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DIABETES AND INSULIN RESISTANCE (M RUTTER, SECTION EDITOR)

Debate: Testosterone Therapy Reduces Cardiovascular Risk in Men with Diabetes. Against the Motion

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Abstract Observationally, men with low testosterone are more vulnerable to type 2 diabetes (T2DM). In meta-analysis of, albeit small, randomized controlled trials (RCTs) giving men testosterone improves glucose metabolism. T2DM predicts cardiovascular disease; improving glucose metabolism could be expected to reduce cardiovascular disease risk. Taken together, trials have not shown clearly that commonly used agents for glucose reduction also reduce cardiovascular risk substantially, although some treatments for T2DM, such as insulin and sulfonylureas may raise testosterone. Testosterone has never been tested as a strategy for cardiovascular disease prevention or treatment in men with T2DM. Meta-analysis of RCTs in men suggests that testosterone administration has no effect on cardiovascular events or increases cardiovascular-related events, perhaps because testosterone promotes coagulability. Regulators have warned of cardiovascular risk on testosterone and/or suggested prescription of testosterone be restricted. As such, testosterone is unlikely to be an effective means of reducing cardiovascular risk in men with T2DM.

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Introduction

Testosterone is an appealing product for men and has been promoted to create a climate of opinion where it is seen in favorable light [1]. Testosterone is also recommended for symptomatic testosterone deficiency by the Endocrine Society [2], albeit largely based on observational rather than experimental evidence. However, regulators highlighted concerns about the cardiovascular safety of testosterone treatment in 2014 and 2015. Specifically, the Food and Drug Administration (FDA) in the USA warned about venous thromboembolism on testosterone in June 2014 [3]. Health Canada warned about "heart attack, stroke, blood clot in the lungs or legs; and increased or irregular heart rate with the use of testosterone replacement products" in July 2014 [4]. The FDA required manufacturers to "add information to the labeling [for testosterone] about a possible increased risk of heart attacks and strokes" in March 2015 and recommended more restricted prescription of testosterone [5]. The European Medicines Agency was more equivocal, but emphasized the need for appropriate use of testosterone [6]. Testosterone prescriptions had been rising steeply into 2014, particularly in the USA [7•], but started falling in 2014 because of concerns about cardiovascular risk [8]. Nevertheless meta-analysis of randomized controlled trials shows that testosterone administration may improve glucose metabolism in hypogonadal men with type 2 diabetes (T2DM) [9], raising the possibility that testosterone treatment may reduce cardiovascular risk in men with T2DM. However, no adequately powered randomized controlled trial (RCT) has ever been implemented to assess the role of testosterone in cardiovascular disease prevention or

treatment in men with T2DM. Testosterone is currently not widely licensed for use in women. Here, focusing on men, we review the association of testosterone with T2DM, the effect of testosterone treatment on glucose metabolism and the link between glucose metabolism and cardiovascular disease. We also review the mechanisms by which testosterone modulates cardiovascular risk and the evidence concerning the effect of testosterone on cardiovascular disease, so as to provide an assessment of the likely role of testosterone as a cardiovascular disease prevention or treatment in men with T2DM.

The Relation of Testosterone with Type 2 Diabetes

Observationally, lower testosterone in men is consistently associated with higher risk for T2DM [10], although the reverse is observed in women [10]. A number of biologically plausible reasons underlie these observations. First, obesity undoubtedly causes T2DM [11]. Obesity in men may also reduce testosterone as interventions that effectively and substantially reduce weight in men also raise testosterone [12–15], probably because of less conversion of testosterone to estrogen in adipose tissue [15]. Correspondingly, testosterone administration in men reduces fat mass, although the effect may be mediated through changes in estrogen [16•]. As such, whether testosterone protects against T2DM by reducing fat mass [16] or whether obesity in men causing both low testosterone and T2DM has generated an apparent association between lower testosterone and T2DM has not been fully clarified. Second, testosterone increases muscle mass [16]. Muscle mass is a sink for glucose disposal [17], consistent with resistance training improving glucose metabolism [18]. Third, testosterone suppresses immune response [19], when inflammation may contribute to the pathogenesis of T2DM [20]. Consistent with these findings, T2DM is well known to be a side effect of androgen deprivation therapy for prostate cancer [21], although this observation has not been confirmed in RCTs. Men with lifelong low androgens due to Klinefelter's syndrome are also more vulnerable to T2DM [22]. These observations raise the question as to whether testosterone treatment would be beneficial in T2DM, both to treat T2DM and to treat other chronic diseases associated with T2DM, such as cardiovascular disease.

The Effect of Testosterone on Glucose Metabolism

A meta-analysis of five RCTs [23–27] in men with T2DM, and often with hypogonadism, showed that testosterone treatment reduced fasting glucose, fasting insulin, and HbA1c [9]. An update of this meta-analysis including two subsequent trials [28, 29] is given in Fig. 1. Testosterone reduced fasting glucose (Fig. 1a) among a total of 574 men. It also reduced fasting insulin (Fig. 1b) among 319 men but had less effect on HbA1c (Fig. 1c) among 466 men. One study of 108 men also showed no effect of testosterone on HbA1c at the end of the trial, but provided insufficient data for inclusion in the meta-analysis [27]. Taken together, the limited evidence currently available from RCTs suggests that testosterone reduces fasting glucose and insulin among men with T2DM although the effect on HbA1c is less clear. Consistent with this updated meta-analysis of testosterone in men with T2DM (Fig. 1), a recent meta-analysis of testosterone treatment in men with T2DM or the metabolic syndrome found that testosterone improved insulin resistance but had no effect on HbA1c [30]; the effects on fasting glucose and fasting insulin were not reported [30]. As such, the key question is how these beneficial effects on glucose metabolism impact cardiovascular risk.

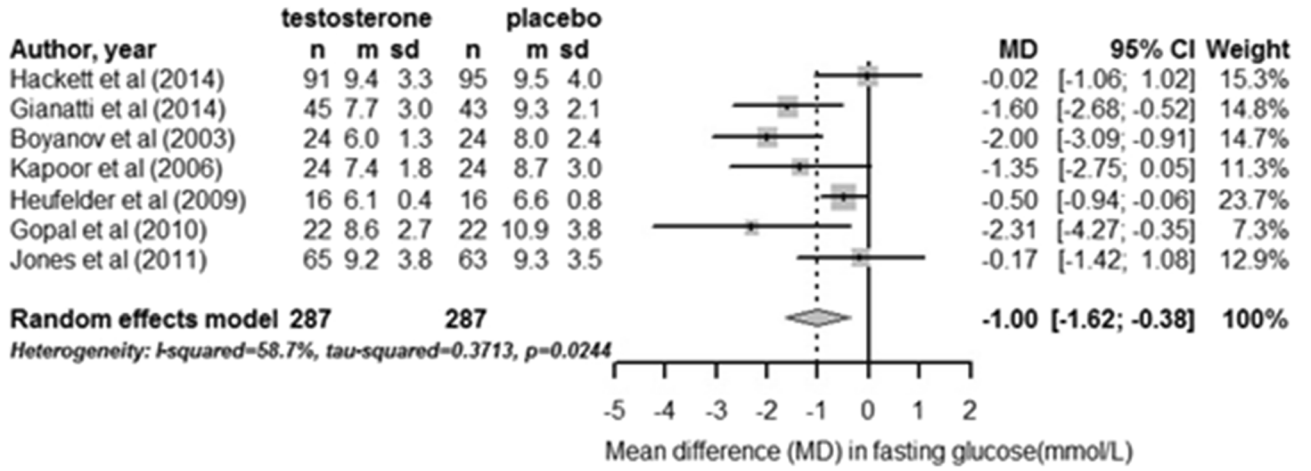
Fig. 1 Forest plots of randomized controlled trials examining the pooled effects of testosterone replacement on **a** fasting glucose, **b** fasting insulin, and **c** HbA1c in men with type 2 diabetes. The mean and standard deviation of HbA1c% in Kapoor et al. was approximated from a figure, as no exact values were provided in the text. The median and interquartile range (IQR) of the outcomes in the study of Gianatti et al. were converted to mean and standard deviation (SD), mean approximated to median and SD approximated to IQR/1.35

Glucose Metabolism in Cardiovascular Disease

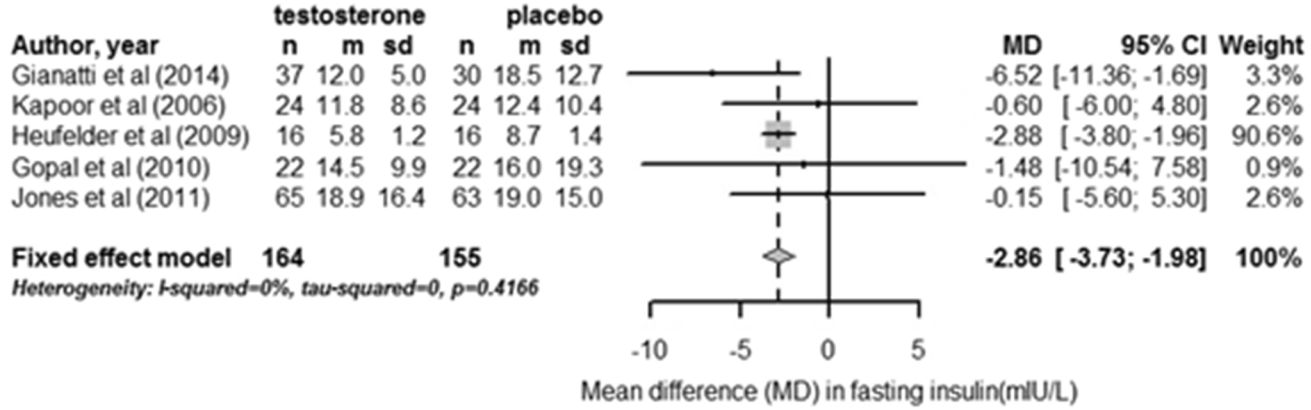
T2DM is a very well-established risk factor for cardiovascular disease, which consistently predicts cardiovascular disease [31]. Men and women with T2DM have a risk of cardiovascular disease approximately twice as high as people without T2DM [31], with a greater risk for women than men [31]. Fasting glucose has a less marked and non-linear association with cardiovascular disease [31]. Similarly, fasting insulin and HbA1c predict ischemic heart disease [32, 33]. As such, testosterone would be expected to reduce the risk of cardiovascular events in men with T2DM, on the basis that impaired glucose metabolism causes cardiovascular disease so an agent, such as testosterone, which improves glucose metabolism also reduces cardiovascular disease. As yet, no large-scale trial has been undertaken to confirm a beneficial effect of testosterone on cardiovascular mortality and morbidity in men with T2DM or in men in the general population, because the Institute of Medicine advised in 2004 that the efficacy of testosterone be established in small-scale trials before conducting any large-scale trials [34]. In the absence of definitive evidence from RCTs, we summarize the rationale for expecting cardiovascular benefits and harms from testosterone treatment in men with T2DM.

A key aspect of this debate is the role of T2DM and glucose metabolism in cardiovascular disease. Although it seems obvious that T2DM causes cardiovascular disease because T2DM predicts cardiovascular disease [31], association is not causation. People with genetically higher nonfasting

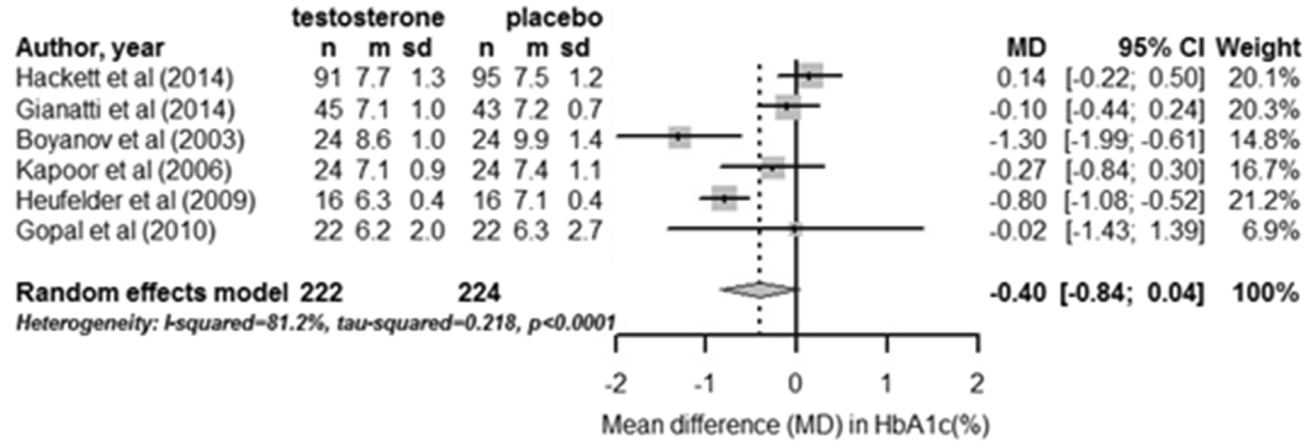
a Fasting glucose (mmol/L) in testosterone and control groups



b Fasting insulin (mIU/L) in testosterone and control groups



c HbA1c% in testosterone and control groups



glucose do also have a higher risk of ischemic heart disease [35], substantiating a causal relation unless the genetic variants predicting nonfasting glucose cause ischemic heart disease by a different mechanism. On the other hand, genome-wide association studies have shown little overlap of the genetic variants associated with T2DM and those associated with ischemic heart disease [36], suggesting little shared genetic architecture of T2DM and cardiovascular disease. From a practical perspective, the acid test of a causal role is reversibility.

Most treatments for T2DM have been approved on the basis of improving surrogate outcomes, so the experimental evidence base for the effect of treatments for T2DM on cardiovascular disease morbidity and mortality is surprisingly limited [37••]. RCTs have shown benefits of T2DM treatments for some cardiovascular outcomes. During the 10-year post-trial follow-up of UKPDS, benefits for myocardial infarction emerged on randomization to sulfonylurea or metformin compared to conventional treatment despite little in mean difference in glycated hemoglobin levels between groups.[38] The PROactive trial of pioglitazone compared to placebo found benefits for the main secondary endpoint (death from any cause, non-fatal myocardial infarction, or stroke), but not for the primary endpoint (secondary endpoint or acute coronary syndrome, leg amputation, coronary revascularization, or revascularization of the leg) [39]. The ACCORD trial found a benefit for non-fatal myocardial infarction, although not for the primary outcome or for death from cardiovascular causes [40]. However, meta-analyses of RCTs show no clear advantage of tight glucose control in people with T2DM [41, 42], such that the value of intensive glycemic control in T2DM, particularly for older patients, has recently been questioned [43]. As regards specific types of treatment for T2DM, the available evidence from RCTs suggests that metformin and insulin glargine have no effect of cardiovascular mortality [44, 45], while evidence for sulfonylureas is insufficient to draw a conclusion [46], the thiazolidinedione rosiglitazone has been withdrawn from some markets because of concerns over cardiovascular safety [47] while pioglitazone has raised fewer concerns [48], two recent trials of dipeptidyl-peptidase-4 inhibitors showed no reduction in cardiovascular events [49, 50]. Further trials are underway to clarify these findings and test new therapies, such as GLP-1 receptor antagonists and SGLT-2 inhibitors [37]. Notably, small intervention studies suggest that some treatments for T2DM, such as insulin [51], sulfonylureas [52], and rosiglitazone [53], may increase testosterone, even without weight change [51, 52]. In contrast, one of the most effective treatments for cardiovascular disease, statins, reduces testosterone [54] and increases the risk of T2DM with a dose-response effect [55], but still reduces cardiovascular events in T2DM patients [56].

None of these pieces of evidence definitively show that testosterone does not protect against cardiovascular disease in men with T2DM. Nevertheless, the available evidence indicates a very complex relation between T2DM and cardiovascular disease, where it cannot be assumed that treatments which improve glucose metabolism, via increasing testosterone or other means, reduce the risk of cardiovascular events or conversely that agents, which cause T2DM, increase the risk of cardiovascular events. Nevertheless, testosterone could protect against cardiovascular disease in T2DM by mechanisms other than glucose metabolism, such as traditional biological risk factors for cardiovascular disease, i.e., lipids, blood pressure, and obesity, or via effects on treatment targets in cardiovascular disease, such as coagulability and arrhythmia.

Effects of Testosterone on Lipids, Blood Pressure, and Obesity

Evidence from meta-analysis of RCTs among men indicates that testosterone reduces HDL-cholesterol [57], but may have a neutral effect on blood pressure and LDL-cholesterol [57]. Testosterone may also reduce body fat [16]. Meta-analysis of RCTs among women indicates that testosterone reduces HDL-cholesterol and raises LDL-cholesterol [58], consistent with evidence of men with genetically higher testosterone having lower HDL-cholesterol and higher LDL-cholesterol [59]. Given that lower HDL-cholesterol, higher LDL-cholesterol, higher blood pressure, and obesity reliably predict cardiovascular disease in the general population [60–62] and in people with T2DM [63], overall, testosterone could have a positive, neutral, or harmful effect on cardiovascular disease, depending on the extent to which testosterone operates on cardiovascular disease by these mechanisms and their relative contribution to causing cardiovascular disease.

Evidence from many sources suggests that HDL-cholesterol does not cause cardiovascular disease. People with genetically lower HDL-cholesterol do not have a higher risk of myocardial infarction [64]. To date, no treatment has been identified which raises HDL-cholesterol and also reduces major cardiovascular diseases [65]. Similarly, the causal effect of obesity on cardiovascular disease has been harder to show than expected. An RCT using lifestyle interventions to reduce weight had no effect on cardiovascular disease in people with T2DM [66], and people with genetically higher body mass index do not appear to have a higher risk of ischemic heart disease [11]. However, these unexpected findings concerning the role of HDL-cholesterol and obesity in cardiovascular disease could reflect the limitations of the available evidence rather than the absence of causal pathways. In contrast, people with genetically higher LDL-cholesterol do have a higher risk of myocardial infarction [64], suggestive of LDL-cholesterol causing myocardial infarction unless the LDL-related genetic variants cause myocardial infarction by another mechanism. Interventions that reduce LDL-cholesterol, such as estrogen,

niacin, fibrates, and CETP inhibitors, do not always prevent cardiovascular disease [67]. In contrast, higher blood pressure undoubtedly causes cardiovascular disease, because blood pressure reduction reduces cardiovascular disease events in people with and without T2DM [68]; however, no experimental evidence suggests testosterone reduces blood pressure [57].

Effects of Testosterone on Coagulability

Both Health Canada and the FDA highlighted a potential thrombotic role of testosterone [3, 4], partially based on evidence from case reports [4]. Little experimental evidence concerning the effects of testosterone on specifically thrombotic factors in men is available. A few RCTs of testosterone have reported effects on some hemostatic factors, but are difficult to interpret because of the small numbers and different factors reported [69–71]. Some clinical studies suggest that testosterone increases platelet reactivity and thromboxane [72, 73]. However, evidence from meta-analysis of RCTs clearly indicates that testosterone raises hematocrit and hemoglobin [57]. Hematocrit has long been thought to increase cardiovascular risk [74], consistent with some limited trial evidence [75, 76]. People genetically predisposed to anemia, through thalassemia minor or G6PD deficiency, have been observed to have a lower risk of cardiovascular disease [77, 78]. However, no large-scale RCT has confirmed effects of hemoglobin manipulation on cardiovascular disease across the normal range or in specific sub-groups, such as people with T2DM.

Effects of Testosterone on Heart Rate and Arrhythmia

Evidence from RCTs concerning the effect of testosterone on heart rate and arrhythmia is limited and inconclusive [79]. Observationally, testosterone is associated with a slower heart rate and/or with shorter corrected QT interval [80, 81], when longer QT interval predicts arrhythmia [82]. However, the observed associations were not evident for a different androgen biomarker [83]. Moreover, men with genetically higher testosterone do not have slower heart rate or shorter QT interval; if anything, the association is in the other direction [79].

Based on the limited experimental evidence available, testosterone might be expected to have little net effect on cardiovascular disease via lipids, blood pressure, or obesity. However, evidence is suggestive that testosterone could have adverse effects on cardiovascular disease via other mechanisms, such as coagulability or arrhythmia. Nevertheless, the possibility that testosterone could have a beneficial effect on cardiovascular disease for men with T2DM remains, because it has not been disproved in a large RCT. In the absence of such "gold-standard" evidence, the available evidence from other types of study design is summarized below.

Testosterone Administration in Diabetes

Evidence from Trials

Observationally, meta-analyses suggest lower testosterone is associated with a higher risk of cardiovascular disease [84, 85], which the authors suggest may be due to testosterone acting as indicator of poor health [84, 85]. Consistent with this interpretation, a meta-analysis of RCTs does not suggest testosterone reduces cardiovascular-related events but might result in an increased risk [86]. The experimental evidence is too limited to determine whether the effects might be different among men with T2DM. Instead, the role of testosterone treatment for cardiovascular disease in men with T2DM can only be assessed from observational studies, which are not always a reliable guide to causality and reversibility.

Evidence from Observational Studies

Observational studies assessing the effects of treatment are open to confounding by need for treatment and issues with retrospectively constructed cohorts. First, patients are given treatment for a reason, which may confound the association of treatment with disease. For example, a treatment given only to very sick patients may appear to be associated with death even if it is in fact protective because it is only given to people more likely to die than anybody else. Second, in retrospectively constructed cohorts, care must be taken to compare rates taking exposure time into account rather to compare the proportion of events; otherwise, "immortal time bias" can occur which normally favors the treatment [87•]. For example, in a cohort where everybody is recruited at the same time and then some of them are given treatment later, it is invalid to compare the proportion of events in the ever-treated and the never-treated groups, because the ever treated have to survive from recruitment to treatment in order to be part of the treated group and thus they have the benefit of immortal time from recruitment to treatment. Immortal time bias can be avoided at the design stage by matching the participants to ensure each treated person is matched to someone else alive at the time treatment is started. Immortal time bias can also be avoided at the analysis stage, by comparing rates, i.e., events per unit of exposure time, or in a Cox proportional hazards model by use of time-varying exposure to treatment. However, blunders are easy to make and hard to spot [87•, 88, 89].

Muraleedharan et al. report on mortality, mainly from cardiovascular disease, in a small cohort (n=238) of men with T2DM and low testosterone [90]. In the untreated group, the proportion of deaths was 20 % (35/174), and in the treated

group, who received testosterone, the proportion of deaths was 9.3 % (6/64), with an adjusted hazard ratio of 2.3 (95 % confidence interval (CI) 1.3 to 3.9) for untreated compared with testosterone treated [90]. However, it is unclear whether treatment started at baseline, no comparison of death rates taking exposure time into account is given, and no use is made of time-varying exposure to treatment in the Cox regression model, so immortal time bias and an inappropriate comparison of proportions rather than rates cannot be ruled out. Few other studies in English giving the association in observational studies of testosterone treatment with overall mortality or cardiovascular events are available for men with T2DM. However, studies in men with and without T2DM are available.

Shores et al. report on mortality, mainly from cardiovascular disease, in a slightly larger cohort (n=1031) of veterans with low testosterone and a high proportion of men with T2DM (39 %) [91]. In the untreated group, the proportion of deaths was 20.7 % (131/633) compared to 10.3 % in the treated group (41/398). Using a Cox model, with time-varying exposure to treatment, the hazard ratio of death for the treated compared with the untreated was 0.61, 95 % CI 0.42 to 0.88 [91].

Vigen et al. reported on the association of testosterone treatment in a cohort of 8709 veterans with low testosterone who were undergoing coronary angiography, in which 55 % had T2DM [92]. In the untreated group, the proportion of men who experience myocardial infarction, stroke, or death was 21 % (1587/7486) compared to 10 % (123/1223) in the treated group; however, taking time-varying exposure to treatment into consideration to give rates not proportions and thereby avoiding "immortal time bias," the hazard ratio on testosterone was 1.29 (95 % CI 1.04 to 1.58) [92]. For reasons which are difficult to understand, Vigen et al. have been repeatedly criticized for presenting results unbiased by immortal time rather than biased results inevitably more favorable to testosterone [93–95].

Baillargeon et al. reported on the association of testosterone with hospitalization for myocardial infarction in 6355 Medicare beneficiaries treated with testosterone compared to 19,065 matched Medicare beneficiaries without such treatment and found a hazard ratio of 0.84 (95 % CI 0.69 to 1.02) favoring testosterone therapy [96]. However, Baillargeon et al. did not use a time-varying exposure in the Cox model and the description of the construction of the cohorts is insufficiently detailed to rule out immortal time bias.

Finally, to avoid confounding and immortal time bias, Finkle et al. compared the incidence rate of myocardial infarction in the 90 days following a testosterone prescription with the rate in the previous year for 55,593 men. This group found a relative risk of 1.36 (95 % CI 1.03 to 1.81) suggesting harm from the intervention [97].

As such, the association of testosterone treatment with a lower risk of death seen in small and possibly flawed studies [90, 91] has not been replicated in larger studies of the association of testosterone treatment with cardiovascular events where testosterone is associated with at best a neutral effect. Similarly, meta-analysis of RCTs indicates that men given androgen deprivation for prostate cancer are not at a higher risk of cardiovascular mortality [98] and might be at lower risk with some forms of androgen deprivation therapy [99]. Currently, no study examining whether men with genetically higher testosterone have a higher or lower risk of cardiovascular events has been published. However, men with lifelong low androgens due to Klinefelter's syndrome have a lower risk of ischemic heart disease mortality [100], despite their vulnerability to T2DM [22].

Of course, it is possible that the effect of testosterone treatment on cardiovascular risk is different for men with T2DM than men without T2DM, even though statins, which reduce testosterone [54], protect against cardiovascular disease in men with and without diabetes [55, 56]. For example, in men with and without T2DM, there could be some synergism or antagonism (here, between testosterone and T2DM) that changes the direction of treatment effect on cardiovascular disease risk factors or events. However, at the moment, no evidence of such a difference has been reported, nor a clear rationale for synergism or antagonism. In the absence of a rationale for synergism or antagonism, causal effects are generally expected to be similar across sub-groups.

Summary

Testosterone supplementation improves aspects of glucose metabolism in men with T2DM. However, many agents which improve glucose metabolism do not unequivocally reduce cardiovascular morbidity and mortality, despite the strong the correlation between T2DM and cardiovascular disease. A priori, no reason exists for testosterone to be any more effective in preventing cardiovascular disease than these other agents which improve glucose metabolism without having strong effects on incident cardiovascular disease. On the contrary, for some T2DM treatments, raising testosterone could be an off-target effect which is offsetting the benefits of improved glucose metabolism. No evidence from RCTs suggests that testosterone reduces cardiovascular risk; instead the trial evidence is, if anything, in the other direction. Moreover, pathways exist, independent of glucose metabolism, via coagulability, by which testosterone could increase the risk of cardiovascular disease in line with warnings from regulators. Although the evidence available is not definitive, taking all the evidence together, it seems unlikely that testosterone treatment would reduce cardiovascular risk for men with T2DM, but more likely that testosterone would increase it.

Compliance with Ethics Guidelines

Conflict of Interest C. Mary Schooling, Lin Xu, and Jie Zhao have no relevant disclosures to report.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

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- •• Of major importance

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