Testosterone replacement therapy and cardiovascular risk

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Abstract | Testosterone is the main male sex hormone and is essential for the maintenance of male secondary sexual characteristics and fertility. Androgen deficiency in young men owing to organic disease of the hypothalamus, pituitary gland or testes has been treated with testosterone replacement for decades without reports of increased cardiovascular events. In the past decade, the number of testosterone prescriptions issued for middle-aged or older men with either age-related or obesity-related decline in serum testosterone levels has increased exponentially even though these conditions are not approved indications for testosterone therapy. Some retrospective studies and randomized trials have suggested that testosterone replacement therapy increases the risk of cardiovascular disease, which has led the FDA to release a warning statement about the potential cardiovascular risks of testosterone replacement therapy. However, no trials of testosterone replacement therapy published to date were designed or adequately powered to assess cardiovascular events; therefore, the cardiovascular safety of this therapy remains unclear. In this Review, we provide an overview of epidemiological data on the association between serum levels of endogenous testosterone and cardiovascular disease, prescription database studies on the risk of cardiovascular disease in men receiving testosterone therapy, randomized trials and meta-analyses evaluating testosterone replacement therapy and its association with cardiovascular events and mechanistic studies on the effects of testosterone on the cardiovascular system. Our aim is to help clinicians to make informed decisions when considering testosterone replacement therapy in their patients.

Testosterone is the main male sex hormone and is essential for the maintenance of secondary sexual characteristics and fertility in men. Organic male hypogonadism is caused by conditions that affect the testes (primary hypogonadism) or the hypothalamus and/or the pituitary gland (secondary hypogonadism)[1]. In young men with organic hypogonadism, in whom serum levels of endogenous testosterone are unequivocally low, physiological testosterone replacement therapy is generally safe and has beneficial effects[2]. In some men, serum testosterone levels decline as a consequence of ageing[3,4], even in the absence of pituitary or testicular disease[5,6]; some studies report that the prevalence of low testosterone level increases from 12% to 49% between the ages of 50 and 80 years[5]. In most of these men, serum testosterone concentrations are not unequivocally low and are often close to the lower limit of the normal range. Importantly, the syndromic prevalence of hypogonadism (low testosterone level plus specific symptoms) among these men is even lower (~2%)[7], and consequently, the benefits and risks of testosterone replacement therapy in men with age-related low testosterone level remain unclear. A large trial of testosterone therapy in older men with age-related low testosterone level and associated symptoms showed improvement in sexual function and mood, whereas no improvement was seen in physical function or vitality[8]. Despite modest benefits, the number of off-label testosterone prescriptions has increased exponentially worldwide, mostly in middle-aged and older men[9–12] as a result of a sophisticated marketing campaign in mass media[13]. This increase in the prescription rate has slowed slightly after the FDA issued a safety warning in 2014 alerting the medical community to possible serious cardiovascular adverse events related to testosterone therapy[14].

Several cohort studies have shown an association between low serum levels of endogenous testosterone and increased risk of cardiovascular disease[15–21]. As sex steroid levels are known to decrease in both acute and subacute illnesses, the low testosterone concentrations present in men with cardiovascular disease might be a consequence of their morbidity rather than the cause. This decline in testosterone level could be an adaptive response, suggesting that testosterone is a biomarker of

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https://doi.org/10.1038/s41569-019-0211-4

Key points

- Population studies suggest that low serum levels of endogenous testosterone are a risk factor for cardiovascular events, although these studies cannot establish causality or exclude reverse causality, and some of these associations might result from residual confounding.
- Although many retrospective studies show no association, some retrospective studies of prescription databases have shown a higher risk of cardiovascular events in men receiving testosterone, with the risk increasing early after treatment initiation.
- Meta-analyses of randomized, controlled trials of testosterone replacement therapy report conflicting findings, probably because the included trials lacked power or the duration was too short to assess cardiovascular events.
- The TRAVERSE trial, the first trial of testosterone therapy that is adequately powered to assess cardiovascular events, began in 2018, and its findings might take a decade to become available.
- Until the results of the TRAVERSE trial are available, clinicians should individualize testosterone treatment after having an informed discussion with their patients about the risks and benefits of testosterone replacement therapy.

health. Furthermore, population studies cannot establish causality, nor can they exclude reverse causality. In contrast to these cohort studies, some retrospective, prescription database studies that evaluated cardiovascular outcomes have shown an increased number of cardiovascular events in men who received testosterone replacement therapy[22–25], whereas other studies found either neutral or beneficial effects[26–38]. These contradictory findings, together with reports of increased cardiovascular events in some studies of testosterone replacement[39,40], have reignited the debate about the cardiovascular safety of testosterone replacement.

As no clinical trials of testosterone replacement published to date have been adequately powered to assess cardiovascular events, the cardiovascular safety of testosterone replacement remains unclear. The TRAVERSE trial[41], a large clinical trial of a transdermal testosterone gel versus a placebo gel, which is powered to assess cardiovascular events, commenced in 2018; however, with a planned intervention duration of 5 years, it will be a decade before its findings are published. In the interim, we hope that this Review will aid clinicians in having an informed discussion with their patients about the potential cardiovascular risks of testosterone therapy. In this Review, we discuss epidemiological studies reporting the association between serum levels of endogenous testosterone and cardiovascular events, cardiovascular-related mortality and all-cause mortality; retrospective studies that evaluated the risk of cardiovascular events and all-cause death in men who received testosterone therapy; randomized, controlled trials of testosterone therapy that reported cardiovascular events; meta-analyses of randomized trials; and mechanistic studies, both preclinical and in humans, that assessed the effects of testosterone on the cardiovascular system.

Effect of endogenous testosterone level

Epidemiological studies

Population studies have consistently shown an association between lower serum concentrations of endogenous testosterone and increased risk of cardiovascular disease, cardiovascular death and all-cause death in middle-aged and older men. In this section, we review large epidemiological studies that assessed cardiovascular outcomes and used reliable assays for the measurement of testosterone levels.

Cardiovascular events. A number of prospective cohort studies have assessed the association between serum concentrations of endogenous testosterone in men and the risk of cardiovascular events (TABLE 1). Before discussing these studies in detail, we highlight some limitations of these population studies that should be considered when evaluating their findings. First, most of these studies measured serum testosterone levels with the use of immunoassays, which are less precise and accurate (particularly at lower testosterone concentrations[42,43]) than the gold-standard method for testosterone measurement[44], liquid chromatography–tandem mass spectrometry. Second, only a single testosterone measurement was made in most of these studies, which is suboptimal given the considerable diurnal variation in serum testosterone levels. Third, most of the studies enrolled men solely on the basis of a numerical testosterone value and did not consider the presence of symptoms of androgen deficiency (that is, syndromic diagnosis was not made).

In a large study of Australian men aged ≥70 years who were followed up for a median of 3.5 years, men with lower total testosterone levels in serum (<337 ng/dl, measured by immunoassay) were twice as likely to experience a stroke or a transient ischaemic attack as men with normal testosterone levels[15], a finding that was later confirmed with the use of mass spectrometry[18]. By contrast, the Cardiovascular Health Study[45] did not find an association between serum total testosterone concentrations and incident ischaemic stroke in 1,032 elderly men. Although the reasons for these contradictory findings remain unclear, differences in study population characteristics and the small number of events overall might have contributed to this discrepancy. In the ARIC study[46], serum total testosterone levels were not associated with increased risk of ischaemic stroke in 1,558 men (79 ischaemic stroke events) during a median follow-up duration of 14.1 years. Interestingly, a study of 2,416 Swedish men aged 69–81 years found a protective effect of testosterone on the risk of acute myocardial infarction (MI), unstable angina, cardiovascular revascularization or death from coronary artery disease; men with a serum total testosterone level ≥550 ng/dl (highest quartile) had a 30% reduced risk of a cardiovascular event[16]. A study of 495 men (aged >65 years) participating in the French Three-City prospective cohort study[17] found a J-shaped association between total testosterone levels in serum and risk of ischaemic arterial disease (112 coronary heart disease and 34 stroke events) at the 4-year follow-up, even after adjusting for cardiovascular risk factors. Men in the lowest (HR 2.23, 95% CI 1.02–4.88) and highest (HR 3.61, 95% CI 1.55–8.45) quintiles of serum testosterone level were at a higher risk of ischaemic arterial disease than men in the second quintile[17], which suggests that testosterone within a certain concentration range provides some cardiovascular protection compared with testosterone concentrations at either extreme.

Table 1 | **Association between endogenous testosterone levels in serum and cardiovascular events**

Study (year)	Testosterone measurement method	*n*	Age (years)	Follow-up duration (years)	Findings	Refs
Association present						
Yeap et al. (2009)	Immunoassay	3,443	≥70	3.5	Lower TT levels associated with higher risk of stroke or transient ischaemic attack after multivariate adjustment (HR 1.99, 95% CI 1.33–2.99)	15
Ohlsson et al. (2011)	LC–MS–MS	2,416	69–81	5	Higher TT levels associated with reduced risk (HR 0.70, 95% CI 0.56–0.88) of CV events (acute MI, unstable angina, revascularization or death from coronary disease)	16
Soisson et al. (2013)	Immunoassay	491	≥65	4	Higher risk of coronary disease or stroke in men with TT levels in lowest and highest quintiles	17
Yeap et al. (2014)	LC–MS–MS	3,690	70–89	6.6	Lower incidence of stroke (HR 0.48, 95% CI 0.31–0.73) but not MI in men with higher TT levels	18
Magnani et al. (2014)	Immunoassay	1,251	≥55	10	Each s.d. decrease in TT level associated with incident AF in men aged 55–69 years (HR 1.30, 95% CI 1.07–1.59) and in men aged >80 years (HR 3.53, 95% CI 1.96–6.37)	47
No association						
Ärnlöv et al. (2006)	Immunoassay	2,084	30–60	10	TT not associated with risk of CV events (MI, angina pectoris, coronary insufficiency, death from coronary disease, congestive heart failure, stroke or peripheral vascular disease)	269
Abbott et al. (2007)	Immunoassay	2,197	71–94	5–7	TT not associated with risk of stroke	270
Vikan et al. (2009)	Immunoassay	1,318	59.6	9.1	TT and FT not associated with risk of first MI	54
Haring et al. (2013)	Immunoassay	254	75.5	10	TT not associated with risk of CV events (MI, angina pectoris, coronary insufficiency, death from coronary disease, congestive heart failure or stroke)	61
Shores et al. (2014)	LC–MS–MS	1,032	66–97	10	TT and FT not associated with risk of stroke	45
Srinath et al. (2016)	LC–MS–MS	1,558	51–76	14.1	TT not associated with incident stroke, percentage white matter hyperintensities, cortical infarcts or subcortical infarcts	46

AF, atrial fibrillation; CV, cardiovascular; FT, free testosterone; LC–MS–MS, liquid chromatography–tandem mass spectrometry; MI, myocardial infarction; TT, total testosterone.

Low endogenous testosterone concentrations in serum have also been linked to increased risk of atrial fibrillation in middle-aged and older men. In a cohort of 1,251 men (mean age 68 years) participating in the Framingham Heart Study[47], 275 men developed incident atrial fibrillation during follow-up. In adjusted hazard models, a significant association was observed between age and testosterone levels: in men aged 55–69 years, each standard deviation decrease in serum testosterone level was associated with a hazard ratio of 1.30 (95% CI 1.07–1.59), whereas in men aged ≥80 years, the hazard ratio was 3.53 (95% CI 1.96–6.37)[47]. Lower androgen levels have also been linked to incident atrial fibrillation incidence in men participating in the Cardiovascular Health Study[48] and the FINRISK97 study[49].

In 2011, a meta-analysis of 19 prospective population-based cohort and nested case–control studies evaluated the association between endogenous testosterone concentrations and risk of cardiovascular disease (atherosclerosis, stroke, MI, ischaemic heart disease and cardiovascular or all-cause death) and found a weak association (relative risk (RR) 0.89, 95% CI 0.83–0.96) between higher serum testosterone levels and lower risk of cardiovascular disease[50]. The analysis also found no association between serum testosterone levels and cardiovascular disease among men aged <70 years, although a weak benefit was observed in studies that enrolled men aged ≥70 years (RR 0.84, 95% CI 0.76–0.92)[50]. Interestingly, a Mendelian randomization analysis of a cohort of 1,882 men (aged 20–79 years)

participating in the Study of Health in Pomerania suggested that associations between serum testosterone concentrations and cardiometabolic risk factors and mortality are not causal[51] and might result from residual confounding or even reverse causation. These findings are in agreement with data from the European Male Ageing Study[3], which did not find a significant association between serum testosterone concentrations and age after adjusting for confounding factors, such as BMI, smoking status, alcohol consumption and comorbidities.

Cardiovascular or all-cause mortality. The association between serum concentrations of endogenous testosterone and cardiovascular mortality or mortality from other causes has been the focus of some cohort studies (TABLE 2). Most of these studies reported a protective effect of testosterone[19–21,30,52–58], whereas other studies did

Table 2 | **Studies on the association between endogenous testosterone levels in serum and mortality**

Study (year)	*n*	Age (years)	Follow-up duration (years)	Findings	Refs
Association present					
Shores et al. (2006)	858	≥40	4.3	Low TT level associated with increased mortality (HR 1.88, 95% CI 1.34–2.63), even after adjusting for confounding factors	52
Khaw et al. (2007)	11,606	40–79	7	Low TT level associated with higher all-cause and CV mortality	19
Laughlin et al. (2008)	794	50–91	11.8	Low TT level associated with higher all-cause mortality (HR 1.40, 95% CI 1.14–1.71) and CV mortality (HR 1.38, 95% CI 1.02–1.85); low BT level also associated with these outcomes	20
Tivesten et al. (2009)	3,014	69–80	4.5	Low TT level associated with higher all-cause mortality (HR 1.65, 95% CI 1.29–2.12)	53
Vikan et al. (2009)	1,568	59.6	11.2	Low FT (but not TT) level associated with higher all-cause mortality in age-adjusted model (HR 1.24, 95% CI 1.01–1.53) and multivariate-adjusted model (HR 1.24, 95% CI 1.01–1.54)	54
Malkin et al. (2010)	930	≥60	6.9	Low BT (but not TT) level associated with higher all-cause or vascular mortality (HR 2.27, 95% CI 1.45–3.6) in men with coronary artery disease	55
Haring et al. (2010)	1,954	20–79	7.2	Low TT level associated with higher all-cause mortality (HR 2.32, 95% CI 1.38–3.89) and CV mortality (HR 2.56, 95% CI 1.15–6.52) after adjusting for confounding factors	21
Menke et al. (2010)	1,114	≥20	18	Higher all-cause mortality with low FT (HR 1.43, 95% CI 1.09–1.87) and low BT (HR 1.52, 95% CI 1.15–2.02) (but not TT) level between baseline and year 9 (but not between years 9 and 18) after adjusting for confounding factors	56
Hyde et al. (2012)	3,637	70–88	5.1	Low FT level associated with higher all-cause mortality (HR 1.62, 95% CI 1.20–2.19) and CV mortality (sub-HR 1.71, 95% CI 1.12–2.62)	57
Muraleedharan et al. (2013)	581	31–88	5.8	Low TT level associated with higher all-cause mortality (HR 2.02, 95% CI 1.2–3.4) in men with type 2 diabetes mellitus in the multivariate-adjusted model	30
Yeap et al. (2014)	3,690	70–89	6.7	Midrange TT levels associated with lower all-cause mortality	58
No association					
Araujo et al. (2007)	1,686	40–70	15.3	TT not associated with all-cause mortality	59
Szulc et al. (2009)	782	≥50	10	TT not a predictor of all-cause mortality	60
Haring et al. (2013)	254	75.5	10	Higher baseline TT level marginally associated with lower all-cause mortality at 5-year follow-up (HR 0.74, 95% CI 0.56–0.98) but not at 10-year follow-up	61
Shores et al. (2014)	1,032	66–97	9	TT not associated with all-cause mortality after adjustment for CV risk factors	62
Chan et al. (2016)	1,804	17–97	14.9	TT not associated with all-cause mortality or CV mortality after adjustment for risk factors	63

BT, bioavailable testosterone; CV, cardiovascular; FT, free testosterone; TT, total testosterone.

not find such an association[59–63]. In the EPIC-Norfolk study[19], a nested case–control study of 2,314 men aged 40–79 years without prevalent cancer or cardiovascular disease, higher serum levels of endogenous testosterone at baseline were associated with lower risk of all-cause death and cardiovascular-related death in multivariate-adjusted analyses. The risk of cardiovascular death decreased with increasing endogenous testosterone levels, with men in the highest quartile having an odds ratio of 0.53 (95% CI 0.32–0.86) compared with men in the lowest quartile. Similarly, all-cause mortality decreased with increasing serum testosterone concentrations (OR 0.59, 95% CI 0.42–0.85 for men in the highest quartile compared with men in the lowest quartile)[19]. A Swedish study of 3,014 elderly men (mean follow-up duration 4.5 years) found that men with total testosterone levels in the lowest quartile (≤336 ng/dl) had a 65% higher risk of all-cause death than men in quartiles 2–4 (HR 1.65, 95% CI 1.29–2.12)[53]. Another study of 3,690 elderly men (median follow-up duration 7.1 years) found a U-shaped association between total testosterone levels in the serum and all-cause mortality: men with total testosterone level in the second and third quartiles (283–454 ng/dl) had a lower risk than men in the lowest or the highest quartiles[58]. In contrast to these findings, total testosterone levels were not associated with mortality in another report[62]. A study of 1,804 men (mean age 50 years) showed that men who died during the follow-up duration of 15 years had lower baseline levels of total testosterone in the serum, although this association was not significant after adjustment for relevant risk factors[63].

A meta-analysis of studies on the association between endogenous testosterone levels and cardiovascular and all-cause mortality found that testosterone had a protective effect[64]. In this meta-analysis, lower serum testosterone levels were associated with a higher risk of cardiovascular death (RR 1.25, 95% CI 0.97–1.60) and all-cause death (RR 1.35, 95% CI 1.13–1.62)[64]. However, because of substantial heterogeneity between the studies included in this analysis (for example, variable duration of follow-up and timing of blood draw) and differences in the characteristics of participants (such as age and testosterone levels), these data should be interpreted with caution.

Summary of epidemiological studies. In summary, although conflicting results have been obtained in epidemiological studies, most studies suggest that low serum levels of endogenous testosterone are a risk factor for cardiovascular events, cardiovascular mortality and all-cause mortality. However, these data must be interpreted with caution because population studies, no matter how methodologically rigorous, cannot establish causality or exclude reverse causality. Indeed, the researchers in some of these studies suggested that this association is not causal[3,51]. Some investigators suggest that testosterone has a beneficial effect on the risk of cardiovascular disease on the basis of studies in men with prostate cancer who received androgen deprivation therapy, which show that low serum testosterone levels are consistently associated with metabolic syndrome, cardiovascular morbidity and mortality[65–69]. However, because serum testosterone levels in these men are in the castrate range, which is profoundly lower than the cut-off thresholds in the above-mentioned population studies, these data must also be interpreted with caution.

Effect of testosterone therapy

Retrospective studies

In the past decade, some retrospective studies of health-care and prescription databases have reported an increased risk of cardiovascular events in men who received testosterone therapy (TABLE 3). In a study reviewing data from 8,709 men who had undergone coronary angiography and had low serum testosterone concentrations (<300 ng/dl), an adjusted analysis revealed that the 1,223 men who received testosterone replacement therapy had a 29% increased risk of a composite of cardiovascular events (MI, stroke or all-cause death) compared with men who did not receive testosterone replacement therapy[22]. However, no adjustment was made for a difference in baseline serum testosterone levels, which were lower in men receiving testosterone therapy (175 ng/dl) than in men who did not receive testosterone (205 ng/dl). Considering these limitations, as well as the fact that this study included only men who were referred for coronary angiography, the findings of the analysis might not be applicable to the general population.

A subsequent study evaluated a large cohort of 55,593 men from an insurance database who were receiving intramuscular testosterone therapy and found that the risk of nonfatal MI in the 90 days following testosterone prescription was 36% higher than during the previous year and increased with age (119% higher in men aged ≥65 years, and 243% higher in men aged ≥75 years)[23]. Importantly, this study showed that the risk of nonfatal MI was higher even among younger men who had prevalent cardiovascular disease. Interestingly, among the men who did not refill their testosterone prescriptions, the risk of nonfatal MI decreased in the 91–180-day post-prescription interval, suggesting that this increased risk was acute and probably due to testosterone administration. In another large, case–control study, an increased risk of MI was associated with first-time exposure, defined as the first testosterone prescription in the past 90 days (RR 1.41, 95% CI 1.06–1.87), whereas current and past testosterone treatment were not associated with increased risk of MI[24]. An important limitation of this study was that the control individuals were not selected on the basis of low serum testosterone levels. By contrast, another study that evaluated 6,355 Medicare beneficiaries aged ≥66 years who were treated with at least one testosterone injection, matched with 19,065 control individuals, found that testosterone treatment was not associated with higher risk of MI (HR 0.84, 95% CI 0.69–1.02) and, indeed, that testosterone treatment was beneficial in men at greatest risk of MI (HR 0.69, 95% CI 0.53–0.92)[31]. Similar findings were observed in the RHYME cohort[70].

A study on the effect of cumulative exposure to testosterone therapy in 10,311 men (median follow-up

Table 3 | **Retrospective studies on testosterone replacement therapy and risk of cardiovascular events and death**

Study (year)	Number of patients (controls)	Age (years)	Hypogonadism criteria	Cohort	Follow-up duration	Findings	Refs
Increased risk							
Vigen et al. (2013)	1,223 (7,486)	63.4	TT <300 ng/dl	Men with hypogonadism who underwent coronary angiography in the VA system	27.5 months	Increased risk of adverse outcomes (all-cause death, MI and ischaemic stroke) in men receiving TTh (HR 1.29, 95% CI 1.05–1.58)	22
Finkle et al. (2014)	55,593 (141,031)	54.3	Not available	Men receiving TTh or PDE5 inhibitor	22–25 months	The rate of nonfatal MI in the 90 days following TTh initiation was higher than in the year before TTh initiation (RR 1.36, 95% CI 1.03–1.81)	23
Etminan et al. (2015)	30,066 (120,264)	45–80	Not available	Patients with new-onset acute MI and matched controls from an insurance database	2.8 years	Initiation of TTh in the past 90 days (but not overall TTh) associated with increased rate of MI (RR 1.41, 95% CI 1.06–1.87)	24
Martinez et al. (2016)	19,215 (909,530)	20–89	Not available	Men with confirmed VTE and age-matched controls	NA	Increased risk of VTE during the first 6 months of TTh (RR 1.63, 95% CI 1.12–2.30), but overall risk was not increased	25
No association							
Baillargeon et al. (2015)	7,643 (22,929)	≥40	Not available	Men with confirmed VTE and matched controls	NA	TTh in the 15 days before the event or index date was not associated with increased risk of VTE (aOR 0.90, 95% CI 0.73–1.12)	26
Li et al. (2016)	2,785 (11,119)	≥18	Not available	Men with confirmed VTE and matched controls	NA	TTh not associated with increased risk of VTE for current exposure (aOR 1.02, 95% CI 0.92–1.13) or past exposure (aOR 0.92, 95% CI 0.82–1.03) to TTh	27
Maggi et al. (2016)	750 (249)	≥18	TT lower than local laboratory reference range	Men with hypogonadism from the RHYME registry	2–3 years	TTh not associated with increased risk of CV events (DVT, VTE, AF, MI, RPs or stroke) (RR 1.18, 95% CI 0.57–2.44)	70
Sharma et al. (2016)	38,362 normal TT with TTh (10,854 not treated)	64	TT lower than local laboratory reference range	Men with hypogonadism in the VA system	6.1	TTh not associated with increased risk of VTE (HR 1.1, 95% CI 0.78–1.54)	28
Reduced risk							
Shores et al. (2012)	398 (633)	>40	TT ≤250 ng/dl	Men with hypogonadism in the VA system	40.5 months	Reduced mortality in men receiving TTh (HR 0.61, 95% CI 0.42–0.88)	29
Muraleedharan et al. (2013)	64 (174)	59.5	TT ≤300 ng/dl	Men with hypogonadism and type 2 diabetes mellitus	5.8 years	Increased mortality in men not receiving TTh (HR 2.3, 95% CI 1.3–3.9)	30
Baillargeon et al. (2014)	6,355 (19,065)	≥66	Not available	Medicare beneficiaries treated with intramuscular testosterone and matched controls	1,495 days	Reduced risk of MI in men receiving TTh and in the highest quartile of the MI prognostic score (HR 0.69, 95% CI 0.53–0.92)	31
Sharma et al. (2015)	40,852 normal TT with TTh (11,957 not treated)	66	TT lower than local laboratory reference range	Men with hypogonadism in the VA system	6 years	Lower risk of all-cause death (HR 0.44, 95% CI 0.42–0.46), MI (HR 0.76, 95% CI 0.63–0.93) and stroke (HR 0.64, 95% CI 0.43–0.96) in men with normalized TT after TTh versus men not receiving TTh	32
Tan et al. (2015)	19,968 (821,725 from MI registry; 117,000 from stroke registry)	20–86	TT <350 ng/dl	Men with hypogonadism who were receiving TTh and matched controls from stroke and MI registries	24.7 months	Reduced risk of MI and stroke in men receiving TTh versus men not receiving TTh	33

Table 3 (cont.) | **Retrospective studies on testosterone replacement therapy and risk of cardiovascular events and death**

Study (year)	Number of patients (controls)	Age (years)	Hypogonadism criteria	Cohort	Follow-up duration	Findings	Refs
Reduced risk (cont.)							
Anderson et al. (2016)	2,241 with normal TT (801 with persistent low TT; 1,694 with high TT)	≥50	TT <212 ng/dl	Men with hypogonadism who were receiving TTh	3 years	Lower 3-year risk of MACE (driven mainly by lower all-cause death: HR 0.65, 95% CI 0.47–0.90), but not MI or stroke, in men with normalized TT levels versus men with persistent low TT level	34
Wallis et al. (2016)	10,311 (28,029)	≥66	Not available	Men receiving TTh and matched controls	5.3 years	Lower all-cause mortality (HR 0.88, 95% CI 0.84–0.93) in men receiving TTh	35
Oni et al. (2017)	8,137 with normal TT on TTh (4,418 with non-normalized TT on TTh)	67	TT lower than local laboratory reference range	Men with hypogonadism in the VA system	6 years	Lower risk of all-cause death (HR 0.53, 95% CI 0.48–0.58) and MI (HR 0.72, 95% CI 0.52–0.99), but not stroke, in nonsmokers with normalized TT level after TTh	36
Cheetham et al. (2017)	8,808 (35,527)	≥40	Coded diagnosis and/or TT <300 ng/dl	Men with hypogonadism	4.2 years	Reduced risk of CV events (acute MI, coronary revascularization, unstable angina, stroke, transient ischaemic attack and sudden cardiac death) in men receiving TTh (HR 0.67, 95% CI 0.62–0.73)	37
Sharma et al. (2017)	40,856 normal TT level with TTh (11,853 not treated)	66	TT lower than local laboratory reference range	Men with hypogonadism in the VA system	6 years	Reduced risk of AF in men with normal TT level after TTh versus untreated men (HR 0.79, 95% CI 0.70–0.89)	38

AF, atrial fibrillation; aOR, adjusted odds ratio; CV, cardiovascular; DVT, deep-vein thrombosis; MACE, major cardiovascular adverse events; MI, myocardial infarction; NA, not applicable; PDE5, phosphodiesterase type 5; RPs, reperfusion procedures (stenting, CABG surgery or percutaneous coronary intervention); RR, relative risk; TT, total testosterone; TTh, testosterone therapy; VA, Veterans Affairs; VTE, venous thromboembolism.

duration 5.3 years) compared with 28,029 matched control individuals showed that testosterone treatment was associated with decreased mortality (HR 0.88, 95% CI 0.84–0.93), particularly in men with longer exposure (median therapy duration 35 months; HR 0.67, 95% CI 0.62–0.73)[35]. In addition, men with longer exposure to testosterone therapy had slightly lower incidence of major cardiovascular events (MI, cerebrovascular accident or venous thromboembolism) than control individuals (HR 0.84, 95% CI 0.72–0.98). By contrast, the risk of cardiovascular events was increased in men with short-term exposure to testosterone therapy (median therapy duration 60 days) compared with controls (HR 1.26, 95% CI 1.09–1.46)[35], confirming the results of other studies[23,24]. These reports indicate that although the risk of cardiovascular events might be higher soon after initiation of testosterone therapy, the risk is attenuated with longer duration of treatment.

Most of the above-mentioned retrospective studies did not report on-treatment serum testosterone concentrations, and consequently, adequacy of treatment could not be assessed. However, one study did evaluate differences in cardiovascular outcomes between men who achieved therapeutic on-treatment serum testosterone concentrations and men who continued to have persistently low on-treatment testosterone levels[32]. The study found that men with persistently low on-treatment testosterone levels had an increased risk of all-cause death, MI and stroke compared with men who achieved therapeutic serum testosterone levels[32]. Moreover, the risk of stroke and MI in men with persistently low on-treatment serum testosterone levels was similar to that in men who did not receive any treatment[32], findings that have been confirmed in other studies[34,36].

The type of testosterone formulation has been proposed to have an influence on cardiovascular outcomes[71]. Among 544,115 men aged ≥18 years who were initiating testosterone replacement therapy, those receiving intramuscular injections were at increased risk of cardiovascular events (MI, unstable angina and stroke; HR 1.26, 95% CI 1.18–1.35), hospitalizations (HR 1.16, 95% CI 1.13–1.19) and death (HR 1.34, 95% CI 1.15–1.56) compared with men receiving transdermal testosterone gel[71]. This study also did not report on-treatment serum testosterone concentrations (an important limitation), although it is possible (but not verifiable) that men on intramuscular testosterone might have achieved supraphysiological circulating testosterone concentrations.

In summary, these retrospective, prescription database studies provide useful information on the cardiovascular effects of testosterone replacement therapy (TABLE 3). However, because of the inherent design limitations of retrospective studies, making

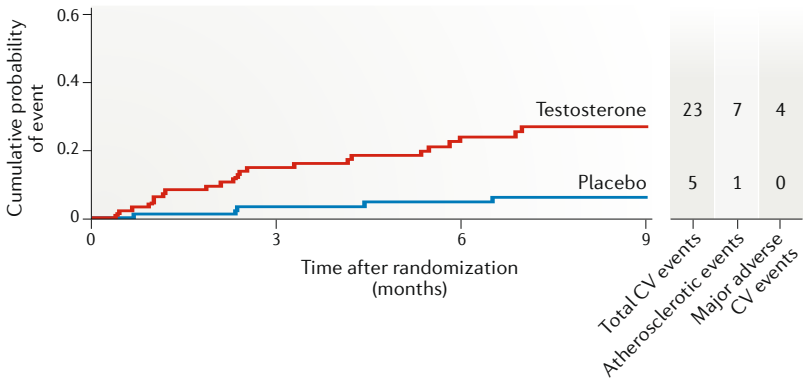

Fig. 1 | **Time-to-event analysis of cardiovascular adverse events in the TOM trial.** The figure depicts Kaplan–Meier estimates of the cumulative probability of incident cardiovascular (CV) adverse events in the TOM trial[39] from randomization to the end of the planned observation phase (9 months after randomization) for the testosterone and placebo groups ($P < 0.001$), which show the early divergence of the curves for each treatment group weeks after randomization. Data are obtained from REF.[39].

firm conclusions about the cardiovascular safety of testosterone therapy is not possible.

Randomized, controlled trials

To date, no published clinical trials have been sufficiently powered to evaluate differences in cardiovascular event rates in individuals receiving testosterone replacement therapy versus placebo. Furthermore, the reporting of adverse events in most trials of testosterone replacement therapy has not followed a standardized format[72]. In this section, we summarize clinical trials of testosterone replacement therapy that reported cardiovascular events and large clinical trials with atherosclerosis progression as the primary outcome.

The Copenhagen Study Group trial. A Danish study published in 1986 that evaluated the effects of testosterone therapy on survival in men with alcoholic cirrhosis was one of the first studies to report an increase in mortality with testosterone treatment. In this study, 221 men received either a daily 600 mg dose of micronized testosterone or placebo[73]. The trial was stopped prematurely (median follow-up duration 28 months) owing to a 17% higher mortality in the testosterone group than in the placebo group. However, only one death was attributed to a cardiovascular event (MI).

The TOM trial. The results from the TOM trial[39] were published in 2010 after the trial was stopped prematurely by the Data and Safety Monitoring Board of the trial because of a higher incidence of cardiovascular-related events in the testosterone group than in the placebo group. The trial included men aged ≥65 years with low levels of total testosterone or free testosterone in the serum and who had mobility limitation. Men were randomly assigned to receive transdermal testosterone gel 100 mg or placebo gel daily for 6 months. A total of 23 cardiovascular-related events occurred in the testosterone group, whereas five events occurred in the placebo group. These events were diverse, included both atherosclerotic and non-atherosclerotic events and were evident within weeks of treatment initiation. Only men in the testosterone group had major adverse cardiovascular events (MACE; a composite of MI, stroke or death related to cardiovascular disease), which included MI in two men, stroke in one man and one death[39]. Men receiving testosterone therapy who experienced cardiovascular events had higher on-treatment serum levels of total testosterone than men who did not have cardiovascular events[39]; secondary analyses demonstrated that these events were associated with changes in serum levels of free testosterone[74].

Of note, the participants in the TOM trial[39] had a high prevalence of comorbidities at baseline: >80% had hypertension, 50% were obese, 25% had diabetes mellitus, and nearly half had pre-existing cardiac disease. This high burden of comorbidities is common in men with frailty[75,76]. In addition, the starting dose of testosterone gel in this trial was higher than the starting dose used in clinical practice. Nonetheless, mean on-treatment serum testosterone levels in the TOM trial[39] participants were similar to serum testosterone concentrations in older men participating in other trials that did not report a higher incidence of cardiovascular events with testosterone treatment.

The diverse nature of cardiovascular-related events reported in the TOM trial suggests that these events were not due to a single mechanism. Furthermore, the rapid divergence (within weeks of treatment initiation) in the frequency of events between the two groups (FIG. 1) suggests that an acute mechanism might be involved in these events. These observations were subsequently confirmed by retrospective studies[23,24].

The TEAAM trial. The TEAAM trial[77] evaluated the effects of testosterone therapy on progression of subclinical atherosclerosis in the common carotid artery (assessed by sonographic measurement of common carotid intima–media thickness) and the coronary arteries (assessed by measurement of coronary artery calcium score by CT scan). The study enrolled 308 men aged ≥60 years with 100–400 ng/dl total testosterone or <50 pg/ml free-testosterone concentration in serum. Men were treated for 3 years with either 75 mg of testosterone gel or placebo gel. After a 3-year intervention, testosterone treatment was not associated with progression of carotid intima–media thickness[77]. In addition, coronary artery calcium scores did not differ between groups, suggesting that progression of calcified coronary plaques was not influenced by testosterone treatment (FIG. 2). Given that atherosclerosis progression is slow, the 3-year intervention period might have been insufficient to determine differences in atherosclerosis progression between the two groups. Few participants experienced cardiovascular events, and the incidence of MACE was similar in the two groups: coronary revascularization was required in five men in the testosterone group and in two men in the placebo group; MI occurred in three men in the testosterone group and in two men in the placebo group; stroke occurred in three men in the testosterone group versus none in the placebo group; and one participant died from a cardiovascular-related event in the testosterone group versus none in the placebo group[77].

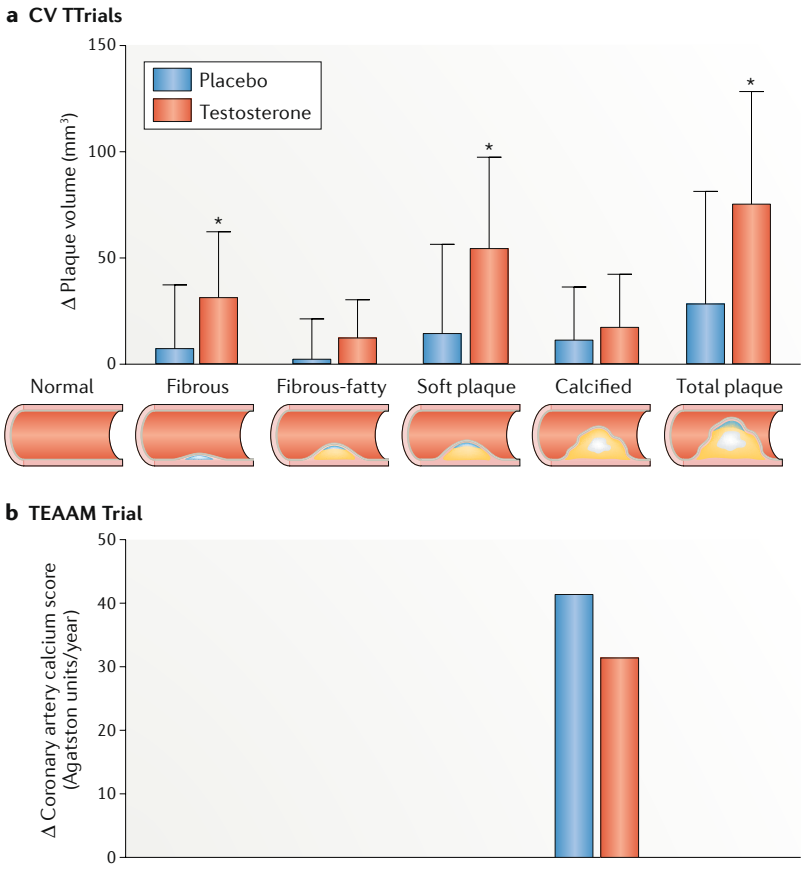

Fig. 2 | **Effects of testosterone treatment on coronary artery plaques in clinical trials.** **a** | Changes from baseline in the volume of coronary artery plaques in the cardiovascular substudy of the TTrials[79] (CV TTrials). The drawings below the graph depict anatomical representations of the plaque subtypes. Compared with placebo, testosterone treatment for 12 months induced a significant increase in fibrous, soft (noncalcified) and total coronary artery plaque volume, as assessed by coronary CT angiography, whereas no significant treatment effect was seen for fibrous-fatty or calcified coronary artery plaque volume. Bars are mean change from baseline to 12 months with testosterone therapy and placebo adjusted for baseline total testosterone level in the serum (≤200 ng/dl or >200 ng/dl), age (≤75 years or >75 years), trial site, participation in the main TTrials, use or nonuse of antidepressants and use or nonuse of phosphodiesterase type 5 inhibitors. Error bars are 95% CI. Data are derived from REF.[79]. *Significant difference ($P < 0.05$) determined by a linear mixed model with all balancing factors and baseline outcome value as covariates and a random effect for participant. **b** | Changes from baseline in coronary artery calcium scores in the TEAAM trial[77]. Compared with baseline, no significant difference was observed between treatment groups in mean estimated yearly change in coronary artery calcium scores, as assessed with multidetector-row CT over 3 years of treatment. Estimates are derived from mixed-effects regression models supplemented by multiple imputation of missing records; 95% CIs were not available. Data are obtained from REF.[77].

The TTrials. The TTrials[8,78–81] were a set of seven, highly coordinated, multicentre, double-blind, placebo-controlled trials that evaluated the effects of testosterone replacement therapy on sexual function, physical function, vitality, anaemia, bone mass, cognition and coronary artery plaque volume (cardiovascular substudy). In the parent trial, 790 men aged ≥65 years with average serum total testosterone level <275 ng/dl (from two measurements) were randomly assigned to receive either a daily dose of 50 mg transdermal testosterone or a placebo gel for 1 year[8]. In the cardiovascular substudy of the TTrials, 138 participants underwent a CT angiography scan to evaluate the progression of non-calcified and calcified coronary artery plaque volume (primary outcome) and coronary artery calcium score (secondary outcome)[79]. After 1 year of intervention, testosterone treatment was not associated with progression of calcified plaque compared with placebo; however, noncalcified plaque volume significantly increased in the testosterone group compared with the placebo group[79] (FIG. 2). The clinical implications of this finding remain unclear. Importantly, no difference in the number of cardiovascular events between the two groups was observed; seven participants in each group experienced a MACE (MI, stroke or death related to cardiovascular disease)[8].

Trials on testosterone as a male contraceptive. Many trials have evaluated the efficacy of testosterone therapy as a male contraceptive in young men. Although some of these trials used doses of testosterone that resulted in supraphysiological serum testosterone concentrations, most studies administered doses that maintained serum total testosterone levels within the normal range for young, healthy men[82–113]. Routes of administration included intramuscular injections[82–101], insertion of subcutaneous pellets[102–110] and transdermal gels and patches[101,111–113]. Treatment duration ranged from 20 days to 30 months. None of these studies reported occurrence of MACE during treatment; however, these studies were not designed to assess cardiovascular events. In addition, most studies also co-administered a progestin to improve suppression of spermatogenesis, which could itself affect the risk of cardiovascular disease. Of note, testosterone treatment resulted in a reduction in serum HDL-cholesterol concentration in some studies[90,93,98,101,110,112].

Meta-analyses of randomized trials

Several meta-analyses of randomized, controlled trials of testosterone replacement therapy have been published in which the researchers have attempted to elucidate the association between testosterone administration and cardiovascular events by pooling the number of reported adverse events (FIG. 3). However, these meta-analyses included trials that were mostly of low-to-medium quality, enrolled men with heterogeneous characteristics, used different testosterone doses and formulations and had variable treatment durations. Most importantly, none of these trials was adequately powered to evaluate cardiovascular events. Consequently, the contradictory findings of these meta-analyses are difficult to reconcile.

The first meta-analysis that evaluated cardiovascular events in randomized, controlled trials of testosterone therapy was published in 2005 (REF.[114]). This analysis pooled 19 trials of men aged ≥45 years with low or low–normal serum testosterone levels at baseline and treatment duration of ≥90 days. The pooled analyses found 18 cardiovascular events (including MI in four men and stroke in three men) among 651 men treated with testosterone therapy, and 16 events were observed among 433 men receiving placebo (including MI in three men and stroke in four men)[114]. Testosterone treatment was not associated with an increased rate of cardiovascular

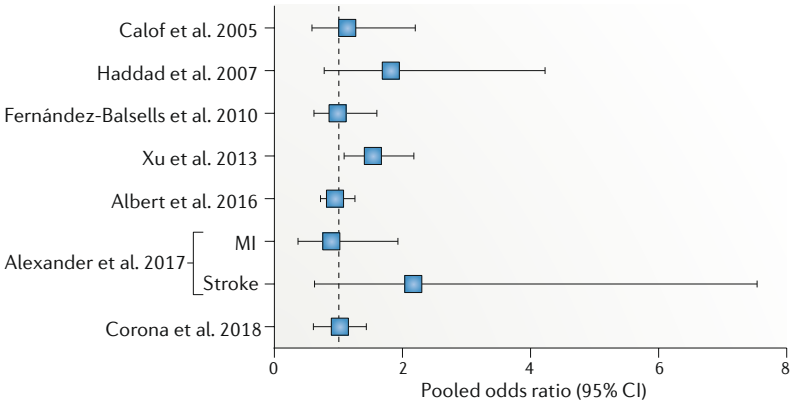

Fig. 3 | **Meta-analyses of clinical trials of testosterone replacement therapy.** The graph depicts the pooled odds ratio of composite cardiovascular events from meta-analyses of clinical trials of testosterone replacement therapy, with the exception of the study by Alexander et al.[118], which reported myocardial infarction (MI) and stroke as separate outcomes. The definition of composite cardiovascular events varied across studies, but all included MI, angina, coronary revascularization and stroke. In addition, the report by Calof et al.[114] included atrial fibrillation, sudden death and other vascular events; Haddad et al.[115] included cardiovascular death and claudication; Fernández-Balsells et al.[116] included death, peripheral vascular events, changes in blood lipid fractions, changes in fasting glucose level, new onset of diabetes mellitus and hypertension; Xu et al.[40] included any events reported by the researchers as cardiac disorders, cardiovascular complaints, cardiovascular events and vascular disorders; Albert et al.[117] included death, syncope, arrhythmia and hospital admission for congestive heart failure; and Corona et al.[119] included cardiovascular-related death and heart failure reported as serious adverse events.

events (OR 1.14, 95% CI 0.59–2.20). However, this meta-analysis was limited by the inclusion of fairly young men. A subsequent study evaluated cardiovascular events in six clinical trials and found that 14 cardiovascular events occurred among 161 men in the testosterone group, whereas seven events occurred among 147 men receiving placebo (OR 1.82, 95% CI 0.78–4.23)[115]. The odds ratio for fatal and nonfatal MI was 2.24 (95% CI 0.50–10.02). Although a higher rate of cardiovascular events was observed in men receiving testosterone, this difference was not significant[115].

A 2010 study analysed 51 randomized and nonrandomized trials that included 2,679 men and found no significant effect of testosterone therapy on mortality or any cardiovascular outcome (including arrhythmias, CABG surgery and MI)[116]. However, the included studies were considered to be of low or medium quality, and only nine studies reported cardiovascular events. By contrast, a subsequent meta-analysis found that testosterone therapy was associated with increased risk of cardiovascular events[40]. In this analysis, 180 cardiovascular-related events were observed in the 2,994 men studied in 27 trials. Testosterone administration was associated with a 54% increased risk of a cardiovascular-related event (OR 1.54, 95% CI 1.09–2.18). Interestingly, this risk was higher in trials that were not funded by the pharmaceutical industry (OR 2.06, 95% CI 1.34–3.17) than in industry-funded studies (OR 0.89, 95% CI 0.50–1.60)[40]. In 2016, an analysis of 45 clinical trials to evaluate the effect of age and testosterone formulation on cardiovascular events found that testosterone treatment was overall not associated with an increased risk of a cardiovascular event compared with placebo (RR 1.10, 95% CI 0.86–1.41)[117]. However, men aged ≥65 years had an increased risk of cardiovascular events (RR 2.90, 95% CI 1.35–6.21), which was evident mainly during the first 12 months of therapy. Interestingly, transdermal testosterone administration was associated with an increased risk of a cardiovascular event compared with placebo (RR 2.80, 95% CI 1.38–5.68), whereas intramuscular testosterone administration had no effect on the risk of a cardiovascular event (RR 0.96, 95% CI 0.46–1.98). These observations contrast with the findings of a retrospective study published in 2015, in which intramuscular administration was associated with a greater risk of a cardiovascular event than testosterone gel[71].

A meta-analysis of 30 randomized trials reported 69 cardiovascular events among 3,230 men who received testosterone therapy, whereas 53 events occurred in 2,221 men receiving placebo[118]. Testosterone treatment was not associated with an increased risk of MI (OR 0.87, 95% CI 0.39–1.93) or death (OR 0.88, 95% CI 0.55–1.41). Although the risk of stroke seemed to be higher with testosterone therapy than with placebo (OR 2.17, 95% CI 0.63–7.54), the difference was not significant[118]. A meta-analysis of 93 randomized trials of testosterone therapy in adult men found that testosterone treatment had no effect on the risk of MACE compared with placebo (OR 0.97, 95% CI 0.64–1.46)[119].

Although these meta-analyses provide useful information, most of the trials that were included were of low-to-medium quality, and none was sufficiently powered to evaluate cardiovascular events; therefore, the applicability of these analyses is limited.

Cardiovascular effects of testosterone

Several epidemiological studies have suggested that men are at higher risk of cardiovascular disease than women[120–122], and sex steroids have been suggested to contribute, at least in part, to this increased risk[123]. Over the past 5 decades, both preclinical studies and research in humans have produced a large body of data on the molecular mechanisms of the effects of testosterone on the cardiovascular system (FIG. 4) and how testosterone replacement therapy might modify the risk of cardiovascular disease.

Studies in vitro and in animals

Preclinical studies have assessed the effect of testosterone on various aspects of cardiovascular biology.

Atherosclerosis. Several studies in animals have shown that testosterone slows atheroma progression. Castrated rabbits fed a high-cholesterol diet show accelerated aortic atherosclerosis[124,125] that is reversed by testosterone administration[124,126,127]. Similarly, administration of 5α-dihydrotestosterone (DHT), a non-aromatizable metabolite of testosterone, also slows atherosclerosis progression[125], which suggests that aromatization to oestrogens is not required for this effect. In mice with testicular feminization (that is, mice with a non-functional androgen receptor and low endogenous testosterone levels) that were fed a cholesterol-rich diet for 28 weeks, testosterone replacement therapy reduced lipid deposition

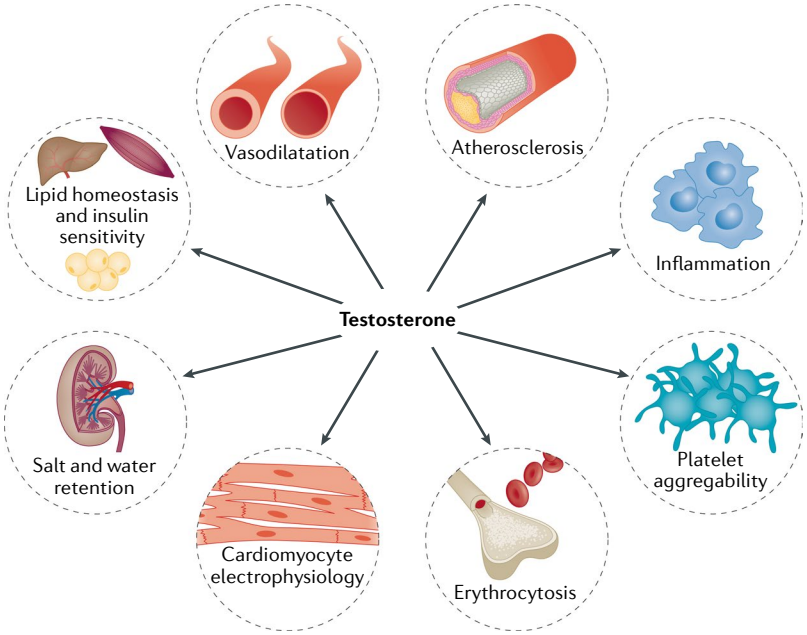

Fig. 4 | **Cardiovascular targets and effects of testosterone.** Studies in vitro, in animals and in humans have examined the effects of testosterone on various organs and processes. Some studies have shown that testosterone modulates vascular tone, increases erythropoiesis (which can induce erythrocytosis in some men) and affects platelet aggregability and cardiomyocyte electrophysiology and contractile activity. In addition, conflicting findings have been reported in studies evaluating the effect of serum levels of endogenous testosterone and testosterone therapy on atherosclerosis progression and inflammation and on metabolic parameters such as insulin sensitivity and lipid homeostasis; these metabolic effects probably involve complex interactions of the liver, skeletal muscle and adipose tissue.

within the aortic root, which was only partially reversed by co-administration of an aromatase inhibitor or an oestrogen receptor-α antagonist[128]. Similarly, knockout of *Ar* (which encodes the androgen receptor) accelerates atherosclerosis in apolipoprotein E-deficient mice[129]. These studies suggest that testosterone itself, rather than oestradiol, is the predominant hormone that inhibits atherogenesis. By contrast, testosterone or oestradiol treatment in castrated, *Ldlr*-knockout mice resulted in a similar magnitude of atheroma regression, and the protective effect of testosterone was lost when an aromatase inhibitor was co-administered, which suggests an important role of oestradiol[130]. Taken together, these studies suggest that androgens and oestrogens have independent roles in slowing progression of atherosclerosis. In addition, in a study in mice with testicular feminization that were fed a high-cholesterol diet, testosterone treatment reduced local inflammation within fatty streak areas of the aortic root[131].

In vitro studies of atherosclerosis have reported conflicting findings. Neointimal plaque development in cultured segments of the aortic ring from male rabbits after endothelial denudation was inhibited by testosterone treatment compared with untreated rings[132]. Testosterone also suppressed the tumour necrosis factor (TNF)-induced expression of vascular cell adhesion protein 1 (VCAM1) in human aortic endothelial cells[133] and human umbilical vein endothelial cells (HUVECs)[134]. Increased expression of VCAM1 facilitates attachment of leukocytes to endothelial surfaces, which is an important step in the initial development of atheroma[135–137]. By contrast, DHT treatment of HUVECs resulted in increased expression of VCAM1 and increased adhesion of human monocytes to endothelial cells, which were both blocked by an androgen-receptor antagonist[138]. Findings from these in vitro studies suggest that aromatization of testosterone to oestradiol is important in mediating the protective effects of testosterone on the expression of adhesion molecules[134]. In addition, binding of testosterone and DHT to the androgen receptor might elicit different receptor conformations that recruit different cofactors and result in distinct cellular responses.

Conflicting findings have also been reported in studies evaluating the effects of androgens on aortic smooth muscle cells. In human aortic smooth muscle cells, testosterone treatment inhibited inorganic phosphate-induced vascular calcification by androgen receptor-dependent transactivation of growth arrest-specific protein 6 (GAS6)[139], which is an important regulator of arterial calcification[140,141]. Conversely, in mouse aortic smooth muscle cells, testosterone and DHT treatment increased inorganic phosphate-induced vascular calcification, and calcification was 50% lower in *Ar*-knockout mice than in wild-type mice[142].

Lipid metabolism. Preclinical data suggest that testosterone has a substantial effect on cholesterol and lipid metabolism. In the HepG2 hepatocyte cell line, testosterone treatment induced a dose-dependent increase in scavenger receptor class B member 1 (SRB1) expression, an important protein that regulates cholesterol uptake by the liver from circulating HDL[143]. A similar effect was also observed in castrated, obese mice treated with DHT[144]. In addition, DHT treatment decreased LDL secretion and the activity of cholesterol 7α-hydroxylase, a crucial enzyme for bile formation and cholesterol removal[144]. These findings suggest that testosterone replacement therapy reduces the concentration of serum cholesterol and LDL by increasing hepatic uptake of cholesterol and suppressing cholesterol excretion.

Testosterone influences the activity of hepatic lipase, an enzyme that removes phospholipids and triacylglycerols from lipoprotein particles. In old, eugonadal men treated with testosterone (600 mg per week) for 3 weeks, hepatic lipase activity increased by 66% from baseline, and HDL-cholesterol level (in particular, subclass 2 and 3) was significantly reduced compared with placebo[145]. Similarly, hepatic lipase activity also increased, and HDL-cholesterol concentrations decreased compared with baseline in men with hypogonadism who were receiving 250 mg of testosterone enanthate every 4 weeks for 12 weeks[146]. This effect of testosterone therapy on hepatic lipase activity is considered to be the main mechanism by which testosterone reduces serum HDL concentrations in some clinical trials. Although this reduction in HDL concentrations is of concern, studies evaluating the effect of testosterone on cholesterol efflux from macrophages in vitro (a metric of HDL function[147]) found that HDL function does not change significantly in response to androgen therapy[148,149].

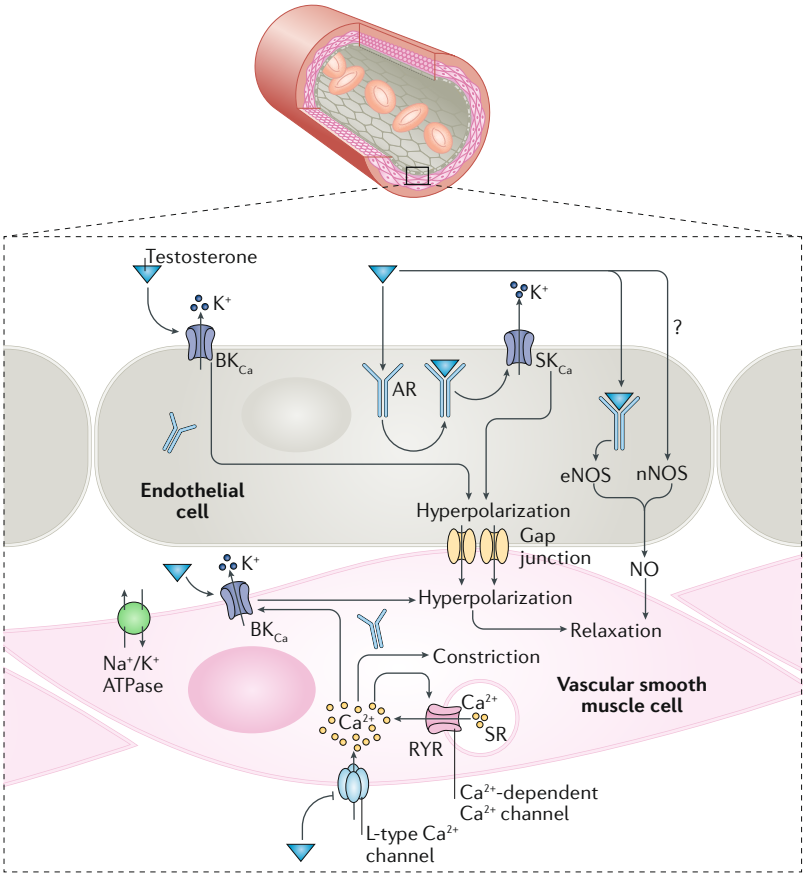

Fig. 5 | **Molecular mechanisms of testosterone modulation of vascular tone.** Testosterone has effects in both vascular endothelial cells and vascular smooth muscle cells, which together produce changes in vascular tone. Testosterone relaxes the vascular smooth muscle by inhibiting the L-type calcium current through voltage-dependent L-type calcium channel subunit α1C ($Ca_v1.2$), independently of the vascular endothelium or the androgen receptor (AR). Testosterone also induces vascular smooth muscle relaxation via the opening of big-conductance calcium-activated and voltage-activated potassium channels (BK_{Ca}) in vascular smooth muscle cell as well as in endothelial cells. In addition, testosterone induces nitric oxide (NO) synthesis in the endothelial cell by endothelial nitric oxide synthase (eNOS) via an AR-dependent, non-transcriptional mechanism; neuronal nitric oxide synthase (nNOS) in the endothelium might also contribute to the testosterone-induced increase in endothelial NO synthesis. Involvement of small-conductance calcium-activated potassium channels (SK_{Ca}) in addition to BK_{Ca} in the endothelium-dependent vasodilatory effects of testosterone has also been suggested. RYR, ryanodine receptor; SR, sarcoplasmic reticulum.

Blood viscosity and platelet function. Testosterone has an important role in the regulation of erythropoiesis in mammals[150–153]. Indeed, excessive erythrocytosis is the most common adverse effect of testosterone replacement therapy in men[2]. This testosterone-induced erythrocytosis is associated with an increase in blood viscosity in both mice and rabbits[154,155], which results in augmentation of blood-flow resistance. Although an increased number of red blood cells might be considered beneficial because it increases the oxygen-transporting capacity, above an erythrocyte concentration threshold, any beneficial effect is off-set by diminished blood flow[156]. Furthermore, in vitro studies have shown a direct correlation between haematocrit and platelet aggregation, which suggests that in addition to reduced blood flow, increased red blood cell numbers also increase the risk of thrombosis[157]. In addition, testosterone treatment directly augments ex vivo platelet aggregability by increasing thromboxane A2 receptor density on human platelets[158]; conversely, both maximum platelet aggregation response and thromboxane A2 receptor density were lower in old, castrated men than in uncastrated men[159]. These data suggest that testosterone might have prothrombotic effects.

Endothelial function. Several studies in vitro and in animals showed that testosterone therapy induces vasodilatation via various mechanisms (FIG. 5). In endothelium-stripped coronary arteries and aortas from adult male rabbits, testosterone induced vasodilatation, suggesting that the vascular action of testosterone is endothelium-independent[160]. This vasodilatation was not attenuated by inhibition of aromatase or nitric oxide synthase (NOS) and was also not blocked by androgen receptor antagonists but was attenuated by a nonspecific inhibitor of potassium channels, suggesting that opening of membrane voltage-sensitive potassium channels (K_V) and calcium-sensitive potassium channels (K_{Ca}) is involved in mediating testosterone-induced vasorelaxation[160]. Both testosterone and DHT treatment induce endothelium-independent relaxation of pig isolated coronary arteries via the opening of the big-conductance calcium-activated and voltage-activated potassium channels (BK_{Ca})[161]. By contrast, data from other studies suggest that this vasodilatory effect of androgens is mediated in part by nitric oxide (NO)-dependent and endothelium-dependent pathways[162]. Intracoronary administration of testosterone in dogs increases coronary blood flow, which is partially reduced by pretreatment with a nonspecific NOS inhibitor[163]. Neuronal NOS has been suggested to have a role in the vasodilatory effect of testosterone[164]. In conscious rats with blocked ganglia, intravenous administration of testosterone induced a dose-dependent hypotensive response that was abolished by pretreatment with a neuronal NOS inhibitor[164]. However, given that neuronal NOS is also expressed in endothelial cells[165], a contribution by the endothelium cannot be excluded. This role of NO in vasorelaxation has also been demonstrated in pig mesenteric, renal and iliac arteries[166].

Physiological concentrations of testosterone potently inhibit voltage-dependent L-type calcium channel subunit α1C ($Ca_v1.2$) expressed in HEK293 human embryonic kidney cells by a mechanism similar to that of dihydropyridine calcium-channel blockers[167]. This antagonism of the activity of calcium channels by androgens was later shown to be independent of the vascular endothelium and the androgen receptor[168]. Physiological levels of testosterone and DHT also induce NO synthesis in human aortic endothelial cells in vitro by an androgen receptor-dependent mechanism[169], and this effect is not abrogated by pretreatment with an aromatase inhibitor or by transfection with a small interfering RNA targeting *ESR1* (encoding the oestrogen receptor 1)[169,170]. Whole-cell, patch–clamp studies also demonstrated an involvement of small-conductance calcium-activated potassium channels in addition to

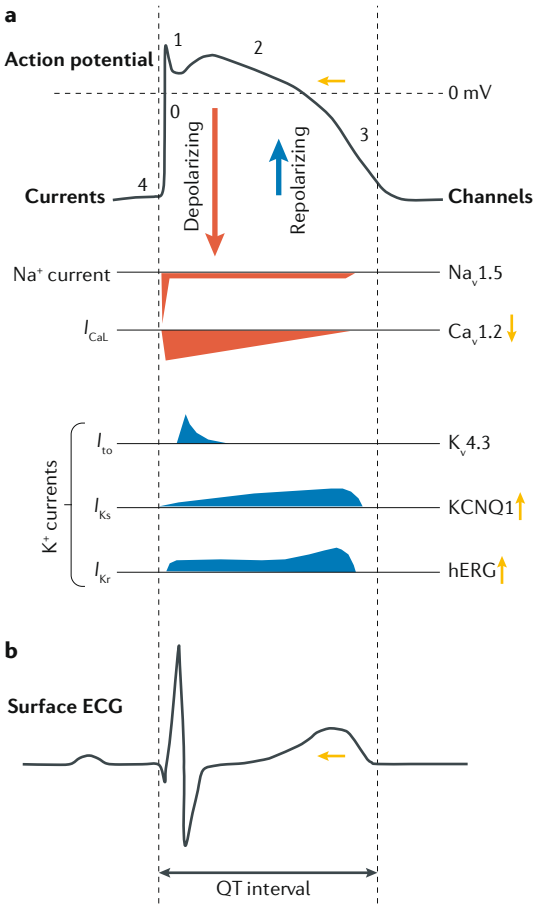

Fig. 6 | **Effects of testosterone on cardiac electrophysiology. a** | Mechanisms by which testosterone affects the action potential of a ventricular cardiomyocyte. Testosterone has been shown to diminish the L-type calcium current (I_{CaL}) through voltage-dependent L-type calcium channel subunit α1C ($Ca_v1.2$) voltage-dependent calcium channels, shortening the plateau phase of the action potential (phase 2). In addition, testosterone increases the slowly (I_{Ks}) and rapidly (I_{Kr}) activating delayed rectifier potassium currents — corresponding to the function of voltage-gated potassium channel subfamily Q member 1 (KCNQ1) and human ether-a-go-go-related gene (hERG) potassium channel activities, respectively — accentuating phase 3 (rapid repolarization) of the action potential. Therefore, testosterone seems to shorten the total duration of the action potential by accelerating the recovery of the resting membrane potential (phase 4) without affecting phase 0 (fast depolarization; resulting from the opening of voltage-gated $Na_v1.5$ sodium channels), phase 1 (transient outward potassium current (I_{to}); through $K_v4.3$ potassium channels) or phase 4. The yellow arrows illustrate the resulting effect of testosterone on each current and on the action potential duration. **b** | Effect of testosterone on surface electrocardiogram (ECG). The cumulative effect of testosterone on the action potential of ventricular cardiomyocytes results in a shorter ventricular repolarization time, which can be seen in the ECG as a shorter corrected QT interval. Vertical dashed lines illustrate the chronological relationship between the action potential of a ventricular cardiomyocyte (part **a**) and the surface ECG (part **b**). Adapted with permission from REF.[268], Elsevier.

BK_{Ca} in the endothelium-dependent vasodilatory effects of testosterone in human coronary arteries[171].

Renal salt and water retention. Androgens have long been known to stimulate salt and water retention. In rats, androgens directly upregulate the proximal tubule renin–angiotensin–aldosterone system[172,173], increase the activity of the Na^+/H^+ exchanger[174] and increase renal mRNA and protein levels of epithelial sodium channel subunits, predominantly in the distal tubules and the collecting ducts[175]. In addition, testosterone increases transcript and protein levels of aquaporin 1 in rat nephrons[176]. These data indicate that androgens increase salt and water absorption by multiple mechanisms, which results in an expansion of extracellular volume.

Cardiomyocyte electrophysiology. Sex steroids influence cardiac electrophysiology, and clear sex-specific differences exist in various parameters of the electrocardiogram (ECG) — in particular, ventricular repolarization[177]. In isolated ventricular cardiomyocytes from guinea pigs, acute testosterone treatment shortens that action potential duration via a non-transcriptional, androgen receptor-mediated pathway by increasing slowly activating delayed rectifier potassium currents (I_{Ks}; an outward repolarizing current) and by inhibiting the inward depolarizing L-type calcium current (I_{CaL})[178]. However, chronic testosterone treatment of rat cardiomyocytes increases $Ca_v1.2$ density and I_{CaL} current through androgen receptor activation[179]. In addition, testosterone treatment increases the human ether-a-go-go-related gene (hERG; also known as KCNH2) potassium channel current, the rapid component of the rapidly activating delayed rectifier potassium current (I_{Kr}; an outward repolarizing current), via androgen receptor activation[180]. On the basis of these data, testosterone is thought to facilitate cardiomyocyte repolarization, which results in the shortening of the action potential duration (FIG. 6).

Cardiac contractility. Testosterone has been shown to affect cardiac contractility and relaxation. In isolated ventricular cardiomyocytes from rats, acute exposure in vitro (24 h) to a high concentration of testosterone (1 μmol/l) increased the rate of cardiomyocyte relaxation[181]. In addition, in cardiomyocytes isolated from rats 2 weeks after orchiectomy (the surgical removal of the testicles), maximum cardiomyocyte shortening was markedly reduced and relaxation substantially delayed compared with cardiomyocytes from control rats or from orchiectomized rats that were treated with physiological doses of testosterone[182]. Similar findings were obtained when orchiectomy was carried out 16 weeks earlier[183]. Interestingly, studies in rat Langendorff-perfused hearts show that short-term (9 weeks) testosterone deficiency induced by orchiectomy does not affect left ventricular function[184]. By contrast, longer periods of testosterone withdrawal (16 weeks) substantially impaired cardiac contractility, which was restored by testosterone treatment[185]. Physiological levels of testosterone increase positive inotropic response and myocardial relaxation to stimulation of α_1-adrenergic receptor and β_1-adrenergic

receptor via the androgen receptor[184]. Testosterone-induced increased cardiomyocyte contractility and faster relaxation are thought to be mediated through changes in cardiomyocyte calcium handling[182], mainly by increasing calcium release via the ryanodine receptor and by more rapid calcium clearance from the cytosol by increasing sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) activity[184,186].

Studies in humans

Coronary arteries. Some studies in humans have reported the testosterone-induced vasodilatation that was observed in animal studies. A 1977 placebo-controlled study in 50 men with ST-segment depression on baseline exercise testing who were randomly assigned to either 200 mg per week intramuscular testosterone cypionate or placebo for 8 weeks demonstrated a 51% reduction in the sum of ST-segment depression with testosterone treatment[187]. This result suggests that testosterone reduces ischaemia, probably by causing coronary vasodilatation. In a subsequent, small study, intracoronary administration of supraphysiological doses of testosterone in 13 men with angiographically proven coronary artery disease resulted in vasodilatation[188]. In another small, placebo-controlled trial of 46 men with a history of stable angina, application of a 5 mg transdermal testosterone patch for 12 weeks improved the time to 1 mm ST-segment depression on the exercise treadmill test[189]. Similar beneficial effects of testosterone on myocardial ischaemia were reported in a placebo-controlled study of 15 men, seven of whom received intramuscular testosterone undecanoate for 12 months[190]. In addition to beneficial changes in ECG parameters, testosterone has also been shown to improve myocardial perfusion. In a randomized, controlled trial of 22 men with coronary heart disease who were treated with oral testosterone undecanoate for 8 weeks, a modest increase in myocardial perfusion in the region supplied by unobstructed coronary arteries was detected by MRI[191]. This finding that testosterone is a coronary vasorelaxant complements observations that androgen deprivation therapy in men with prostate cancer results in increased stiffness of large arteries[192,193]. Taken together, these findings suggest that testosterone might have beneficial effects on the coronary vasculature; however, these trials enrolled small numbers of participants, and some studies used routes of testosterone administration that are not practical in clinical practice (such as intracoronary infusion); therefore, these findings should be interpreted with caution.

Fluid retention. The results of studies in humans have confirmed the finding in animals that testosterone administration results in salt and water retention and expansion of extracellular water volume[194]. Whereas pressure natriuresis limits volume overload in healthy men, men with underlying renal or cardiac disease might not be able to excrete excess water efficiently, thereby predisposing them to fluid overload. Indeed, some clinical trials of testosterone therapy in old men have reported exacerbation of congestive heart failure[39,74].

Cardiac electrophysiology. Human studies on the effects of testosterone therapy on cardiac electrophysiology complement the data from animal studies (FIG. 6). Male and female newborn babies have a similar corrected QT (QTc) interval[195], and this similarity remains until the age of 10 years[196]. However, after puberty, the QTc interval is significantly shorter in boys than in girls[197], which suggests that testosterone has a direct effect on the cardiac conduction system. In 2,942 men who were enrolled in the Third National Health and Nutrition Examination survey, serum levels of endogenous testosterone were negatively correlated with the QTc interval[198]. Similarly, evaluation of ECGs in 2,755 men participating in the Health 2000 study showed that men with an early repolarization pattern with a rapidly ascending ST segment had higher serum levels of endogenous testosterone than men without this pattern[199]. Indeed, the age-related prolongation of the QTc interval in men has been suggested to be influenced, at least in part, by the age-related decline in serum testosterone levels[200]. Secondary analyses of randomized trials of testosterone administration reported that testosterone treatment results in shortening of the QTc interval duration in community-dwelling men[201] and men with chronic heart failure[202]. The influence of testosterone on the cardiac conduction system was further confirmed by a study showing that androgen deprivation therapy in men with prostate cancer was associated with significant prolongation of the QTc interval[203]. Prolongation of the QTc interval is associated with increased risk of ventricular tachyarrhythmias (in particular, torsades de pointes) and sudden cardiac death[204–206]; therefore, these findings suggest that testosterone might have antiarrhythmic properties. Indeed, a study analysing data from the European pharmacovigilance database concluded that male hypogonadism was associated with torsades de pointes, which could be treated or prevented by testosterone replacement therapy[207].

Cardiac function. Although women have a higher ejection fraction at rest than men[208], men develop greater increments in ejection fraction in response to exercise[209,210]. In addition, testosterone replacement in older men attenuates the age-related decline in aerobic capacity[211,212].

A few studies have evaluated the effects of testosterone treatment in men with congestive heart failure. In a small trial evaluating the acute haemodynamic effects of testosterone in 12 men with moderate-to-severe left ventricular dysfunction, daily oral administration of 60 mg of testosterone for 2 days improved cardiac output, which was mediated, at least in part, by the testosterone-induced improvement in peripheral vascular resistance[213]. Some trials have evaluated the effect of chronic testosterone treatment on cardiac function in men with congestive heart failure. In a study of 76 men with a mean ejection fraction of 32.5%, treatment with a 5 mg transdermal testosterone patch for 12 months significantly improved functional capacity (as assessed by the incremental shuttle-walk test) compared with placebo[214]. In addition, 13 men from the testosterone group (35%) had an improvement of at

least one NYHA functional class compared with only three men in the placebo group (8%)[214]. These findings were corroborated by a 24-week study of 70 elderly men with congestive heart failure (NYHA class II or III) and left ventricular ejection fraction <40% who were randomly assigned to receive 1,000 mg of testosterone undecanoate or placebo[215]. Testosterone treatment significantly improved aerobic capacity, as assessed by the 6-min walking test[215], and improved arterial baroreflex sensitivity, which is commonly suppressed in patients with congestive heart failure and is associated with poor prognosis[216].

Atherosclerosis progression. Many population studies have evaluated the association of testosterone levels with indices of atherosclerosis. In the Tromsø study, low serum testosterone levels were inversely associated with carotid artery intima–media thickness in 1,482 men aged 25–84 years, even after adjustment for age and traditional cardiovascular risk factors[217]. At the 7-year follow-up of 1,101 of these men, no association was found between serum testosterone concentrations and progression of carotid intima–media thickness[218]. A smaller study that prospectively followed up 196 old men for 4 years found that serum free-testosterone concentration was inversely related to the progression of intima–media thickness of the common carotid artery[219]. The inverse association between testosterone and atherosclerosis progression seems to be stronger in older men who also have low-grade inflammation (serum levels of C-reactive protein (CRP) ≥2 mg/l)[220].

A few studies have evaluated the association between coronary and aortic atherosclerosis and serum testosterone levels[221–225]. In a small study of 90 men, 60 men with coronary artery disease, assessed by angiography, had lower bioavailable testosterone levels than healthy men, even after adjusting for age and BMI[225]. Bioavailable testosterone levels were also inversely associated with coronary calcium score in 105 middle-aged, non-obese, Korean men without a history of cardiovascular disease[222]. In a study of 803 men who had undergone elective coronary angiography, men with lower total testosterone levels had more severe coronary artery disease[221]. An inverse association between serum total testosterone levels and coronary and aortic calcification was reported for 1,654 community-dwelling men from the Offspring and Third Generation cohorts of the Framingham Heart Study, although this association was no longer significant after adjustment for cardiovascular risk factors[223]. An inverse association between testosterone concentration and peripheral artery disease has also been reported[226].

Although these population studies suggest that serum testosterone levels are inversely associated with atherosclerosis progression, findings from randomized, controlled trials do not support this association. In the TEAAM trial[77], testosterone treatment was not associated with changes in the carotid intima–media thickness (FIG. 2) or coronary artery calcium scores. Moreover, in the cardiovascular substudy of the TTrials[79], the volume of noncalcified plaques increased more in the group receiving testosterone therapy after a 12-month intervention than in the placebo group (FIG. 2). The conflicting findings of population studies and published clinical trials about testosterone and atherosclerosis progression necessitate that future trials be adequately powered to evaluate cardiovascular events.

Lipoprotein profile. Higher endogenous testosterone concentrations are associated with lower levels of total cholesterol, LDL cholesterol and triglycerides in the serum[227–229], although multiple studies have also reported an association with reduced levels of HDL cholesterol[227–230]. In clinical trials, the effects of testosterone replacement on circulating lipids have been inconsistent. In a randomized trial of 108 old men, transdermal testosterone treatment for 3 years had no effect on any lipoprotein[231]; similar findings were reported in the TEAAM trial[77]. A meta-analysis of 19 studies that evaluated the effects of intramuscular testosterone administration in 272 men concluded that testosterone replacement therapy was associated with reduced levels of total cholesterol and LDL cholesterol in the serum and a small decrease in HDL-cholesterol levels, whereas serum triglyceride levels were unchanged[232]. Similar findings were reported in the TOM trial[39] and the TTrials[233]. Given that testosterone treatment is associated with reductions in the levels of both 'bad' lipoproteins (total cholesterol, LDL cholesterol and triglycerides) and 'good' lipoproteins (HDL cholesterol), the relative contribution of these lipid changes to the risk of cardiovascular disease remains unclear. Furthermore, as previously discussed, testosterone does not seem to affect HDL function substantially[148,149]. In a trial in men with type 2 diabetes and/or metabolic syndrome and a serum total testosterone level ≤317 ng/dl, those with metabolic syndrome receiving testosterone therapy for 6 months had a mean reduction in lipoprotein(a) levels of 21% from baseline (−0.31 μmol/l, 95% CI −0.543 to −0.082 μmol/l) compared with men who received placebo[234]; the clinical relevance of these observations remains unclear.

Glucose metabolism. Higher endogenous serum testosterone levels in men have consistently been associated with a reduced risk of metabolic syndrome[235–237] and diabetes[238–240]. In addition, acute withdrawal of testosterone replacement therapy in men with hypogonadism reduces insulin sensitivity[241]. Similarly, men receiving androgen deprivation therapy for prostate cancer are also at increased risk of developing metabolic syndrome and diabetes[65,67,192,242–246]. These data suggest that testosterone modulates insulin sensitivity in men.

In cross-sectional studies, the association between endogenous testosterone levels and risk of incident diabetes is independent of adiposity[238,239], which suggests that a low testosterone level itself is a risk factor for diabetes. However, in a population-based, prospective cohort study, the increased risk of incident diabetes in men with a total testosterone concentration in the two lower quartiles was dependent on waist circumference[240], which suggests that the protective effects of testosterone might be mediated through its effects on central obesity. Another

cross-sectional study confirmed these findings[247]. In a study of 1,651 men aged 49–79 years[248], men with low testosterone levels were more likely to develop metabolic syndrome (OR 1.64, 95% CI 1.41–1.90) than those with normal testosterone levels, and the risk remained higher even after adjustments for age, lifestyle factors and sex hormone-binding globulin (SHBG) levels[248]. By contrast, another study found no association between total testosterone or free-testosterone level and insulin resistance or risk of incident diabetes[249]. Findings in randomized trials have also been inconsistent. Small mechanistic studies have showed improvement in insulin sensitivity with testosterone therapy, as assessed by the hyperinsulinaemic–euglycaemic clamp method[250,251], but randomized trials have obtained conflicting results, with some studies indicating that testosterone therapy has beneficial effects on insulin resistance[234,252] and others showing no effect[253,254]. In summary, the effect of testosterone replacement therapy on glycaemic parameters remains unclear.

Inflammation. Inflammation is a risk factor for cardiovascular disease[255,256], and pro-inflammatory markers, such as serum CRP and IL-6 levels, are prognostic of future cardiovascular events[257,258]. Furthermore, increased serum levels of TNF in asymptomatic men are also associated with clinical and subclinical cardiovascular disease[259]. Studies evaluating the association between endogenous testosterone concentrations and inflammatory markers have obtained conflicting results[260–266]; whereas some studies have shown an inverse association between serum concentrations of endogenous testosterone and serum IL-6 (REF.[260]) and CRP[260–264] levels, others did not confirm these findings[265,266]. In a small, randomized, crossover trial, testosterone replacement therapy reduced the serum levels of the pro-inflammatory cytokines TNF and IL-1β and increased the levels of an anti-inflammatory cytokine, IL-10, in serum[267], which suggests that testosterone therapy might have anti-inflammatory effects; however, these results are not supported by the findings of the TTrials[233].

The TRAVERSE trial

The TRAVERSE trial[41] is the first randomized, controlled trial that is adequately powered to evaluate the incidence of cardiovascular events with testosterone replacement therapy. Commencing in 2018, the investigators started to randomly assign a planned sample of approximately 6,000 men aged 45–80 years at high risk of cardiovascular disease and with a serum total testosterone level <300 ng/dl to receive either testosterone gel or placebo. The planned treatment duration is 5 years, and the primary end point is time to MACE (nonfatal MI, nonfatal stroke or death from cardiovascular causes). Secondary outcomes include time to occurrence of the composite cardiovascular end point (nonfatal MI, nonfatal stroke, death from cardiovascular causes or cardiac revascularization procedures including percutaneous coronary intervention and CABG surgery). The findings of this trial will provide more definitive evidence about the cardiovascular safety of testosterone replacement therapy.

Conclusions

Testosterone has an important role in cardiovascular physiology and metabolic health. Although epidemiological data are conflicting, some retrospective studies support a beneficial effect of testosterone replacement therapy on mortality, whereas other reports suggest that testosterone might increase the risk of serious cardiovascular events, a finding that is also supported by some randomized trials. Meta-analyses also report conflicting results and are limited by the inclusion of low-to-medium quality trials. Furthermore, to date, no published trials of testosterone replacement therapy have been adequately powered to assess cardiovascular events. Therefore, the TRAVERSE trial[41] will go a long way towards enabling an assessment of the cardiovascular safety of testosterone therapy. In the interim, clinicians should guide their patients in making informed decisions by having an open discussion about the current evidence on the cardiovascular safety of testosterone replacement therapy.

Published online: 23 May 2019

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Author contributions
The authors contributed equally to all aspects of the article.

Competing interests
S.B. has previously consulted for AbbVie, Eli Lilly and Regeneron Pharmaceuticals. T.G.-J. declares no competing interests.

Publisher's note

Review criteria
MEDLINE and PubMed were searched for original articles that focused on testosterone and were published before October 2018. The search terms used were "testosterone deficiency", "androgen deficiency", "late-onset hypogonadism", "male hypogonadism", "testosterone" and "testosterone replacement" in combination with "myocardial infarction", "arrhythmia", "stroke", "sudden death", "atherosclerosis", "cardiovascular disease", "metabolic syndrome", "diabetes", "lipids", "endothelium", "endothelial function", "hypertension", "inflammation", "heart failure", "cardiovascular disease", "cardiovascular events", "retrospective study", "randomized trial", "meta-analysis", "adverse effects", "hematocrit", "erythrocytosis", "thrombosis" and "thromboembolism". All identified articles were published in English. The authors also searched the reference lists of identified articles as well as their citation data from Web of Science for related papers.