

## ANDROGENS AND CARDIOVASCULAR DISEASE IN MEN

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#### 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 metabolites bioactive 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 casecontrol 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 of risk 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 bodv 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  $5\alpha$ -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 methoddependent 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  $\geq$ 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	Size (n	Follow-	Age	Summary of results				
and year	of men)	up (yr)	(yr)					
Smith GD, 2005	2,512	16.5	45-59	No association of total T with IHD events or				
(46)				deaths.				
Arnlov J, 2006	2,084	10	56	Higher total E2 at baseline associated with				
(47)				lower incidence of CVD events, total T was not				
				associated.				
Abbott RD, 2007	2,197	≤7	71-93	Baseline total E2 in top quintile (≥125 pmol/L)				
(48)				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	3,443	3.5	≥70	Total and free T in the lowest quartiles (<11.7				
(50)				nmol/liter and <222 pmol/liter) predicted				
				increased incidence of stroke or TIA.				
* Ohlsson C, 2011	2,416	5	69-81	Men with total T <sup>a</sup> in highest quartile (≥19 mol/L)				
(51)				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	254	5, 10	75.5	No associations of baseline total T or total E2				
(53)				with incident CVD events.				
Soisson V, 2013	495; 146	4	>65	Total T in lowest and highest quintiles				
(54)				associated with CHD or stroke.				

* Shores MM,	1,032	9	76	DHT <sup>b</sup> <1.7 or >2.6 nmol/L associated with
2014 (55)				cardiovascular events. Total T was not
				associated.
* Shores MM,	1,032	10	76	Non-linear association of DHT <sup>b</sup> with stroke with
2014 (56)				lowest risk in men with DHT 1.7-2.6 nmol/L.
				Total T <sup>o</sup> was not associated with stroke. DHT
+ )/ DD 0044	0.000	0.0	70.00	<0.86 nmol/L associated with CVD mortality.
~ Yeap BB, 2014	3,690	0.0	70-89	Higher total 1° (>12.6 nmol/L) or DH1 (>1.34
(57)				atroke T <sup>c</sup> DHT and E2 were not approxisted
				with MI
* Srinath R 2015	1 558	12.8	63.1	T <sup>d</sup> was not associated with incidence of CHD
(58)	1,000	12.0	00.1	events or cardiac-related mortality
Holmegard HN	4 602	20	57	Total T in lowest decile (0-10 <sup>th</sup> percentile)
2016 (59)	1,002	20	01	associated with stroke.
* Chan YX. 2016	1.804	14.9	50.3	Total T <sup>c</sup> . DHT and E2 were not associated with
(60)				CVD events.
* Srinath R, 2016	1,558	14.1	63.1	T <sup>d</sup> was not associated with stroke.
(61)				
Wang A, 2019	5,553	6	63.5	Neither total T nor free T were associated with
(62)				CVD events.
* Gyawali P, 2019	1,492	4.9	54.2	Higher total T <sup>c</sup> associated with lower risk of
(63)				incident CVD events, but not with CVD
	0.4.0	10	10.1	mortality. E2 not associated.
Hatami H, 2020	816	12	46.1	Total T was not associated with risk of CVD
(64) 7h D - 0000 (05)	4 4 0 7	40.0	00.0	events.
Znao D, 2020 (65)	4,107	19.2	63.Z	Lower total 1 associated with increased risk of
	552	74	70 /	Total T <sup>d</sup> not accorded with CVD events
(66)	552	1.4	12.4	Total T Thot associated with CVD events.
* Boden WE 2020	2 118	3	>40	643 men with total $T^{b} < 10.4$ nmol/L had higher
(67)	2,110	U	-10	risk of combined endpoint of CHD death MI or
(0))				stroke, compared with 1.475 men with total T
				≥10.4 nmol/L.
Shafer S, 2021	3,865	13.8	48.2	Lower total T not associated with incident heart
(68)				failure.
Yeap BB, 2022	210,700	9	58	Total T not associated with incident MI, stroke,
(69)				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. <sup>a</sup>T and E2 assayed using gas chromatographymass spectrometry (GC-MS), <sup>b</sup>T and DHT assayed using liquid chromatography-tandem mass spectrometry (LC-MS), <sup>c</sup>T, DHT and E2 assayed by LC-MS, <sup>d</sup>T 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 nonfatal 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). from the Atherosclerosis Risk Analyses 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:

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 Т 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	Size (n	Follow-	Age (yr)	Summary of results				
and year	of men)	up (yr)						
Khaw K-T, 2007	825 and	≤10	40-79	Total T inversely related to mortality from				
(72)	1489			all causes, CVD and cancer.				
Araujo AB, 2007	1,686	15.3	40-70	Lower free T associated with lower IHD				
(73)				mortality. Equivocal association of lower				
				DHT with IHD mortality.				
Laughlin GA,	794	11.8	50-91	Total T in the lowest quartile (<8.4				
2008 (74)				nmol/L) predicted increased mortality				
				from all causes and from CVD and				
				respiratory causes.				
* Tivesten A,	3,014	4.5	75	Total T <sup>a</sup> and E2 levels in the lowest				
2009 (75)				quartiles predicted all-cause and non-				
				CVD mortality. T and E2 were not				
				associated with CVD mortality.				

**IMMUNOASSAY RESULTS** 

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 90 <sup>th</sup> and
Haring R, 2010 (77)	1,954	7.2	20-79	10 <sup>th</sup> percentiles for free T and total E2) 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 T <sup>b</sup> (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 T <sup>c</sup> <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 T <sup>d</sup> over time associated with all-cause but not CVD mortality. Decrease in total E2 <sup>d</sup> was associated with all-cause and CVD mortality.
* Chasland L, 2017 (83)	1,649	20	49.8	Higher physical activity and total T <sup>b</sup> , 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. <sup>a</sup>T and E2 measured using gas chromatography-mass spectrometry (GC-MS). <sup>b</sup>T, DHT and E2 measured using liquid chromatography-tandem mass spectrometry (LC-MS), <sup>c</sup>T measured using GC-MS, <sup>d</sup>T 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 allcause 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 plague 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 noncastrated 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 plague in apolipoprotein E (ApoE)-null mice, even as T had a neutral effect on plague volume and DHT decreased plague 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 plague, 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 p27<sup>kip1</sup> 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

smooth muscle cells contribute Vascular 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 plague 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 plague 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 Creactive protein (CRP) concentrations, reducing the risk of cardiovascular events in a primary prevention setting in adults with LDL <3.4 mmol/L and highsensitivity CRP ≥2.0 mg/L (108). Recently, antiinflammatory intervention utilizing canakinumab, a monoclonal antibody targeting interleukin-1ß, in a secondary prevention setting in adults with highsensitivity 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- $\alpha$  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-a and interleukin-1ß, 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-a, 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 postexercise 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 inMiddle-Aged and Older Men Reporting Outcomes Related to Angina (A), Artery Health (B), andCardiovascular 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	T cypionate 200 mg weekly	25	25	8 weeks	Decreased postexercise ST segment depression in T-
	age 58 years)					treated but not placebo group

English KM, 2000 (130) Webb CM, 2008 (131)	Menwithcoronary arterydisease (meanage 62 years)Menwithangiographicallyprovencoronary artery	Transdermal patch 5 mg Oral T undecanoate 80 mg bd	22	24	12 weeks 8 weeks, cross- over	Increased time to 1- mm ST- segment depression during treadmill exercise No difference in angina or endothelial function,
	disease, 40-75 years					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
В						
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	Mean age 66	Transdermal gel,	41, 43	40	12	No effect of T on
KL, 2018	years, T 6.9-	titrated to 13.9-			months	endothelial function
(141)	12.1 nmol/L	19.1 or 20.8-34.7				or on CIMT
		nmol/L				
С						
Basaria S,	≥65 years, T	Transdermal gel	106	103	6 months	Trial stopped
2010 (172)	3.5-12.1	100 mg daily				prematurely due to
	nmol/L or free					excess
	T <173 pmol/L,					cardiovascular
	mobility					events in T arm
	limitation					
Srinivas-	≥65 years, T	Transdermal gel	138	136	6 months	T improved muscle
Shankar U,	≤12 nmol/L or	50 mg daily				strength and
2010 (173)	free T ≤250					physical function,
	pmol/L, frail or					no signal for
	intermediate					cardiovascular
	frail					adverse events
Snyder P,	≥65 years, T	Transdermal gel	395	395	12	Modest benefit of T
2016 (174)	<9.5 nmol/L,	50 mg daily	(A	(A 229,	months	on sexual function,
	sexual		230,	B 238,		no signal for
	dysfunction		B 236,	C 197)		cardiovascular
	(A), diminished		C 193)			adverse events
	vitality (B)					
	and/or mobility					
	limitations (C)					
Wittert GA,	50-74 years,	Depot	504	503	24	All men received
2021 (175)	waist ≥95 cm, T	intramuscular			months	background
	≤14 nmol/L,	testosterone				lifestyle
	and impaired	undecanoate				intervention
	glucose	1000 mg every 3				(Weight Watchers).
	tolerance or	months				T reduced risk of
	newly					type 2 diabetes at 2
	diagnosed type					years by 40%.
	2 diabetes					

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 Tinduced 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 noninvasively 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  $\geq 2$  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 plague area during follow-up, possibly due to increased use of antihypertensive 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 communitydwelling 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 plague in men with CAD (161). One study found that higher E2 was associated with the presence of lipid core in carotid plagues 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 Ttreated group experienced a greater increase in noncalcified 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 noncalcified 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 plague 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  $\geq$ 95 cm and baseline T  $\leq$ 14.0 nmol/L ( $\leq$ 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 (VO<sub>2</sub>peak), 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 VO<sub>2</sub> 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, placebotreated men showed a decline in VO<sub>2</sub>peak 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 metaanalyses 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 charact	teristics			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% Cl 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 metaanalysis, Elliott et al. (2017) included RCTs that enrolled men with baseline T  $\leq$ 12 nmol/L ( $\leq$ 346 ng/dl) or free T  $\leq$ 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 Prescriptio	<mark>ns with (</mark>	<mark>Cardiovas</mark>	<mark>cular Eve</mark>	ents and Mortality in Middle-Aged and Older Men				
Study characte	ristics			Results				
Study author	Size	Follow-	Age	Favors no T	Favors T			
and year	(n of	up (yr)	(yr)					
	men)							
Shores MM,	1,031	3.4	62.1		Male veterans with total T			
2012 (197)					≤8.7 nmol/L, T prescribed in			
					398. T supplementation			
					associated with lower			
					mortality.			
Muraleedharan	581	5.8	59.5		Men with Type 2 diabetes,			
V, 2013 (198)					238 with total T ≤10.4			
					nmol/L. T supplementation			
					associated with lower			
) <i>(</i> , , , , , , , , , , , , , , , , , , ,	0 700		00.4		mortality.			
Vigen R, 2013	8,709	2.3	63.4	Male veterans who had				
(199)				coronary anglography				
				and total $1 \le 10.4$				
				nmoi/L. I prescription				
				increased rick of death				
				MI or stroke				
Finkle WD	55 593	90 days	54 4	Men prescribed T				
2014 (200)	00,000		0	Higher rate of non-fatal				
				MI in 90 days following				
				prescription compared				
				to preceding 1 year.				
Baillargeon J,	6,355;	4.1; 3.3	≥66		6,355 men prescribed T vs			
2014 (201)	19,065				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			
	00.040	0.0						
Snarma R,	83,010	0.2;	00		IVIALE VETERANS WITH IOW I.			
2013 (202)		4.0, 4.7			normalization of circulating			
					T $(n=43.931)$ was			
					associated with lower risk of			
					associated with lower lisk 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)	204,85 7	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). (	Curre	ent int	ramu	scular
				T use	not	asso	ciated	d with
				risk for	cor	nposit	e en	dpoint
				in me	n v	vithou	t or	with
				prevale	nt	CVD	(HR:	=0.91,
				95% CI 0.80-1.04; HR=0.98,				
				95%	C		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 nonrandomized 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 Т 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 nonuse. 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 metaanalyses 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 plague 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 auestion 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 а 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

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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 likely outweigh risks of treatment putative 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 preexisting 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.

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