gyawali P, Martin SA, Heilbronn LK, et al. Higher serum sex hormone-binding globulin levels are associated with incident cardiovascular disease in men. J Clin Endocrinol Metab 2019; 104: 6301-6315.
64. Hatami H, Parizadeh D, Yarandi RB, et al. Endogenous testosterone does not improve prediction of incident cardiovascular disease in a community-based cohort of adult men: results from the Tehran Lipid and Glucose Study. Aging Male 2020; 23:243-250.
65. Zhao D, Guallar E, Ballantyne CE, et al. Sex hormones and incident heart failure in men and postmenopausal women: the Atherosclerosis Risk in Communities Study. J Clin Endocrinol Metab. 2020; 105: e3789-e3807.
66. Collett T-H, Ewing SK, Ensrud KE, et al. Endogenous testosterone levels and the risk of cardiovascular events in elderly men: the MrOS Prospective Study. J Endocr Soc 2020; 4: bvaa038.
67. Boden WE, Miller MG, McBride R, et al. Testosterone concentrations and risk of cardiovascular events in androgen-deficient men with atherosclerotic cardiovascular disease. Am Heart J 2020; 224: 65-76.
68. Schafer S, Aydin MA, Appelbaum S, et al. Low testosterone concentrations and prediction of future heart failure in men and in women: evidence from the large FINRISK97 study. ESC Heart Failure 2021; 8:2485-2491.
69. Yeap BB, Marriott RJ, Antonio L, et al. Associations of serum testosterone and sex hormone-binding globulin with incident cardiovascular events in middle-aged to older men. Ann Intern Med 2022; 175: 159-170.
70. Dittadi R, Matteucci M, Meneghetti E, Ndreu R. Reassessment of the Access Testosterone chemiluminescence assay and comparison with LC-MS method. J Clin Lab Anal. 2018; 32: e22286.
71. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. Am J Epidemiol 2017; 186: 1026-1034.
72. Khaw K-T, Dowsett M, Folkerd E, et al. Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men. European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) prospective population study. Circulation 2007; 116: 2694-2701.
73. Araujo AB, Kupelian V, Page ST, et al. Sex steroids and cause-specific mortality in men. Arch Intern Med 2007; 167: 1252-1260.
74. Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. J Clin Endocrinol Metab 2008; 93: 68-75.
75. Tivesten A, Vandenput L, Labrie F, et al. Low serum testosterone and estradiol predict mortality in elderly men. J Clin Endocrinol Metab 2009; 94: 2482-2488.
76. Menke A, Guallar E, Rohrmann S, et al. Sex steroid concentrations and risk of death in US men. Am J Epidemiol 2010; 171: 583-592.
77. Haring R, Volzke H, Steveling A, et al. Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20-79. Eur Heart J 2010; 31: 1494-1501.
78. Hyde Z, Norman PE, Flicker L, et al. Low free testosterone predicts mortality from cardiovascular disease but not other causes: the Health In Men Study. J Clin Endocrinol Metab 2012; 97: 179-189.
79. Yeap BB, Alfonso H, Chubb SAP, et al. In older men an optimal plasma testosterone is associated with reduced all-cause mortality, and higher dihydrotestosterone with reduced ischaemic heart disease mortality, while estradiol levels do not predict mortality. J Clin Endocrinol Metab 2014; 99: E9-E18.
80. Pye SR, Huhtaniemi IT, Finn JD, et al. Late-onset hypogonadism and mortality in ageing men. J Clin Endocrinol Metab 2014; 99: 1357-1366.
81. Holmboe SA, Vradi E, Jensen TK, et al. The association of reproductive hormone levels and all-cause, cancer, and cardiovascular disease mortality in men. J Clin Endocrinol Metab 2015; 100: 4472-4480.
82. Hsu B, Cumming RG, Naganathan V, et al. Temporal changes in androgens and estrogens are associated with al-cause and cause-specific mortality in older men. J Clin Endocrinol Metab 2016; 101: 2201-2210.
83. Chasland LC, Knuiman MW, Divitini ML, et al. Greater physical activity and higher androgen concentrations are independently associated with lower cardiometabolic risk in men. Clin Endocrinol 2017; 87: 466-474.
84. Yeap BB, Marriott RJ, Antonio L, et al. Serum testosterone is inversely and sex hormone-binding globulin is directly associated with all-cause mortality in men. J Clin Endocrinol Metab 2021; 106: e625-e637.
85. Takov K, Wu J, Denvir MA, Smith LB, Hadoke PWF. The role of androgen receptors in atherosclerosis. Mol Cell Endocrinol 2018; 465: 82-91.
86. Jones TH, Kelly DM. Randomized controlled trials – mechanistic studies of testosterone and the cardiovascular system. Asian J Androl 2018; 20: 120-130.
87. Larsen BA, Nordestgaard BG, Stender A, Kjeldsen K. Effect of testosterone on atherogenesis in cholesterol-fed rabbits with similar plasma cholesterol levels. Atherosclerosis 1993; 99: 79-86.
88. Bruck B, Brehme U, Gugel N, et al. Gender-specific differences in the effects of testosterone and estrogen on the development of atherosclerosis in rabbits. Arterioscler Thromb Vasc Biol 1997; 17: 2192-2199.
89. Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H, Christiansen C. Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. Circ Res 1999; 84: 813-819.
90. Li SJ, Li XY, Li Y. Regulation of atherosclerotic plaque growth and stability by testosterone via influence of inflammatory reaction. Vascul Pharmacol 2008; 49: 14-18.
91. Deng L, Fu D, Zhu L, Huang J, Ling Y, Cai Z. Testosterone deficiency accelerates early stage atherosclerosis in miniature pigs fed a high-fat and high-cholesterol diet: urine 1H NMR metabolomics targeted analysis. Mol Cell Biochem 2021; 476: 1245-1255.
92. Nathan L, Shi W, Dinh H, et al. Testosterone inhibits early atherogenesis by conversion to estradiol: Critical role of aromatase. PNAS 2001; 98: 3589-3593.
93. McRobb L, Handelsman DJ, Heather AK. Androgen-induced progression of arterial calcification in Apolipoprotein E-null mice is uncoupled from plaque growth and lipid levels. Endocrinol 2009; 150: 841-848.
94. Nettleship JE, Jones TH, Channer KS, Jones RD. Physiological testosterone replacement therapy attenuates fatty streak formation and improves high-density lipoprotein cholesterol in the Tfm mouse. Circulation 2007; 116: 2427-2434.
95. Bourghardt J, Wilhelmson ASK, Alexanderson C, et al. Androgen receptor-dependent and independent atheroprotection by testosterone in male mice. Endocrinol 2010; 151: 5428-5437.
96. Hanke H, Lenz C, Hess B, Spindler KD, Weidemann W. effect of testosterone on plaque development and androgen receptor expression in the arterial wall. Circulation 2001; 103: 1382-1385.
97. Tharp DL, Masseau I, Ivey J, Ganjam VK, Bowles DK. Endogenous testosterone attenuates neointima formation after moderate coronary balloon injury in male swine. Cardiovasc Res 2009; 82: 152-160.
98. Wu J, Hadoke PWF, Mair I, et al. Modulation of neointimal lesion formation by endogenous androgens in independent of vascular androgen receptor. Cardiovasc Res 2014; 103: 281-190.
99. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. Circ Res 2014; 114: 1852-1866.
100. Rana K, Lee NKL, Zajac JD, MacLean HE. Expression of androgen receptor target genes in skeletal muscle. Asian J Androl 2014; 16: 675-683.
101. Rana K, Chiu MWS, Russel PK, et al. Muscle-specific androgen receptor deletion shows limited actions in myoblasts but not in myofibers in different muscles *in vivo*. J Mol Endocrinol 2016; 57: 125-138.
102. Williams MRI, Ling S, Dawood T, et al. Dehydroepiandrosterone inhibits human vascular smooth muscle cell proliferation independent of ARs and ERs. J Clin Endocrinol Metab 2002; 87: 176-181.
103. Lopes RAM, Neves KB, Pestana CR, et al. Testosterone induces apoptosis in vascular smooth muscle cells via extrinsic apoptotic pathway with mitochondria-generated reactive oxygen species involvement. Am J Physiol Heart Circ Physiol 2014; 306: H1485-H1494.
104. Huang C-K, Pang H, Wang L, et al. New therapy in targeting androgen receptor in monocytes/macrophages to battle atherosclerosis. Hypertension 2014; 63: 1345-1353.
105. Zhu D, Hadoke PWF, Wu J, et al. Ablation of the androgen receptor from vascular smooth muscle cells demonstrate a role for testosterone in vascular calcification. Sci Rep 2016; 6: 24807.
106. Libby P, Loscalzo J, Ridker P, et al. Inflammation, immunity, and infection in atherothrombosis. J Am Coll Cardiol 2018; 72: 2071-2081.
107. Moriya J. Critical roles of inflammation in atherosclerosis. J Cardiol 2019; 73: 22-27.
108. Ridker PM, Danielson E, Fonseca FAH, et al. Rosuvastatin to prevent events in men and women with elevated C-reactive protein. N Engl J Med 2008; 359: 2195-2207.
109. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. New Eng J Med 2017; 377: 1119-1131.
110. Ridker PM, Everett BM, Pradhan A, et al. Low-dose methotrexate for the prevention of atherosclerotic events. New Eng J Med 2018; doi:10.1056/NEJMoa1809798.
111. Tardiff J-C, Kouz S, Waters DD, et al. Efficacy and safety of low-dose colchicine after myocardial infarction. N Engl J Med 2019; 381: 2497-2505.
112. Nidorf SM, Fiolet ATL, Mosterd A, et al. Colchicine in patients with chronic coronary coronary disease. N Engl J Med 2020; 383: 1838-1847.
113. Li ZG, Danis VA, Brooks PM. Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells in vitro. Clin Exp Rheumatol 1993; 11: 157-162.
114. D’Agostino P, Milano S, Barbera C, et al. Sex hormones modulate inflammatory mediators produced by macrophages. Ann NY Acad Sci 1999; 876: 426-429.
115. Hatakeyama H, Nishizawa M, Nakagawa A, Nakano S, Kigoshi T, Uchida K. Testosterone inhibits tumor necrosis factor-α-induced vascular cells adhesion moleculre-1 expression in human aortic endothelial cells. FEBS Lett 2002; 530: 129-132.
116. Wilhelmson AS, Rodriguez ML, Eriksson ES, et al. Testosterone protects against atherosclerosis in male mice by targeting thymic epithelial cells-brief report. Arterioscler Thromb Vasc Biol 2018; 38: 1519-1527.
117. Khosla S, Atkinson EJ, Dunstan CR, O’Fallon WM. Effect of estrogen versus testosterone on circulating osteoprotegerin and other cytokine levels in normal elderly men. J Clin Endocrinol Metab 2001; 87: 1550-1554.
118. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. J Clin Endocrinol Metab 2004; 89: 3213-3318.
119. Kapoor D, Clarke S, Stanworth R, Channer KS, Jones TH. The effect of testosterone replacement therapy on adipocytokines and C-reactive protein in hypogonadal men with type 2 diabetes. Eur J Endocrinol 2007; 156: 595-602.
120. Mohler ER, Ellenbery SS, Lewis CE, et al. The effect of testosterone on cardiovascular biomarkers in the Testosterone Trials. J Clin Endocrinol Metab 2018; 103: 681-688.
121. Ng MK, Liu PY, Williams AJ, et al. Prospective study of effect of androgens on serum inflammatory markers in men. Arterioscler Thromb Vasc Biol 2002; 22: 1136-1141.
122. Aversa A, Bruzziches R, Francomano D, et al. Effects of testosterone undecanoate on cardiovascular risk factors and atherosclerosis in middle-aged men with late-onset hypogonadism and metabolic syndrome: results from a 24-month, randomized, double-blind, placebo-controlled trial. J Sex Med 2010; 7: 3495-3503.
123. Khripun I, Vorobyev S, Belousov I, Kogan M, Zitzmann M. Influence of testosterone substitution on glycemic control and endothelial markers in men with newly diagnosed functional hypogonadism and type 2 diabetes mellitus: a randomized controlled trial. Aging Male 2019; 22: 241-249.
124. Lesser MA. The treatment of angina pectoris with testosterone propionate. New Engl J Med 1942; 226: 51-54.
125. Levine SA, Likoff WB. The therapeutic value of testosterone propionate in angina pectoris. New Engl J Med 1943; 229; 770-772.
126. Lesser MA. Testosterone propionate therapy in one hundred cases of angina pectoris. J Clin Endocrinol (Metab) 1946; 6: 549-557.
127. The Coronary Drug Project Research Group. Initial findings leading to modifications of its research protocol. JAMA 1970; 214: 1303-1313.
128. Yeap BB, Grossmann M, McLachlan RI, et al. Endocrine Society of Australia position statement on male hypogonadism (part 2): treatment and therapeutic considerations. Med J Aust 2016; 205: 228-231.
129. Jaffe MD. Effect of testosterone cypionate on postexercise ST segment depression, Brit Heart J 1977; 39: 1217-1222.
130. English KM, Steeds RP, Hugh Jones T, et al. Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina. A randomized, double-blind, placebo-controlled study. Circulation 2000; 102: 1906-1911.
131. Webb CM, Elkington AG, Kraidly MM, et al. Effects of oral testosterone treatment on myocardial perfusion and vascular function in men with low plasma testosterone and coronary heart disease. Am J Cardiol 2008; 101: 618-624.
132. Mathur A, Malkin C, Saeed B, et al. Long-term benefits of testosterone replacement therapy on angina threshold and atheroma in men. Eur J Endocrinol 2009; 161: 443-449.
133. Cornoldi A, Caminiti G, Marazzi G, et al. Effects of chronic testosterone administration on myocardial ischemia, lipid metabolism and insulin resistance in elderly male diabetic patients with coronary artery disease. Int J Cardiol 2010; 142: 50-55.
134. Takase B, Uebata A, Akima T, et al. Endothelium-dependent flow-mediated vasodilatation in coronary and brachial arteries in suspected coronary artery disease. Am J Cardiol 1998; 82: 1535-1539.
135. Ben-Shlomo Y, Spears M, Boustred C, et al. Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. J Am Coll Cardiol 2014; 63: 636-646.
136. Vlachopoulos C, Aznaouridis K, Stefanidis C. Aortic stiffness for cardiovascular risk prediction: just measure it, just do it! J Am Coll Cardiol 2014; 63: 647-649.
137. Sader MA, Griffiths KA, Skilton MR, Wishart SM, Handelsman DJ, Celermajer DS. Physiological testosterone replacement and arterial endothelial function in men. Clin Endocrinol 2003; 59: 62-67.
138. Shoskes DA, Tucky B, Polackwich AS. Improvement of endothelial function following initiation of testosterone replacement therapy. Trans Androl Urol 2016; 5: 819-823.
139. Yaron M, Greenman Y, Rosenfeld JB, et al. Effects of testosterone replacement therapy in arterial stiffness in older hypogonadal men. Eur J Endocrinol 2009; 160: 839-846.
140. Groti K, Zuran I, Antonic B, Forsnaric L, Pfeifer M. The impact of testosterone replacement therapy on glycemic control, vascular function, and components of the metabolic syndrome in obese hypogonadal men with type 2 diabetes. Aging Male 2018; 21: 158-169.
141. Hildreth KL, Schwartz RS, van de Griend J, Kohrt WM, Blatchford PJ, Moreau KL. Effects of testosterone and progressive resistance exercise on vascular function in older men. J Appl Physiol 2018; 125: 1693-1701.
142. Chasland LC, Naylor LH, Yeap BB, Maiorana AJ, Green DJ. Testosterone and exercise in middle-to-older aged men: combined and independent effects on vascular function. Hypertension 2021; 77: 1095-1105.
143. Yu J, Akishita M, Eto M, et al. Androgen receptor-dependent activation of endothelial nitric oxide synthase in vascular endothelial cells: role of phosphatidylinositol 3-kinase/Akt pathway. Endocrinology 2010; 151: 1822-1828.
144. Isidori A, Giannetta E, Greco EA, et al. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. Clin Endocrinol 2005; 63: 280-293.
145. Guo C, Gu W, Liu M, et al. Efficacy and safety of testosterone replacement therapy in men with hypogonadism: a meta-analysis study of placebo-controlled trials. Exp Ther Med 2016; 11: 853-863.
146. Corona G, Giagulli VA, Maseroli E, et al. Testosterone supplementation an body composition: results from a meta-analysis study. Eur J Endocrinol 2016; 174: R99-R116.
147. Fernandez-Balsells MM, Murad MH, Lane M, et al. Adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis. J Clin Endocrinol metab. 2010; 95: 2560-2575.
148. Khera AV, Cuchel M, de la Llera-Moya M, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. N Engl J Med 2011; 364: 127-135.
149. Rohatgi A, Khera A, Berry JD, et al. HDL cholesterol efflux capacity and incident cardiovascular events. N Engl J Med 2014; 371: 2383-2393.
150. Rubinow KB, Vaisar T, Chao JH, Heinecke JW, Page ST. Sex steroids mediate discrete effects on HDL efflux capacity and particle concentration in healthy men. J Clin Lipidol 2018; 12: 1072-1082.
151. Makinen JI, Perheentupa A, Irjala K, et al. Endogenous testosterone and serum lipids in middle-aged men. Atherosclerosis 2008; 197: 688-693.
152. Haring R, Baumeister SE, Volzke H, et al. Prospective association of low total testosterone concentrations with adverse lipid profiles and increased incident dyslipidemia. Eur J Cardiovasc Prev Rehab 2011; 18: 86-96.
153. Lorenz MW, Markus HS, Bots ML, Rosvall M, Sitzer M. Prediction of clinical cardiovascular events with carotid intima-media thickness. Circulation 2007; 115: 459-467.
154. Van den Beld AW, Bots ML, Janssen JAMLL, Pols HAP, Lamberts SWJ, Grobbee DE. Endogenous hormones and carotid atherosclerosis in elderly men. Am J Epidemiol. 2003; 157: 25-31.
155. Svartberg J, von Muhlen D, Mathiesen E, Joakimsen O, Bonaa KH, Stensland-Bugge E. Low testosterone levels are associated with carotid atherosclerosis in men. J Intern Med 2006; 259: 576-582.
156. Muller M, van den Beld AW, Bots ML, Grobbee DE, Lamberts SWJ, van der Schouw YT. Endogenous sex hormones and progression of carotid atherosclerosis in elderly men. Circulation 2004; 109: 2074-2079.
157. Tivesten A, Hulthe J, Wallenfeldt K, Wikstrand J, Ohlsson C, Fagerberg B. Circulating estradiol is an independent predictor of progression of carotid artery intima-media thickness in middle-aged men. J Clin Endocrinol Metab 2006; 91: 4423-4437.
158. Vikan T, Johnsen SH, Schirmer H, Njolstad I, Svartberg J. Endogenous testosterone and the prospective association with carotid atherosclerosis in men: the Tromso study. Eur J Epidemiol 2009; 24: 289-295.
159. Soisson V, Brailly-Tabard S, Empana J-P, et al. Low plasma testosterone and elevated carotid intima-media thickness: importance of low-grade inflammation in elderly men. Atherosclerosis 2012; 223: 244-249.
160. Dorr M, Wallaschofski H, Friedrich N. Association of low total testosterone levels and prevalent carotid plaques: result of the study of health in Pomerania. Eur J Epidemiol 2009; 24: 389-391.
161. Chan YX, Knuiman MW, Hung J, et al. Testosterone, dihydrotestosterone and estradiol are differentially associated with carotid intima-media thickness and the presence of carotid plaque in men with and without coronary artery disease. Endocr J 2015; 62: 777-786.
162. Glisic M, Mujaj B, Rueda-Ochoa OL, et al. Associations of endogenous estradiol and testosterone levels with plaque composition and risk of stroke in subjects with carotid atherosclerosis. Circ Res 2018; 122: 97-105.
163. van Koeverden ID, de Bakker M, Haitjema S, et al. Testosterone to oestradiol ratio reflects systemic and plaque inflammation and predicts future cardiovascular events in men with severe atherosclerosis. Cardiovasc Res 2019; 115: 453-462.
164. Basaria S, Harman SM, Travison TG, et al. Effects of testosterone administration for 3 years on subclinical atherosclerosis progression in older men with low or low-normal testosterone levels: a randomized clinical trial. JAMA 2015; 314: 570-581.
165. Hulten EA, Carbonaro S, Petrillo SP, Mitchell JD, Villines TC. Prognostic value of cardiac computed tomography angiography. J Am Coll Cardiol 2011; 57: 1237-1247.
166. Bamberg F, Summer WH, Hoffmann V, et al. meta-analysis and systematic review of the long-term predictive value of assessment of coronary atherosclerosis by contrast-enhanced coronary computed tomography angiography. J Am Coll Cardiol 2011; 57: 2426-2436.
167. Nakazato R, Arsanjani R, Achenbach S, et al. Age-related risk of major adverse cardiac event risk and coronary artery disease extent and severity by coronary CT angiography: results from 15,187 patients from the International Multisite CONFIRM Study. Eur Heart J Cardiovasc Imaging 2014; 15: 586-594.
168. Budoff MJ, Ellenberg SS, Lewis CE, et al. Testosterone treatment and coronary artery plaque volume in older men with low testosterone. JAMA 2017; 317: 708-716.
169. Yeap BB, Page ST, Grossmann M. Testosterone treatment in older men: clinical implications and unresolved questions from the Testosterone Trials. Lancet Diabetes Endocrinol 2018; 6: 659-672.
170. Dhindsa S, Wilson MF, Dandona P. Letter to the Editor on “Changes in coronary artery plaque with testosterone therapy”. JAMA 2017; 317: 2450.
171. Oikonomou EK, Marwan M, Desai M, et al. Non-invasive detection of coronary inflammation using computed tomography and prediction of residual cardiovascular risk (the CRISP CT study): a post-hoc analysis of prospective outcome data. Lancet 2018; 392: 929-939.
172. Basaria S, Coviello AD, Travison TG, et al. Adverse events associated with testosterone administration. New Engl J Med 2010; 363: 109-122.
173. Srinivas-Shankar U, Roberts SA, Connolly MJ, et al. Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, double-blind, placebo-controlled study. J Clin Endocrinol Metab 2010: 95: 639-650.
174. Snyder PJ, Bhasin S, Cunningham GR, et al. Effects of testosterone treatment in older men. N Engl J Med 2016; 374: 611-624.
175. Wittert G, Bracken K, Robledo KP, et al. Testosterone treatment to prevent or revert type 2 diabetes in men enrolled in a lifestyle programme (T4DM): a randomised, double blind, placebo-controlled, 2-year, phase 3b trial. Lancet Diabetes Endocrinol 2021; 9: 32-45.
176. Snyder PJ, Bhasin S, Cunningham GR, et al. Lessons from the Testosterone Trials. Endocr Rev 2018; 39: 369-386.
177. Blair SN, Kampert JB, Kohl HW, et al. Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. JAMA 1996; 276: 205-10.
178. Sui X, LaMonte MJ, Laditka JN, et al. Cardiorespiratory fitness and adiposity as mortality predictors in older adults. JAMA 2007; 298: 2507-2516.
179. Kodama S, Saito K, Tanaka S, et al. Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in health men and women: a meta-analysis. JAMA 2009; 301: 2024-2035.
180. Ross R, Blair SN, Arena R, et al. Importance of assessing cardiorespiratory fitness in clinical practice: a case for fitness as a clinical vital sign: a scientific statement from the American Heart Association. Circulation 2016; 134: e653-e699.
181. Chasland LC, Yeap BB, Maiorana AJ, et al. Testosterone and exercise: effects on fitness, body composition, and strength in middle-to-older aged men with low normal serum testosterone levels. Am J Physiol Heart Circ Physiol 2021; 320: H1985-H1998.
182. Traustadottir T, Harman SM, Tsitouras P, et al. Long-term testosterone supplementation in older men attenuates age-related decline in aerobic capacity. J Clin Endocrinol Metab 2018; 103: 2861-2869.
183. Bhasin S, Woodhouse L, Casaburi R, et al. Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle. J Clin Endocrinol Metab 2005; 90: 678-688.
184. Page ST, Amory JK, Bowman FD, et al. Exogenous testosterone (T) alone or with finasteride increases physical performance, grip strength, and lean body mass in older men with low serum T. J Clin Endocrinol Metab 2005; 90: 1502-1510.
185. Haddad RM, Kennedy CC, Caples SM, et al. Testosterone and cardiovascular risk in men: a systematic review and meta-analysis of randomized placebo-controlled trials. Mayo Clin Proc 2007; 82: 29-39.
186. Xu L, Freeman G, Cowling BJ, Schooling CM. Testosterone therapy and cardiovascular events among men: a systematic review and meta-analysis of placebo-controlled randomized trials. BMC Med 2013; 11: 108.
187. Ruige JB, Ouwens DM, Kaufman J-M. Beneficial and adverse effects of testosterone on the cardiovascular system in men. J Clin Endocrinol Metab 2013; 98: 4300-4310.
188. Corona G, Maseroli E, Rastrelli G, et al. Cardiovascular risk associated with testosterone-boosting medications: a systematic review and meta-analysis. Expert Opin Drug Saf 2014; 13: 1327-1351.
189. Borst SE, Shuster JJ, Zou B, et al. Cardiovascular risks and elevation of serum DHT vary by route of testosterone administration: a systematic review and meta-analysis. BMC Medicine 2014; 12: 211.
190. Alexander GC, Iyer G, Lucas E, Lin D, Singh S. Cardiovascular risks of exogenous testosterone use among men: a systematic review and meta-analysis. Am J Med 2017; 130: 293-305.
191. Elliott J, Kelly SE, Millar AC, et al. Testosterone therapy in hypogonadal men: a systematic review and network meta-analysis. BMJ Open 2017; 7: e015284.
192. Corona G, Rastrelli G, Di Pasquale G, et al. Testosterone and cardiovascular risk: meta-analysis of interventional studies. J Sex Med 2018; 15: 820-838.
193. Diem SJ, Greer NL, MacDonald R, et al. Efficacy and safety of testosterone treatment in men: an evidence report for a clinical practice guideline by the American College of Physicians. Ann Intern Med 2020; 172: 105-118.
194. Hudson J, Cruickshank M, Quinton R, et al. Adverse cardiovascular events and mortality in men during testosterone treatment: an individual patient and aggregate data data meta-analysis. Lancet Healthy Longev 2022; 3: e381-393.
195. Bhasin S, Lincoff AM, Basaria S, et al. Effects of long-term testosterone treatment on cardiovascular outcomes in men with hypogonadism: Rationale and design of the TRAVERSE study. Am J Heart 2022; 245: 41-50.
196. Shores MM. Testosterone treatment and cardiovascular events in prescription database studies. Asian J Androl 2018; 20: 138-144.
197. Shores MM, Smith NL, Forsberg CW, et al. Testosterone treatment and mortality in men with low testosterone levels. J Clin Endocrinol Metab 2012; 97: 2050-2058.
198. Muraleedharan V, Marsh H, Kapoor D, et al. Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. Eur J Endocrinol 2013; 169: 725-733.
199. Vigen R, O’Donnell CI, Baron AE, et al. Association of testosterone therapy with mortality, myocardial infarction, and stroke in men with low testosterone levels. JAMA 2013; 310: 1829-1836.
200. Finkle WD, Greenland S, Ridgeway GK, et al. Increased risk of non-fatal myocardial infarction following testosterone therapy prescription in men. PLoS One 2014; 9: e85805.
201. Baillargeon J, Urban RJ, Kuo Y-F, et al. Risk of myocardial infarction in older men receiving testosterone therapy. Ann Pharmacother 2014; 48: 1138-1144.
202. Sharma R, Oni OA, Gupta K, et al. Normalization of testosterone level is associated with reduced incidence of myocardial infarction and mortality in men. Eur Heart J 2015; 36: 2706-2715.
203. Anderson JL, May HT, Lappe DL, et al. Impact of testosterone replacement therapy on myocardial infarction, stroke and death in men with low testosterone concentrations in an integrated health care system. Am J Cardiol 2016; 117: 794-799.
204. Wallis CJD, Lo K, Lee Y, et al. Survival and cardiovascular events in men treated with testosterone replacement therapy: an intention-to-treat observational cohort study. Lancet Diabetes Endocrinol 2016; 4: 498-506.
205. Cheetham TC, An JJ, Jacobsen SJ, et al. Association of testosterone replacement with cardiovascular outcomes among men with androgen deficiency. JAMA Intern Med 2017; 177: 491-499.
206. Loo SY, Azoulay L, Nie R, et al. Cardiovascular and cerebrovascular safety of testosterone replacement therapy among aging men with low testosterone levels: a cohort study. Am J Med 2019; 132: 1069-1077.
207. Oni OA, Dehkordi SHH, Jazayeri M-A, et al. Relation of testosterone normalization to mortality and myocardial infarction in men with previous myocardial infarction. Am J Cardiol 2019; 124: 1171-1178.
208. Shores MM, Walsh TJ, Korpak A, et al. Association between testosterone treatment and risk of incident cardiovascular events among US male veterans with low testosterone levels and multiple medical comorbidities. J Am Heart Assoc 2021; 10: e020562.
209. Morgentaler A, Lunenfeld B. Testosterone and cardiovascular risk: world’s experts take unprecedented action to correct misinformation. Aging Male 2014; 17: 63-65.
210. Vigen R, O’Donnell CI, Baron AE, et al. Association of testosterone therapy with mortality, myocardial infarction, and stroke in men with low testosterone levels. JAMA 2013; 310: 1829-1836. Erratum published in JAMA 2014; 311: 967.
211. Swerdloff RS, Pak Y, Wang C, et al. Serum testosterone (T) level variability in T gel-treated older hypogonadal men: treatment monitoring implications. J Clin Endocrinol Metab 2015; 100: 3280-3287.
212. Layton JB, Meier CR, Sharpless JL, et al. Comparative safety of testosterone dosage forms. JAMA Intern Med 2015; 175: 1187-1196.
213. Martinez C, Suissa S, Rietbrock S, et al. Testosterone treatment and risk of venous thromboembolism. BMJ 2016; 355: i5968.
214. Houghton DE, Alsawas M, Barrioneuvo P, et al. Testosterone therapy and venous thromboembolism: a systematic review and meta-analysis. Thromb Res 2018; 172: 94-102.
215. Nieschlag E, Vorona E. Medical consequences of doping with anabolic androgenic steroids: effects in reproductive functions. Eur J Endocrinol 2015; 173: R47-R58.
216. Goldman AL, Pope HG, Bhasin S. The health threat posed by the hidden epidemic of anabolic steroid use and body image disorders among young men. J Clin Endocrinol Metab 2018; doi:10.1210/jc.2018-01706.
217. Baggish AL, Weiner RB, Kanayama G, et al. Cardiovascular toxicity of illicit anabolic-androgenic steroid use. Circulation 2017; 135: 1991-2002.
218. Ng MTF, Hoermann R, Bracken K, et al. Effect of testosterone treatment on bone microarchitecture and bone mineral density in men: a 2-year RCT. J Clin Endocrinol Metab 2021; 106: e3143-3158.