The Effect of Testosterone on Cardiovascular Disease and Cardiovascular Risk Factors in Males: A Review of Clinical and Pre-Clinical Data

Hargun Kaur , Geoff H. Werstuck PhD

PII: S2589-790X(21)00133-5
DOI: https://doi.org/10.1016/j.cjco.2021.05.007
Reference: CJCO 348

To appear in: *CJC Open*

Received date: 27 April 2021
Accepted date: 9 May 2021

Please cite this article as: Hargun Kaur , Geoff H. Werstuck PhD , The Effect of Testosterone on Cardiovascular Disease and Cardiovascular Risk Factors in Males: A Review of Clinical and Pre-Clinical Data, *CJC Open* (2021), doi: https://doi.org/10.1016/j.cjco.2021.05.007

Title: The Effect of Testosterone on Cardiovascular Disease and Cardiovascular Risk Factors in Males: A Review of Clinical and Pre-Clinical Data

Short Title: Effect of Testosterone on Cardiovascular Disease and Risk

Authors: Hargun Kaur,[a] Geoff H. Werstuck, PhD[b, c*]

[a] *Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada*

[b] *Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Ontario, Canada*

[c] *Department of Medicine, McMaster University, Hamilton, Ontario, Canada*

*Corresponding Author: Geoff H. Werstuck, Thrombosis and Atherosclerosis Research Institute, 237 Barton Street East, Hamilton, Ontario L8L 2X2, Canada. Telephone: 905 521-2100 ext 40747. E-mail: geoff.werstuck@taari.ca

Word Count: 4691

Abstract

Cardiovascular disease (CVD) is the leading cause of death worldwide. The effects of testosterone, the primary male sex hormone, on cardiovascular risk have been of special interest due to the increased risk of CVD in males. While it is well-established that testosterone levels decline and cardiovascular mortality increases with age, the association between testosterone and CVD remains unclear. Observational and randomized studies on the effects of endogenous and exogenous testosterone have produced conflicting data and meta-analyses have been inconclusive, suggesting significant study heterogeneity. Despite a lack of adequately powered RCTs, large observational studies in the early 2010s led to advisories on the use of testosterone replacement therapy. Similar advisories have been mandated for certain types of androgen deprivation therapy. Additional research suggests that testosterone shortens the QTc interval, improves glycemic control, induces vasodilation, is pro-thrombotic, and has anti-obesity effects, while associations with atherosclerosis and inflammation are less clear. Despite inconclusive evidence on cardiovascular risk and inconsistencies among clinical practice guidelines, millions of males continue to use testosterone replacement and androgen deprivation therapy. In addition to summarizing clinical and pre-clinical data, this review provides insight on potential mechanisms of action of testosterone on CVD, applications of this knowledge to clinical settings, and avenues for future research.

Brief Summary

The effect of testosterone on cardiovascular disease has long been subject to debate. Observational and interventional studies have been conflicting and meta-analyses suggest significant heterogeneity and low-quality data. Research indicates that testosterone shortens the QTc interval, improves glycemic control, induces vasodilation, is pro-thrombotic, and has anti-obesity effects. This review summarizes existing data and provides insight on potential mechanisms of action of testosterone on CVD, applications to clinical settings, and avenues for future research.

Introduction

Cardiovascular disease (CVD) is the leading cause of death globally.[1] Various factors increase the risk of CVD, including diabetes, obesity, hypertension, dyslipidemia, and increasing age. Epidemiological studies have suggested that males face an increased risk of CVD compared to females[2] and evidence supports a role for sex hormones in the modulation of CVD pathogenesis in males and females. While the protective effect of estrogen on cardiovascular health is well-established,[3] the effect of testosterone is less clear.

An increased risk of premature cardiovascular events in males initially led to the belief that testosterone had detrimental effects on cardiovascular health. Some large observational and randomized studies have supported this conclusion, while others have suggested a cardioprotective role for testosterone. Systematic reviews and meta-analyses have generally reported low-quality evidence and hence have been inconclusive. With testosterone therapies being used in the treatment of conditions that affect millions of males worldwide,[4] its relationship with cardiovascular risk and disease must be better understood to inform guidelines for their use. This review summarizes recent clinical and preclinical studies with the aim to better understand the effect, and possible mechanisms of action, of testosterone on cardiovascular risk. The effect of testosterone on specific cardiovascular risk factors will also be assessed.

Molecular Biology of Testosterone

Biosynthesis and Metabolism of Testosterone

Testosterone is the primary sex hormone in males. It is essential for the development of the male reproductive system and secondary sex characteristics.[5] Following stimulation by the luteinizing hormone (LH), testosterone is synthesized from cholesterol through steroidogenesis.[5] This occurs primarily in testicular Leydig cells and, in smaller quantities, in the adrenal glands.[5] Its synthesis is regulated by the hypothalamic-pituitary-testicular axis, with increasing testosterone levels activating a negative feedback loop that inhibits the release of gonadotropic releasing hormone (GnRH), follicle-stimulating hormone (FSH), and LH.[6]

Secreted testosterone circulates in the blood in its free form or bound to carrier proteins. Sex-hormone binding globulin (SHBG) is the major carrier protein of testosterone,[6] with approximately 60% of testosterone bound to SHBG and an additional 40% bound to albumin.[6] Only 1-2% of testosterone is unbound or free.[7] Although only free testosterone was historically considered to be biologically available, albumin-bound testosterone is now also accepted as bioavailable, due to its lower binding affinity.[7]

Bioavailable testosterone can directly exert its effects on androgen receptors (AR). Alternatively, it may be metabolized to other steroid hormones, such as dihydrotestosterone (DHT) or 17β-estradiol (E2), by 5α-reductase and aromatase, respectively.[5] DHT amplified the effects of testosterone as its highly active metabolite with a greater binding capacity and signalling induction potency.[6] In males, E2 is produced locally through conversion by aromatase,[5] an enzyme expressed in multiple tissues including adipose tissue, bone, and brain.

Testosterone is largely metabolized to androsterone and aetiocholanolone and conjugated with glucuronic or sulphuric acid prior to excretion in the urine.[6] After the age of 60, the metabolic clearance rate of testosterone decreases rapidly.[8] Concurrently, with age, the levels of free and albumin-bound testosterone also decline, while SHBG-bound testosterone levels increase.[8] This results in decreased free testosterone levels and bioavailability. The effect of age on total testosterone concentration is not clear.

Physiological Effects of Testosterone

Most physiological effects of testosterone are mediated through its interaction with the AR, a ligand-dependant nuclear receptor. The AR gene spans eight exons and 90 kb at locus Xq11-12.[9] It has three major functional domains each with a unique role in mediating the molecular mechanisms of androgens: N-terminal transcriptional regulatory domain, DNA-binding domain, and C-terminal ligand-binding domain.[10] While gene transcription is affected by cell-type and age, the AR is expressed in most cell types and tissues, with the exception of the spleen.[10]

Androgens have diverse effects on multiple organ systems (**Figure 1).** These effects can occur through classical and non-classical mechanisms.[11,12] Classical or DNA-binding dependant signalling involves androgen binding-induced conformational changes in the AR, which dissociate chaperone proteins and expose the AR nuclear location sequence.[11] The AR/androgen complex then translocates to the nucleus and forms dimers that bind to specific androgen response promoter elements (ARE) to modulate gene transcription.[11] Non-classical or non-DNA-binding dependant signalling occurs within seconds or minutes and does not directly involve transcriptional changes.[12] While the specific mechanism is unclear, 2nd messenger pathway activation involving MAPK, Akt, and ERK has been implicated, as well as possible indirect gene repression through sequestration of transcription factors.[12]

Testosterone and Cardiovascular Risk

Endogenous Testosterone Levels and Cardiovascular Risk

The Massachusetts Male Aging Study established that testosterone levels peak around age 30, followed by a decline of 1-2% annually.[8] This observation led to an interest in exploring the association between low testosterone concentrations and cardiovascular risk. Speculation on the hormone's effects has led to decades of observational studies and reviews.

Many population-based studies have found an inverse correlation between endogenous testosterone levels and all-cause and cardiovascular mortality, especially in older males. A prospective study of elderly males by Laughlin et al.[13] found that males in the lowest quartile of total testosterone levels had a 40% increased likelihood of 20-year mortality compared to those with higher levels, which could not be explained by several comorbidities and risk factors, including age (HR 1.40 [95% CI 1.14-1.71]). These results were in accordance with an 8-year prospective cohort study of male veterans (HR 1.88 [95% CI 1.34-2.63]).[14] Laughlin et al.[13] also associated lower testosterone with increased cardiovascular mortality (HR 1.38 [95% CI 1.02-1.85]). This is consistent with the prospective Rotterdam study which reported an inverse association between testosterone levels in older males and risk and progression of severe aortic atherosclerosis.[15] EPIC-Norfolk, a nested case-control study, similarly reported an inverse relationship between endogenous testosterone concentrations and all-cause mortality and CVD.[15]

In contrast, other studies have found no statistically significant association between testosterone and cardiovascular mortality. The longitudinal Cardiovascular Health Study failed to find an association between total and free testosterone and incident CVD or mortality in older males. [16] DHT, however, was associated with these outcomes.[16] A recent prospective study of 552 elderly male subjects found no relationship between endogenous testosterone levels and risk of coronary artery disease, cerebrovascular, and peripheral arterial disease events.[17] Interestingly, data from the prospective French Three-City study suggested a "J-shaped" association between serum total testosterone and risk of ischemic arterial disease in elderly males.[18] Individuals in the highest and lowest quintiles had an increased risk of ischemic artery disease as compared to those in the second quintile.[18] This is in direct contrast to previous studies which found that males with the lowest testosterone levels had the highest cardiovascular risk.[13–15]

Some systematic reviews and meta-analyses have suggested that the conflicting associations, and subsequent lack of firm conclusions, may be due to study heterogeneity and low-quality evidence. [19] A 2018 meta-analysis of observational studies by Corona et al.[19] suggested low baseline endogenous testosterone levels predicted overall and cardiovascular mortality. However, the authors indicated that the data may have been influenced by publication bias.[19] A meta-analysis by Araujo et al.[20] also found an association between testosterone and overall mortality; however, significant heterogeneity between studies suggested effects may be driven by cohort differences. Ruige et al.[21] reported a weak pooled protective effect of total testosterone on CVD in healthy males. Similar to Araujo et al.,[20] the authors found significant heterogeneity ($p < 0.0001$), with population age and publication year identified as sources.[21] Following stratification by age, an inverse association was found between total testosterone and CVD in males above the age of 70, while no association was found in younger males.[21] This indicates that the contradicting results of observational studies may be due to varying baseline population characteristics. However, it is important to note the limitations associated with observational studies, including increased susceptibility for bias and confounding. Further, many studies only conducted a

single testosterone measurement which may have been impacted by significant diurnal variation.[22] Most studies also did not consider the clinical presentation of testosterone deficiency.

Thus, while many studies have found inverse associations between endogenous testosterone levels and cardiovascular risk and mortality, conflicting results and heterogeneous study populations have prevented firm conclusions from being drawn.

Testosterone Replacement Therapy

Despite a lack of clarity on the relationship between endogenous testosterone and cardiovascular risk, testosterone replacement therapy (TRT) is widely used, especially in older males with low serum testosterone levels. TRT does have benefits such as improved sexual function, increased skeletal muscle mass, and increased bone mineral density.[23] The Copenhagen Study Group conducted one of the first RCTs reporting an increased mortality in males treated with testosterone, although the effect was not statistically significant (RR 1.17 [95% CI 0.65-2.15]).[24] The infeasibility "to demonstrate—in the foreseeable future—a beneficial effect of testosterone by continuing the study" led to the premature end of the trial.[24] Despite this, testosterone sales increased 100-fold from the 1980s to 2010s, with a 40-fold in Canada from 2000 to 2011.[25]

In the early 2010s, certain large observational and randomized studies found an increased cardiovascular risk to be associated with testosterone therapy. [26] In 2010, the Testosterone in Older Men (TOM) trial had to be stopped prematurely due to the increased incidence of cardiovascular events in the intervention group.[26] The trial involved 209 elderly community-dwelling males with limited mobility, randomized to placebo or testosterone.[26] Males in the highest quartile of testosterone levels were at elevated risk for cardiovascular events (HR 2.4; p = 0.05) compared to other subjects.[26] Vigen et al.[27] later conducted a retrospective cohort study to determine the effects of testosterone therapy in veterans undergoing coronary angiography with pre-existing low testosterone. Cox proportional hazard models indicated an increased risk of adverse cardiovascular outcomes in males receiving testosterone therapy

(HR 1.29 [95% CI 1.04-1.58]).[27] In accordance with these results, a retrospective cohort study by Finkle et al.[28] reported a statistically significant elevation of myocardial infarction rates post-prescription of testosterone compared to pre-prescription, with an especially pronounced effect in males over the age of 75 (RR 3.43 [95% CI 1.54-7.66]). In males under 65, the excess risk was limited to those with a history of heart disease (RR 2.90 [95% CI 1.49-5.62]).[28] Further supporting these results, the T Trials found a statistically significant 1-year increase in noncalcified plaque volume (estimated difference 41 mm^3 [95% CI 14 to 67 mm^3]) in hypogonadal elderly males receiving testosterone therapy compared to the placebo group.[29] No statistically significant difference was found in the number of cardiovascular events or calcified plaque progression between the intervention and control group.[29]

The indication of an association between testosterone therapy and risk for adverse cardiovascular events prompted the FDA to issue a safety warning on testosterone therapy for older males, which was followed by a reduction in testosterone prescriptions.[30] The safety warning cautioned against the use of testosterone therapy for aging-related decline and reinforced the current approval of testosterone products for hypogonadal males only.[30] However, it is important to note that the methodology and reliability of the aforementioned studies have since been questioned. The TOM Trials lacked predetermined cardiovascular endpoints as the trial was not designed to investigate cardiovascular health.[26] Further, despite having a sample with a high prevalence of comorbidities, including hypertension, obesity, diabetes mellitus, and pre-existing CVD, only 4 major adverse cardiovascular events (MACE) occurred, although all were in the testosterone group.[26] These results were not replicated in a later trial with a similar population and testosterone administration technique.[31] Both Vigen et al.[27] and Finkle et al.[28] were retrospective in nature, which poses inherent design limitations that make it difficult to draw definitive conclusions from the data. Questions have also been raised with the methodological validity and statistical analysis techniques of Vigen et al.[27]

In contrast to these studies, others have reported a protective effect of testosterone therapy on cardiovascular health. Cheetham et al.[32] found a lower risk of cardiovascular outcomes in androgen-deficient males who had received TRT (HR 0.67 [95% CI 0.62-0.73]), analyzed retrospectively for a median of 3.4 years. In a recent matched cohort study, short-term testosterone therapy increased the risk of mortality and cardiovascular events in males over the age of 65, while longer-term therapy was associated with reduced risk of mortality, adverse cardiovascular events, and prostate cancer.[33]

As a result of these conflicting results, a recent meta-analysis found no significant association between testosterone therapy and cardiovascular events and mortality and reported low-quality evidence due to bias, inconsistencies, and imprecision.[34] This has led to inconsistencies between clinical practice guidelines. While all acknowledge the possible cardiovascular risks of testosterone therapy, there is disagreement on the minimum amount of time following a major cardiovascular event that an individual may receive testosterone therapy.[35]

Adequately powered randomized clinical trials designed to assess cardiovascular events are required to definitively determine the effect of testosterone therapy on cardiovascular risk. The TRAVERSE trial is an ongoing clinical trial designed to measure the time to MACE in hypogonadal males, aged 45 to 80 years, with increased risk or evidence of CVD.[36] The study commenced in May 2018 and is expected to complete in June 2022, with 6000 planned participants randomized to topical testosterone or placebo.[36] This clinical trial will play an important role in determining the safety of TRT in hypogonadal males.

Androgen Deprivation Therapy

In contrast to the use of TRT in hypogonadal males, androgen deprivation therapy (ADT) is commonly used in the treatment of advanced prostate cancer. Prostate cancer is the second most frequent malignancy in males worldwide.[4] In 1941, Huggins and Hodges[37] were the first to demonstrate the beneficial effects of castration and estrogen injections in males with metastatic prostate cancer. Since then, the introduction of chemical castration and hormonal therapy has resulted in the declined use of physical castration. GnRH agonists downregulate the GnRH receptors, thus decreasing the release of LH and blocking the stimulation of testosterone secretion.[38] They lead to castration levels of testosterone after a couple of weeks, but the GnRH agonists-induced stimulation of LH causes an initial increase in testosterone.[38] GnRH antagonists may be used in those for whom GnRH agonist therapy is not appropriate, although certain GnRH antagonists have been associated with incident anaphylaxis.[39] Other drug classes are also used including antiandrogens, 5α-reductase inhibitors, and adrenal ablating drugs.[40]

There remain valid concerns on the possibility of testosterone therapy increasing the risk of prostate cancer. While the relationship between testosterone therapy and the incidence of prostate cancer is still debated, the Endocrine Society recommends against testosterone therapy in males with an increased risk of prostate cancer.[41] In contrast, the American Urological Association advises clinicians to "inform patients of the absence of evidence linking testosterone therapy to the development of prostate cancer," citing RCTs that have found no significant difference in prostate cancer diagnosis in testosterone-deficient males treated with testosterone compared to placebo.[42] Observational studies, including Ory et al.,[43] reported similar conclusions.

The effects of the artificial lowering of testosterone levels by ADT on an individual's overall health has also been extensively studied. Using Surveillance, Epidemiology and End Results (SEER) Medicare data, Keating et al.[44] found that prostate cancer patients aged 66 or older receiving GnRH agonists had an elevated risk of diabetes (HR 1.44 [95% CI 1.34-1.55]), coronary artery disease (HR 1.16 [95% CI 1.10-1.21]), myocardial infarction (HR 1.11 [95% CI 1.01-1.21]), and sudden cardiac death (HR 1.16 [95% CI 1.05-1.27]). Interestingly, these same effects were not noticed in the orchiectomy group, although that group was underpowered.[44] A similar retrospective study using SEER-Medicare data reported a 20% increased risk of serious cardiovascular morbidity in newly diagnosed prostate cancer patients who received ADT for at least 1 year as compared to patients without ADT.[45] Another study based on data from the Cancer of Prostate Strategic Urologic Research Endeavor (CaPSURE) database associated ADT with significantly increased risk of cardiovascular mortality in patients also receiving radial proctectomy (HR 2.6 [95% CI 1.4-4.7]).[46] These retrospective nature of these studies is inherently prone to increased bias, preventing definitive conclusions from being drawn. In the same year, however, D'Amico et al.[47] pooled data from three RCTs examining short-term androgen suppression therapy and found older males who received androgen suppression therapy had shorter times to fatal myocardial infarctions than those not receiving therapy. Cumulative incidence did not differ among the two groups.[47] These studies led the American Health Association, the American Cancer Society, and the American Urological Association[48] to issue a joint statement in 2010, declaring it to be "appropriate to state that there may be a relation between ADT and cardiovascular risk." Soon after, the FDA also mandated the addition of warnings of increased risk of diabetes and CVD as a result of GnRH agonist use in males with prostate cancer.[49]

Since these advisories, additional observational studies have been conducted to assess the impact of ADT on cardiovascular outcomes. The results have been mixed. A retrospective matched cohort of nearly 40,000 older males with prostate cancer found no association of ADT with acute myocardial infarction (HR 0.91 [95% CI 0.84-1.00]) or sudden cardiac death (HR 0.96 [95% CI 0.83-1.10]).[50] However, a prospective study did find an increased risk of heart failure in males with localized prostate cancer and without pre-existing CVD (adjusted HR 1.81 [95% CI 1.40-2.32]).[51] This association was not replicated in males with pre-existing CVD[51] and is in contrast with previous studies which have found an association only in patients with comorbidities[52] and nonsignificant associations of risk factors with incident myocardial infarction during ADT.[53]

Meta-analyses on the topic have also been mixed, with significant differences between those of RCTs and observational studies. Carneiro et al.[54] found a significant association between ADT and myocardial infarction in observational studies (OR 2.01 [95% CI 1.90-2.13]), but only an association with nonfatal cardiovascular events for RCTs (OR 1.55 [95% CI 1.09-2.20]). Similarly, meta-analysis of RCTs did not find an association of ADT with cardiovascular mortality.[55] It should be noted, however, that the cardiovascular effects associated with ADT differ based on the type of therapy. Much of the research has been with GnRH agonists which have been associated with increased cardiovascular risk. [56] This increased risk may be due to the stimulation of T-cell proliferation and subsequent differentiation into the pro-inflammatory phenotype or initial increase of FSH, a hormone that has been associated with fat accumulation and acceleration of lipogenesis and lipid droplet formation.[57] The PRONOUNCE Trial, examining the cardiovascular safety of GnRH agonist compared to GnRH antagonists, is currently underway.[58]

Molecular Mechanisms of the Action of Testosterone on Cardiovascular Risk Factors

The Effect of Testosterone on Cardiovascular Physiology

Testosterone has a variety of effects on cardiovascular physiology, which may impact the hormone's effect on CVD. Clinical data strongly suggests that low testosterone levels are associated with longer heart-rate-corrected QT (QTc) intervals and TRT results in interval shortening.[59] Prolonged QTc intervals can result in an increased ventricular arrhythmias incidence and subsequent sudden cardiac death.[60]

The majority of pre-clinical studies have found testosterone to have vasodilatory effects. It is believed that this process involves the downregulation of L-type voltage-gated calcium channels[61] and upregulation of calcium-activated potassium channels.[62] The immediacy of the vasodilation has raised questions as to whether the underlying mechanism involves non-genomic actions of testosterone. Further, testosterone has been shown to increase cardiac contractility[61] and cardiomyocyte relaxation speed.[63] It is not clear whether these vascular effects are dependent on the endothelium and/or AR. It is important to note that these findings oppose other studies reporting that testosterone intensifies vasocontraction.[64]

The Association of Testosterone with Atherosclerosis and Thrombosis

Nettleship et al.[65] found that testosterone slows atheroma development and reverses lipid deposition in the artery wall. While the effect of E2 on atheroma progression is debated, a possible mechanism includes oestradiol-dependant suppression of TNF-α induced vascular cell adhesion protein 1 (VCAM-1) expression.[66]

Some observational studies, including Makinen et al.,[67] have reported inverse correlations between testosterone and intima-media thickness (IMT), a surrogate marker for atherosclerosis. However, due to the nature of the studies, reverse causality cannot be ruled out. While there is a lack of RCTs reporting directly on atherosclerosis, some have reported on carotid IMT (CIMT) and plaque calcification. A small RCT found significant beneficial effects of testosterone on CIMT,[68] while larger trials, such as the TTrials, have failed to support this conclusion.[29] Testosterone may also impact plaque stability through effects on endothelial progenitor cells (EPCs), which are related to vessel integrity maintenance and inversely associated with CIMT. Not only do hypogonadal males exhibit lower levels of EPCs, but EPCs appear to increase in proliferation and migration in an AR-dependant manner.[69]

Dyslipidemia, including elevated LDL and total cholesterol, is a major risk factor for atherosclerosis progression. Many trials, including the TOM[26] and TTrials,[29] have indicated that TRT results in lower total and LDL-C. While the effect on HDL-C is unclear, it is hypothesized that prolonged testosterone administration may re-stabilize levels following cholesterol transport normalization.[70] Because of the varying effects on lipoproteins, the overall effect of testosterone on lipid profile and cardiovascular risk is unknown. The effect of testosterone on these parameters in various populations has been subject to debate in many meta-analyses.[71]

Testosterone has been found to have pro-thrombotic effects, increasing the risk of myocardial infarction or stroke following atherosclerotic plaque rupture. Proposed mechanisms include haematocrit stimulation induced platelet aggregation and increased thromboxane A2 receptor density on platelets.[72] However, clinical trials have not found corresponding effects on coagulation.[73]

The Association of Testosterone with Diabetes

In 1978, Shahwan et al.[74] established that male diabetics have lower levels of endogenous testosterone as compared to nondiabetic males. While most clinical data has since supported this conclusions, even after controlling for obesity, the direction of the relationship is less clear.[75] Some studies have found a beneficial effect of testosterone administration on glycemic control, including improved insulin sensitivity, HbA1c, and fasting blood glucose.[76] Further, research on prostate cancer patients found that ADT is associated with hyperglycemia and impaired β-cell function.[77]

Possible mechanisms include non-genomic activation of insulin receptor signalling factors, including Akt, Erk, and mTOR, increased expression of insulin-receptor-β, insulin receptor substrate-1, Akt-2, and GLUT4 transporter, or increased expression of glycolysis enzymes.[70] Knockout models have indicated that deficiencies in 5α-reductase and/or the AR may result in hepatic steatosis and insulin resistance.[78] The effect of testosterone on pancreatic β-cells is less clear; some studies have reported increased AR-dependant hyperglycemic decomposition[79] while others have found a protective effect.[80] Despite these studies, the Endocrine Society has maintained that testosterone therapy is not recommended for improving glycemic control in males with type 2 diabetes.[41]

Ballester et al.[81] found that the administration of insulin restores testosterone, LH, and FSH levels, indicating that diabetes may cause low testosterone. Clinical data has also found Leydig cell response and LH production to be lower in males with insulin resistance.[82] These studies indicate a more complex bidirectional relationship between diabetes and testosterone is perhaps more likely.

The Association of Testosterone with Obesity

Unlike other cardiovascular risk factors, the inverse association of testosterone with obesity is well-established. Various mechanisms have been proposed to underlie this relationship. Pre-clinical studies suggest that testosterone promotes the differentiation of pluripotent stem cells to the myogenic linage and inhibits their commitment to the adipogenic lineage.[83] At a later stage, testosterone may affect the Wnt-signalling pathway and β-catenin, inhibiting further differentiation of certain preadipocytes.[84] Testosterone may also decrease abdominal fat through the stimulation of lipolysis and inhibition of adipogenesis.[85]

The opposite directional relationship has also been suggested, such that adiposity may decrease testosterone production. Increased stimulation of leptin receptors on Leydig cells can attenuate LH stimulation and thus lower testosterone production.[86] The use of aromatase inhibitors or DHT has resulted in the attenuation of the hormone's beneficial effects, indicating an important role of E2. As aromatase is primarily located in adipose tissue, excess adiposity can lead to increased conversion of testosterone into E2 and lowered serum testosterone levels.[87] However, it is important to note that the lower testosterone levels eventually lead to lower E2 levels resulting in increased visceral fat and insulin resistance; this is known as the hypogonadism-obesity cycle.[88]

Adiponectin is an adipocytokine whose levels are inversely related to cardiovascular risk. Adiponectin levels are also inversely associated with testosterone, although it is unclear if this is a direct effect or indirectly mediated through reduced adipocyte count.[89]All in all, these studies indicate a complex, likely bidirectional association between obesity and testosterone regulated by androgen and estrogen receptors.

The Association of Testosterone with Inflammation

As inflammation is a known risk factor of atherosclerosis and CVD, there has been interest in exploring the effects of testosterone on inflammation. Studies comparing the levels of high sensitivity C-reactive protein (hs-CRP) and inflammatory cytokines have largely been conflicting.[87] Whether any observed effects are direct or indirect due to age or obesity is unknown.[87] While Crisostomo et al.[90] indicated that signalling proteins p38 and SPAK/JNK, associated with myocardial inflammation, are involved in testosterone-induced exacerbation of inflammation, Rettew et al.[91] found toll-like receptor-4 to be related to protective effects of testosterone. Some studies have reported anti-inflammatory effects through the suppression of pro-inflammatory cytokines and enhancement of anti-inflammatory cytokines.[92] Others, however, have found an increase or no significant changes in hs-CRP, IL-6, and IL-β levels.[70] Pre-clinical studies have also indicated that IL-6 and TNF-α are capable of reducing testosterone levels.[93] As such, it is important to note that if an association does exist, it may be bidirectional.

The Interplay Between Testosterone and Physical Activity

Extensive data have shown an association between low physical activity and cardiovascular risk factors, including metabolic syndrome,[94] type 2 diabetes,[95] obesity,[96] and hypertension.[97] The American Heart Association has declared sedentary behaviour to be a modifiable risk factor for CVD and diabetes mellitus,[98] and many other organizations recommend physical activity to increase cardiorespiratory fitness.[99] Interestingly, in multiple RCTs, this effect of exercise on improved metabolic profile, including fat-free muscle mass and glycemic control, was enhanced by testosterone administration in hypogonadal males.[100, 101]

The relationship between physical activity and testosterone levels is still unclear. While some observational studies have found a positive association between testosterone levels and activity in younger and older males,[102, 103] others have failed to find an association.[104] Many intervention studies specifically designed to assess the effect of physical activity have found increases in testosterone levels following a physical activity intervention.[105, 106] Age further affects this relationship, as the increase in free testosterone following resistance exercise is significantly diminished in older and middle-aged males, compared to younger age groups.[107]

Given the correlation of physical activity with various cardiovascular risk factors, it is unclear whether any observed associations with testosterone are direct or indirectly mediated by one or more of the risk factors. However, it is important to further understand the interplay between both variables in mediating risk and affecting the success of targeted interventions in males involving testosterone therapies and/or physical activity.

Conclusion and Future Directions

Given the prevalence and morbidity of CVD, it is important to clarify potential risk factors, especially in males as they face higher cardiovascular risk than females. Testosterone, the major sex hormone in males, has been a primary candidate in studies of cardiovascular risk. Despite decades of research on the topic, clinical and pre-clinical data on the effects of exogenous and endogenous testosterone have produced contradictory and/or inconclusive results. In spite of this lack of clarity, many males are currently undergoing testosterone replacement or androgen deprivation therapy.

It is thus imperative to conduct adequately powered RCTs, such as the TRAVERSE[36] and PRONOUNCE[58] trials, designed to study the effect of testosterone on cardiometabolic health to conclusively determine the cardiovascular effects and safety of associated therapies. While these trials are assessing outcomes in males with hypogonadism and prostate cancer, it is also important to study effects in older males without these conditions, as they have an increased likelihood of using testosterone therapies. Although this review is primarily focused on the role of testosterone in males, possible differential effects of the hormone in females must be considered in future studies. Moreover, there must be further investigation into mechanisms of action of testosterone. In the meantime, however, it is important for patients to be advised of the possible cardiovascular risk associated with testosterone therapies and encouraged to make informed decisions while being mindful of study limitations.

Acknowledgements

Figures created with Biorender.com.

Funding Sources

This work was supported by an operational grant from the Canadian Institutes for Health Research (PJT-166092). GHW holds the ISTH-McMaster Chair in Thrombosis and Haemostasis Research as is supported by an HSFC Ontario Mid-Career Investigator Award.

Disclosures

None. The authors have no competing interests to declare.

References

1. Cardiovascular diseases (CVDs). Accessed February 10, 2021. https://www.who.int/en/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)

2. Kappert K, Böhm M, Schmieder R, et al. Impact of sex on cardiovascular outcome in patients at high cardiovascular risk: Analysis of the telmisartan randomized assessment study in ACE-intolerant subjects with cardiovascular disease (TRANSCEND) and the ongoing telmisartan alone and in combination with ramipril global end point trial (ONTARGET). *Circulation*. 2012;126(8):934-941. doi:10.1161/CIRCULATIONAHA.111.086660

3. Zhao D, Guallar E, Ouyang P, et al. Endogenous Sex Hormones and Incident Cardiovascular Disease in Post-Menopausal Women. *Journal of the American College of Cardiology*. 2018;71(22):2555-2566. doi:10.1016/j.jacc.2018.01.083

4. Rawla P. Epidemiology of Prostate Cancer. *World Journal of Oncology*. 2019;10(2):63-89. doi:10.14740/wjon1191

5. Li L, Zirkin BR, Papadopoulos V. Leydig cell androgen synthesis. In: *Encyclopedia of Reproduction*. Elsevier; 2018:215-221. doi:10.1016/B978-0-12-801238-3.64583-X

6. Brooks R v. 1 Androgens. *Clinics in Endocrinology and Metabolism*. 1975;4(3):503-520. doi:10.1016/S0300-595X(75)80045-4

7. TTFB - Clinical: Testosterone, Total, Bioavailable, and Free, Serum. Accessed February 10, 2021. https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/83686

8. Gray A, Feldman HA, Mc kinlay JB, Longcope C. Age, Disease, and Changing Sex Hormone Levels in Middle-Aged Men: Results of the Massachusetts Male Aging Study. *Journal of Clinical Endocrinology and Metabolism*. 1991;73(5):1016-1025. doi:10.1210/jcem-73-5-1016

9. Brown CJ, Goss SJ, Lubahn DB, et al. Androgen receptor locus on the human X chromosome: Regional localization to Xq11-12 and description of a DNA polymorphism. *American Journal of Human Genetics*. 1989;44(2):264-269. Accessed February 10, 2021. /pmc/articles/PMC1715398/?report=abstract

10. Lindzey J, Kumar MV, Grossman M, Young C, Tindall DJ. Molecular Mechanisms of Androgen Action. In: *Vitamins and Hormones*. Vol 49. Vitam Horm; 1994:383-432. doi:10.1016/S0083-6729(08)61151-6

11. Eder IE, Culig Z, Putz T, Nessler-Menardi C, Bartsch G, Klocker H. Molecular biology of the androgen receptor: From molecular understanding to the clinic. *European Urology*. 2001;40(3):241-251. doi:10.1159/000049782

12. Kallio PJ, Poukka H, Moilanen A, Jänne OA, Palvimo JJ. Androgen receptor-mediated transcriptional regulation in the absence of direct interaction with a specific DNA element. *Molecular Endocrinology*. 1995;9(8):1017-1028. doi:10.1210/mend.9.8.7476976

13. Laughlin GA, Barrett-Connor E, Bergstrom J. Low Serum Testosterone and Mortality in Older Men. *The Journal of Clinical Endocrinology & Metabolism*. 2008;93(1):68-75. doi:10.1210/jc.2007-1792

14. Shores MM, Matsumoto AM, Sloan KL, Kivlahan DR. Low serum testosterone and mortality in male veterans. *Archives of Internal Medicine*. 2006;166(15):1660-1665. doi:10.1001/archinte.166.15.1660

15. Khaw KT, Dowsett M, Folkerd E, et al. Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men: European prospective investigation into cancer in Norfolk (EPIC-Norfolk) prospective population study. *Circulation*. 2007;116(23):2694-2701. doi:10.1161/CIRCULATIONAHA.107.719005

16. Shores MM, Biggs ML, Arnold AM, et al. Testosterone, dihydrotestosterone, and incident cardiovascular disease and mortality in the cardiovascular health study. *Journal of Clinical Endocrinology and Metabolism*. 2014;99(6):2061-2068. doi:10.1210/jc.2013-3576

17. Collet T-H, Ewing SK, Ensrud KE, et al. Endogenous Testosterone Levels and the Risk of Incident Cardiovascular Events in Elderly Men: The MrOS Prospective Study. *Journal of the Endocrine Society*. 2020;4(5):1-15. doi:10.1210/jendso/bvaa038

18. Soisson V, Brailly-Tabard S, Helmer C, et al. A J-shaped association between plasma testosterone and risk of ischemic arterial event in elderly men: The French 3C cohort study. *Maturitas*. 2013;75(3):282-288. doi:10.1016/j.maturitas.2013.04.012

19. Corona G, Rastrelli G, di Pasquale G, Sforza A, Mannucci E, Maggi M. Endogenous Testosterone Levels and Cardiovascular Risk: Meta-Analysis of Observational Studies. *Journal of Sexual Medicine*. 2018;15(9):1260-1271. doi:10.1016/j.jsxm.2018.06.012

20. Araujo AB, Dixon JM, Suarez EA, Murad MH, Guey LT, Wittert GA. Endogenous testosterone and mortality in men: A systematic review and meta-analysis. *Journal of*

Clinical Endocrinology and Metabolism. 2011;96(10):3007-3019. doi:10.1210/jc.2011-1137

21. Ruige JB, Mahmoud AM, de Bacquer D, Kaufman JM. Endogenous testosterone and cardiovascular disease in healthy men: A meta-analysis. *Heart*. 2011;97(11):870-875. doi:10.1136/hrt.2010.210757

22. Brambilla DJ, Matsumoto AM, Araujo AB, McKinlay JB. The Effect of Diurnal Variation on Clinical Measurement of Serum Testosterone and Other Sex Hormone Levels in Men. *The Journal of Clinical Endocrinology & Metabolism*. 2009;94(3):907-913. doi:10.1210/jc.2008-1902

23. Spitzer M, Huang G, Basaria S, Travison TG, Bhasin S. Risks and benefits of testosterone therapy in older men. *Nature Reviews Endocrinology*. 2013;9(7):414-424. doi:10.1038/nrendo.2013.73

24. Gluud C. Testosterone treatment of men with alcoholic cirrhosis: A double-blind study. *Hepatology*. 1986;6(5):807-813. doi:10.1002/hep.1840060502

25. Handelsman DJ. Global trends in testosterone prescribing, 2000-2011: Expanding the spectrum of prescription drug misuse. *Medical Journal of Australia*. 2013;199(8):548-551. doi:10.5694/mja13.10111

26. Basaria S, Coviello AD, Travison TG, et al. Adverse Events Associated with Testosterone Administration. *New England Journal of Medicine*. 2010;363(2):109-122. doi:10.1056/nejmoa1000485

27. Vigen R, O'Donnell CI, Barón AE, et al. Association of testosterone therapy with mortality, myocardial infarction, and stroke in men with low testosterone levels. *JAMA - Journal of the American Medical Association*. 2013;310(17):1829-1836. doi:10.1001/jama.2013.280386

28. Finkle WD, Greenland S, Ridgeway GK, et al. Increased risk of non-fatal myocardial infarction following testosterone therapy prescription in men. *PLoS ONE*. 2014;9(1). doi:10.1371/journal.pone.0085805

29. Budoff MJ, Ellenberg SS, Lewis CE, et al. Testosterone treatment and coronary artery plaque volume in older men with low testosterone. *JAMA - Journal of the American Medical Association*. 2017;317(7):708-716. doi:10.1001/jama.2016.21043

30. FDA Drug Safety Communication: FDA cautions about using testosterone products for low testosterone due to aging; requires labeling change to inform of possible increased risk of heart attack and stroke with use | FDA. Accessed February 10, 2021. https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-cautions-about-using-testosterone-products-low-testosterone-due

31. O'Connell MDL, Roberts SA, Srinivas-Shankar U, et al. Do the Effects of Testosterone on Muscle Strength, Physical Function, Body Composition, And Quality of Life Persist Six Months after Treatment in Intermediate-Frail and Frail Elderly Men? *The Journal of Clinical Endocrinology & Metabolism*. 2011;96(2):454-458. doi:10.1210/jc.2010-1167

32. Cheetham TC, An JJ, Jacobsen SJ, et al. Association of testosterone replacement with cardiovascular outcomes among men with androgen deficiency. *JAMA Internal Medicine*. 2017;177(4):491-499. doi:10.1001/jamainternmed.2016.9546

33. Wallis CJD, Lo K, Lee Y, et al. Survival and cardiovascular events in men treated with testosterone replacement therapy: An intention-to-treat observational cohort study. *The Lancet Diabetes and Endocrinology*. 2016;4(6):498-506. doi:10.1016/S2213-8587(16)00112-1

34. Alexander GC, Iyer G, Lucas E, Lin D, Singh S. Cardiovascular Risks of Exogenous Testosterone Use Among Men: A Systematic Review and Meta-Analysis. *American Journal of Medicine*. 2017;130(3):293-305. doi:10.1016/j.amjmed.2016.09.017

35. Pelzman DL, Hwang K. Testosterone therapy: where do the latest guidelines agree and differ? *Current opinion in endocrinology, diabetes, and obesity*. 2020;27(6):397-403. doi:10.1097/MED.0000000000000581

36. A Study to Evaluate the Effect of Testosterone Replacement Therapy (TRT) on the Incidence of Major Adverse Cardiovascular Events (MACE) and Efficacy Measures in Hypogonadal Men - Full Text View - ClinicalTrials.gov. Accessed February 10, 2021. https://www.clinicaltrials.gov/ct2/show/NCT03518034

37. Huggins C, Hodges C v. Studies on prostatic cancer i. the effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Research*. 1941;1(4):293-297. doi:10.3322/canjclin.22.4.232

38. Tolis G, Ackman D, Stellos A, et al. Tumor growth inhibition in patients with prostatic carcinoma treated with luteinizing hormone-releasing hormone agonists. *Proceedings of the National Academy of Sciences of the United States of America*. 1982;79(5):1658-1662. doi:10.1073/pnas.79.5.1658

39. Koechling W, Hjortkjaer R, Tankó LB. Degarelix, a novel GnRH antagonist, causes minimal histamine release compared with cetrorelix, abarelix and ganirelix in an ex vivo model of human skin samples. *British Journal of Clinical Pharmacology*. 2010;70(4):580-587. doi:10.1111/j.1365-2125.2010.03730.x

40. Sharifi N, Gulley JL, Dahut WL. Androgen deprivation therapy for prostate cancer. *Journal of the American Medical Association*. 2005;294(2):238-244. doi:10.1001/jama.294.2.238

41. Bhasin S, Brito JP, Cunningham GR, et al. Testosterone Therapy in Men with Hypogonadism: An Endocrine Society. *Journal of Clinical Endocrinology and Metabolism*. 2018;103(5):1715-1744. doi:10.1210/jc.2018-00229

42. Mulhall JP, Trost LW, Brannigan RE, et al. Evaluation and Management of Testosterone Deficiency: AUA Guideline. doi:10.1016/j.juro.2018.03.115

43. Ory J, Flannigan R, Lundeen C, Huang JG, Pommerville P, Goldenberg SL. Testosterone Therapy in Patients with Treated and Untreated Prostate Cancer: Impact on Oncologic Outcomes. *Journal of Urology*. 2016;196(4):1082-1089. doi:10.1016/j.juro.2016.04.069

44. Keating NL, O'Malley AJ, Smith MR. Diabetes and cardiovascular disease during androgen deprivation therapy for prostate cancer. *Journal of Clinical Oncology*. 2006;24(27):4448-4456. doi:10.1200/JCO.2006.06.2497

45. Saigal CS, Gore JL, Krupski TL, Hanley J, Schonlau M, Litwin MS. Androgen deprivation therapy increases cardiovascular morbidity in men with prostate cancer. *Cancer*. 2007;110(7):1493-1500. doi:10.1002/cncr.22933

46. Tsai HK, D'Amico A v., Sadetsky N, Chen MH, Carroll PR. Androgen deprivation therapy for localized prostate cancer and the risk of cardiovascular mortality. *Journal of the National Cancer Institute*. 2007;99(20):1516-1524. doi:10.1093/jnci/djm168

47. D'Amico A v., Denham JW, Crook J, et al. Influence of androgen suppression therapy for prostate cancer on the frequency and timing of fatal myocardial infarctions. *Journal of Clinical Oncology*. 2007;25(17):2420-2425. doi:10.1200/JCO.2006.09.3369

48. Levine GN, D'Aamico A v., Berger P, et al. Androgen-deprivation therapy in prostate cancer and cardiovascular risk: A science advisory from the American heart association, American cancer society, and American urological association. *Circulation*. 2010;121(6):833-840. doi:10.1161/CIRCULATIONAHA.109.192695

49. FDA Drug Safety Communication: Update to Ongoing Safety Review of GnRH Agonists and Notification to Manufacturers of GnRH Agonists to Add New Safety Information to Labeling Regarding Increased Risk of Diabetes and Certain Cardiovascular Diseases | FDA. Accessed February 10, 2021. https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-update-ongoing-safety-review-gnrh-agonists-and-notification

50. Alibhai SMH, Duong-Hua M, Sutradhar R, et al. Impact of androgen deprivation therapy on cardiovascular disease and diabetes. *Journal of Clinical Oncology*. 2009;27(21):3452-3458. doi:10.1200/JCO.2008.20.0923

51. Haque R, Ulcickasyood M, Xu X, et al. Cardiovascular disease risk and androgen deprivation therapy in patients with localised prostate cancer: A prospective cohort study. *British Journal of Cancer*. 2017;117(8):1233-1240. doi:10.1038/bjc.2017.280

52. Nanda A, Chen MH, Braccioforte MH, Moran BJ, D'Amico A v. Hormonal therapy use for prostate cancer and mortality in men with coronary artery disease-induced congestive heart failure or myocardial infarction. *JAMA - Journal of the American Medical Association*. 2009;302(8):866-873. doi:10.1001/jama.2009.1137

53. Keating NL, O'Malley AJ, Freedland SJ, Smith MR. Does comorbidity influence the risk of myocardial infarction or diabetes during androgen-deprivation therapy for prostate cancer? *European Urology*. 2013;64(1):159-166. doi:10.1016/j.eururo.2012.04.035

54. Carneiro A, Sasse AD, Wagner AA, et al. Cardiovascular events associated with androgen deprivation therapy in patients with prostate cancer: a systematic review and meta-analysis. *World Journal of Urology*. 2015;33(9):1281-1289. doi:10.1007/s00345-014-1439-6

55. Nguyen PL, Je Y, Schutz FAB, et al. Association of androgen deprivation therapy with cardiovascular death in patients with prostate cancer: A meta-analysis of randomized trials. *JAMA - Journal of the American Medical Association*. 2011;306(21):2359-2366. doi:10.1001/jama.2011.1745

56. Abufaraj M, Iwata T, Kimura S, et al. Differential Impact of Gonadotropin-releasing Hormone Antagonist Versus Agonist on Clinical Safety and Oncologic Outcomes on Patients with Metastatic Prostate Cancer: A Meta-analysis of Randomized Controlled Trials. *European Urology*. 2021;79(1):44-53. doi:10.1016/j.eururo.2020.06.002

57. Zareba P, Duivenvoorden W, Leong DP, Pinthus JH. Androgen deprivation therapy and cardiovascular disease: What is the linking mechanism? *Therapeutic Advances in Urology*. 2016;8(2):118-129. doi:10.1177/1756287215617872

58. Melloni C, Slovin SF, Blemings A, et al. Cardiovascular Safety of Degarelix Versus Leuprolide for Advanced Prostate Cancer. *JACC: CardioOncology*. 2020;2(1):70-81. doi:10.1016/j.jaccao.2020.01.004

59. Charbit B, Christin-Maître S, Démolis JL, Soustre E, Young J, Funck-Brentano C. Effects of Testosterone on Ventricular Repolarization in Hypogonadic Men. *American Journal of Cardiology*. 2009;103(6):887-890. doi:10.1016/j.amjcard.2008.11.041

60. Nielsen JB, Graff C, Pietersen A, et al. J-shaped association between QTc interval duration and the risk of atrial fibrillation: Results from the copenhagen ECG study. *Journal of the American College of Cardiology*. 2013;61(25):2557-2564. doi:10.1016/j.jacc.2013.03.032

61. Jones RD, English KM, Pugh PJ, Morice AH, Jones TH, Channer KS. Pulmonary vasodilatory action of testosterone: Evidence of a calcium antagonistic action. *Journal of Cardiovascular Pharmacology*. 2002;39(6):814-823. doi:10.1097/00005344-200206000-00006

62. Cairrão E, Álvarez E, Santos-Silva AJ, Verde I. Potassium channels are involved in testosterone-induced vasorelaxation of human umbilical artery. *Naunyn-Schmiedeberg's Archives of Pharmacology*. 2008;376(5):375-383. doi:10.1007/s00210-007-0213-3

63. Golden KL, Marsh JD, Jiang Y, Moulden J. Acute actions of testosterone on contractile function of isolated rat ventricular myocytes. *European Journal of Endocrinology*. 2005;152(3):479-483. doi:10.1530/eje.1.01845

64. Ceballos G, Figueroa L, Rubio I, et al. Acute and Nongenomic Effects of Testosterone on Isolated and Perfused Rat Heart. *Journal of Cardiovascular Pharmacology*. 1999;33(5):691-697. doi:10.1097/00005344-199905000-00003

65. Nettleship JE, Jones TH, Channer KS, Jones RD. Physiological testosterone replacement therapy attenuates fatty streak formation and improves high-density lipoprotein cholesterol in the Tfm mouse: An effect that is independent of the classic androgen receptor. *Circulation*. 2007;116(21):2427-2434. doi:10.1161/CIRCULATIONAHA.107.708768

66. Mukherjee TK, Dinh H, Chaudhuri G, Nathan L. Testosterone attenuates expression of vascular cell adhesion molecule-1 by conversion to estradiol by aromatase in endothelial cells: Implications in atherosclerosis. *Proceedings of the National Academy of Sciences of the United States of America*. 2002;99(6):4055-4060. doi:10.1073/pnas.052703199

67. Mäkinen J, Järvisalo MJ, Pöllänen P, et al. Increased carotid atherosclerosis in andropausal middle-aged men. *Journal of the American College of Cardiology*. 2005;45(10):1603-1608. doi:10.1016/j.jacc.2005.01.052

68. Mathur A, Malkin C, Saeed B, Muthusamy R, Hugh Jones T, Channer K. Long-term benefits of testosterone replacement therapy on angina threshold and atheroma in men. *European Journal of Endocrinology*. 2009;161(3):443-449. doi:10.1530/EJE-09-0092

69. Zitzmann M. Testosterone deficiency, insulin resistance and the metabolic syndrome. *Nature Reviews Endocrinology*. 2009;5(12):673-681. doi:10.1038/nrendo.2009.212

70. Jones Th, Kelly D. Randomized controlled trials – mechanistic studies of testosterone and the cardiovascular system. *Asian Journal of Andrology*. 2018;20(2):120. doi:10.4103/aja.aja_6_18

71. Oskui PM, French WJ, Herring MJ, Mayeda GS, Burstein S, Kloner RA. Testosterone and the cardiovascular system: a comprehensive review of the clinical literature. *Journal of the American Heart Association*. 2013;2(6). doi:10.1161/JAHA.113.000272

72. Ajayi AAL, Halushka P v. Castration reduces platelet thromboxane A2 receptor density and aggregability. *QJM - Monthly Journal of the Association of Physicians*. 2005;98(5):349-356. doi:10.1093/qjmed/hci054

73. Webb CM, Elkington AG, Kraidly MM, Keenan N, Pennell DJ, Collins P. Effects of Oral Testosterone Treatment on Myocardial Perfusion and Vascular Function in Men With Low Plasma Testosterone and Coronary Heart Disease. *American Journal of Cardiology*. 2008;101(5):618-624. doi:10.1016/j.amjcard.2007.09.114

74. Shahwan MM, Spathis GS, Fry DE, Wood PJ, Marks V. Differences in pituitary and testicular function between diabetic patients on insulin and oral anti-diabetic agents. *Diabetologia*. 1978;15(1):13-17. doi:10.1007/BF01219321

75. Corona G, Monami M, Rastrelli G, et al. Type 2 diabetes mellitus and testosterone: a meta-analysis study. *International Journal of Andrology*. 2011;34(6pt1):528-540. doi:10.1111/j.1365-2605.2010.01117.x

76. Heufelder AE, Saad F, Bunck MC, Gooren L. Fifty-two-Week Treatment With Diet and Exercise Plus Transdermal Testosterone Reverses the Metabolic Syndrome and Improves

Glycemic Control in Men With Newly Diagnosed Type 2 Diabetes and Subnormal Plasma Testosterone. *Journal of Andrology*. 2009;30(6):726-733. doi:10.2164/jandrol.108.007005

77. Inaba M, Otani Y, Nishimura K, et al. Marked hyperglycemia after androgen-deprivation therapy for prostate cancer and usefulness of pioglitazone for its treatment. *Metabolism: Clinical and Experimental*. 2005;54(1):55-59. doi:10.1016/j.metabol.2004.07.010

78. Dowman JK, Hopkins LJ, Reynolds GM, et al. Loss of 5α-Reductase Type 1 Accelerates the Development of Hepatic Steatosis but Protects Against Hepatocellular Carcinoma in Male Mice. *Endocrinology*. 2013;154(12):4536-4547. doi:10.1210/en.2013-1592

79. Paik SG, Michelis MA, Kim YT, Shin S. Induction of insulin-dependent diabetes by streptozotocin. Inhibition by estrogens and potentiation by androgens. *Diabetes*. 1982;31(8):724-729. doi:10.2337/diab.31.8.724

80. Palomar-Morales M, Morimoto S, Mendoza-Rodríguez CA, Cerbón MA. The Protective Effect of Testosterone on Streptozotocin-Induced Apoptosis in β Cells Is Sex Specific. *Pancreas*. 2010;39(2):193-200. doi:10.1097/MPA.0b013e3181c156d9

81. Ballester J. Tungstate Treatment Improves Leydig Cell Function in Streptozotocin-Diabetic Rats. *Journal of Andrology*. 2005;26(6):706-715. doi:10.2164/jandrol.04156

82. Pitteloud N, Hardin M, Dwyer AA, et al. Increasing insulin resistance is associated with a decrease in Leydig cell testosterone secretion in men. *Journal of Clinical Endocrinology and Metabolism*. 2005;90(5):2636-2641. doi:10.1210/jc.2004-2190

83. Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF, Bhasin S. Androgens Stimulate Myogenic Differentiation and Inhibit Adipogenesis in C3H 10T1/2 Pluripotent Cells

through an Androgen Receptor-Mediated Pathway. *Endocrinology*. 2003;144(11):5081-5088. doi:10.1210/en.2003-0741

84. Singh R, Artaza JN, Taylor WE, et al. Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: Nuclear translocation of androgen receptor complex with β-catenin and T-cell factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors. *Endocrinology*. 2006;147(1):141-154. doi:10.1210/en.2004-1649

85. Gupta V, Bhasin S, Guo W, et al. Effects of dihydrotestosterone on differentiation and proliferation of human mesenchymal stem cells and preadipocytes. *Molecular and Cellular Endocrinology*. 2008;296(1-2):32-40. doi:10.1016/j.mce.2008.08.019

86. Caprio M, Isidori AM, Carta AR, Moretti C, Dufau ML, Fabbri A. Expression of functional leptin receptors in rodent Leydig cells. *Endocrinology*. 1999;140(11):4939-4947. doi:10.1210/endo.140.11.7088

87. Oskui PM, French WJ, Herring MJ, Mayeda GS, Burstein S, Kloner RA. Testosterone and the cardiovascular system: a comprehensive review of the clinical literature. *Journal of the American Heart Association*. 2013;2(6). doi:10.1161/JAHA.113.000272

88. Lorigo M, Mariana M, Lemos MC, Cairrao E. Vascular mechanisms of testosterone: The non-genomic point of view. *Journal of Steroid Biochemistry and Molecular Biology*. 2020;196:105496. doi:10.1016/j.jsbmb.2019.105496

89. Kapoor D, Clarke S, Stanworth R, Channer KS, Jones TH. The effect of testosterone replacement theraphy on adipocytokines and C-reactive protein in hypogonadal men with

type 2 diabetes. *European Journal of Endocrinology*. 2007;156(5):595-602. doi:10.1530/EJE-06-0737

90. Crisostomo PR, Wang M, Wairiuko GM, Morrell ED, Meldrum DR. Brief exposure to exogenous testosterone increases death signaling and adversely affects myocardial function after ischemia. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*. 2006;290(5):R1168-R1174. doi:10.1152/ajpregu.00833.2005

91. Rettew JA, Huet-Hudson YM, Marriott I. Testosterone Reduces Macrophage Expression in the Mouse of Toll-Like Receptor 4, a Trigger for Inflammation and Innate Immunity. *Biology of Reproduction*. 2008;78(3):432-437. doi:10.1095/biolreprod.107.063545

92. Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, Jones TH. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *Journal of Clinical Endocrinology and Metabolism*. 2004;89(7):3313-3318. doi:10.1210/jc.2003-031069

93. Hong CY, Park JH, Ahn RS, et al. Molecular Mechanism of Suppression of Testicular Steroidogenesis by Proinflammatory Cytokine Tumor Necrosis Factor Alpha. *Molecular and Cellular Biology*. 2004;24(7):2593-2604. doi:10.1128/mcb.24.7.2593-2604.2004

94. Rennie K, McCarthy N, Yazdgerdi S, Marmot M, Brunner E. Association of the metabolic syndrome with both vigorous and moderate physical activity. *International Journal of Epidemiology*. 2003;32(4):600-606. doi:10.1093/ije/dyg179

95. Kriska AM. Physical Activity, Obesity, and the Incidence of Type 2 Diabetes in a High-Risk Population. *American Journal of Epidemiology*. 2003;158(7):669-675. doi:10.1093/aje/kwg191

96. Lahti-Koski M, Pietinen P, Heliövaara M, Vartiainen E. Associations of body mass index and obesity with physical activity, food choices, alcohol intake, and smoking in the 1982–1997 FINRISK Studies. *The American Journal of Clinical Nutrition*. 2002;75(5):809-817. doi:10.1093/ajcn/75.5.809

97. Hu G, Barengo NC, Tuomilehto J, Lakka TA, Nissinen A, Jousilahti P. Relationship of Physical Activity and Body Mass Index to the Risk of Hypertension: A Prospective Study in Finland. *Hypertension*. 2004;43(1):25-30. doi:10.1161/01.HYP.0000107400.72456.19

98. Young DR, Hivert MF, Alhassan S, et al. Sedentary behavior and cardiovascular morbidity and mortality: A science advisory from the American Heart Association. *Circulation*. 2016;134(13):e262-e279. doi:10.1161/CIR.0000000000000440

99. Martin BJ, Arena R, Haykowsky M, et al. Cardiovascular fitness and mortality after contemporary cardiac rehabilitation. *Mayo Clinic Proceedings*. 2013;88(5):455-463. doi:10.1016/j.mayocp.2013.02.013

100. Heufelder AE, Saad F, Bunck MC, Gooren L. Fifty-two-week treatment with diet and exercise plus transdermal testosterone reverses the metabolic syndrome and improves glycemic control in men with newly diagnosed type 2 diabetes and subnormal plasma testosterone. *Journal of Andrology*. 2009;30(6):726-733. doi:10.2164/jandrol.108.007005

101. Bhasin S, Storer TW, Berman N, et al. The Effects of Supraphysiologic Doses of Testosterone on Muscle Size and Strength in Normal Men. *New England Journal of Medicine*. 1996;335(1):1-7. doi:10.1056/NEJM199607043350101

102. Shiels MS, Rohrmann S, Menke A, et al. Association of cigarette smoking, alcohol consumption, and physical activity with sex steroid hormone levels in US men. *Cancer Causes and Control*. 2009;20(6):877-886. doi:10.1007/s10552-009-9318-y

103. Muller M, den Tonkelaar I, Thijssen JHH, Grobbee DE, van der Schouw YT. Endogenous sex hormones in men aged 40-80 years. *European Journal of Endocrinology*. 2003;149(6):583-589. doi:10.1530/eje.0.1490583

104. Camacho EM, Huhtaniemi IT, O'Neill TW, et al. Age-associated changes in hypothalamic-pituitary-testicular function in middle-aged and older men are modified by weight change and lifestyle factors: longitudinal results from the European Male Ageing Study. *European journal of endocrinology / European Federation of Endocrine Societies*. 2013;168(3):445-455. doi:10.1530/EJE-12-0890

105. Kumagai H, Zempo-Miyaki A, Yoshikawa T, Tsujimoto T, Tanaka K, Maeda S. Increased physical activity has a greater effect than reduced energy intake on lifestyle modification-induced increases in testosterone. *Journal of Clinical Biochemistry and Nutrition*. 2016;58(1):84-89. doi:10.3164/jcbn.15-48

106. Kumagai H, Yoshikawa T, Zempo-Miyaki A, et al. Vigorous Physical Activity is Associated with Regular Aerobic Exercise-Induced Increased Serum Testosterone Levels in Overweight/Obese Men. *Hormone and Metabolic Research*. 2018;50(1):73-79. doi:10.1055/s-0043-117497

107. Kraemer WJ, Häkkinen K, Newton RU, et al. Acute hormonal responses to heavy resistance exercise in younger and older men. *European Journal of Applied Physiology and Occupational Physiology*. 1998;77(3):206-211. doi:10.1007/s004210050323

Figure Legends

Figure 1. Physiological Effects of Testosterone. Testosterone has effects on multiple organs, many of which have direct and/or indirect implications on cardiovascular health. * *indicates inconclusive research on the specific effect.*

Figure 2. Biochemical Pathway of Testosterone. Testosterone, synthesized from cholesterol following LH stimulation, travels through the bloodstream from Leydig cells to target cells. Testosterone can be converted to DHT or E2. Testosterone and DHT bind to the AR to regulate androgen-responsive genes. Testosterone can also act via non-genomic pathways. *AR: androgen receptor, ARE: androgen response element. CR: coregulator, ER: estrogen receptor, HSP: heat shock protein, SHBG: sex-hormone binding globulin, T: testosterone.*

Figure 3. Effect of Testosterone on Cardiovascular Risk. Graphical depiction of the hypothesized mechanisms of action of testosterone on various cardiovascular risk factors.* *indicates research in the area is inconclusive.* ⇔ *indicates a likely bidirectional relationship.*

Figure 1

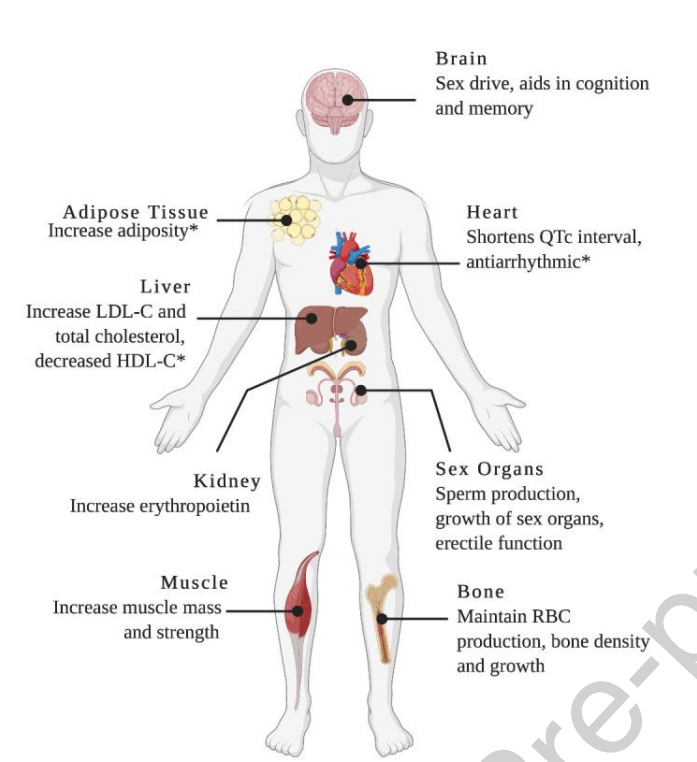

Figure 2

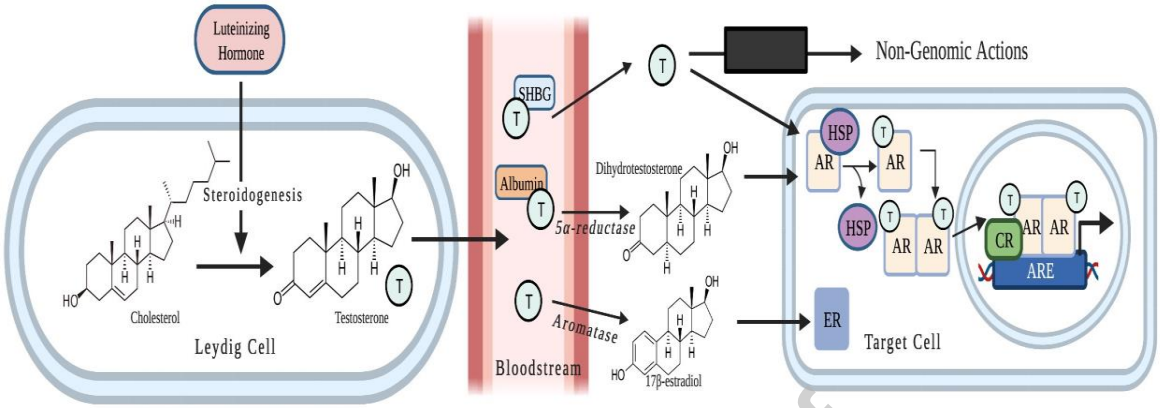

Figure 3