Randomized controlled trials-mechanistic studies of testosterone and the cardiovascular system

JONES, TH and KELLY, Daniel <http://orcid.org/0000-0002-7463-0692>

Asian Journal of Andrology (2018) 20, 120–130
www.asiaandro.com; www.ajandrology.com

Open Access

INVITED REVIEW

Male Endocrinology

Randomized controlled trials – mechanistic studies of testosterone and the cardiovascular system

T Hugh Jones[1,2], Daniel M Kelly[2,3]

Testosterone deficiency is common in men with cardiovascular disease (CVD), and randomized placebo-controlled trials (RCTs) have reported beneficial effects of testosterone therapy on exercise-induced cardiac ischemia in chronic stable angina, functional exercise capacity, maximum oxygen consumption during exercise (VO_{2max}) and muscle strength in chronic heart failure (CHF), shortening of the Q-T interval, and improvement of some cardiovascular risk factors. Testosterone deficiency is associated with an adverse CV risk profile and mortality. Clinical and scientific studies have provided mechanistic evidence to support and explain the findings of the RCTs. Testosterone is a rapid-onset arterial vasodilator within the coronary circulation and other vascular beds including the pulmonary vasculature and can reduce the overall peripheral systemic vascular resistance. Evidence has demonstrated that testosterone mediates this effect on vascular reactivity through calcium channel blockade (L-calcium channel) and stimulates potassium channel opening by direct nongenomic mechanisms. Testosterone also stimulates repolarization of cardiac myocytes by stimulating the ultra-rapid potassium channel-operated current. Testosterone improves cardiac output, functional exercise capacity, VO_{2max} and vagally mediated arterial baroreceptor cardiac reflex sensitivity in CHF, and other mechanisms. Independent of the benefit of testosterone on cardiac function, testosterone substitution may also increase skeletal muscle glucose metabolism and enhance muscular strength, both factors that could contribute to the improvement in functional exercise capacity may include improved glucose metabolism and muscle strength. Testosterone improves metabolic CV risk factors including body composition, insulin resistance, and hypercholesterolemia by improving both glucose utilization and lipid metabolism by a combination of genomic and nongenomic actions of glucose uptake and utilization expression of the insulin receptor, glucose transporters, and expression on regulatory enzymes of key metabolic pathways. The effect on high-density lipoprotein-cholesterol (HDL-C) differs between studies in that it has been found to fall, rise, or have no change in levels. Testosterone replacement can suppress the levels of circulating pro-inflammatory cytokines and stimulate the production of interleukin-10 (IL-10) which has anti-inflammatory and anti-atherogenic actions in men with CVD. No effect on C-reactive protein has been detected. No adverse effects on clotting factors have been detected. RCTs have not clearly demonstrated any significant evidence that testosterone improves or adversely affects the surrogate markers of atherosclerosis such as reduction in carotid intima thickness or coronary calcium deposition. Any effect of testosterone on prevention or amelioration of atherosclerosis is likely to occur over years as shown in statin therapy trials and not months as used in testosterone RCTs. The weight of evidence from long-term epidemiological studies supports a protective effect as evidenced by a reduction in major adverse CV events (MACEs) and mortality in studies which have treated men with testosterone deficiency. No RCT where testosterone has been replaced to the normal healthy range has reported a significant benefit or adverse effect on MACE nor has any recent meta-analysis.

Asian Journal of Andrology (2018) **20**, 120–130; doi: 10.4103/aja.aja_6_18; published online: 9 February 2018

Keywords: cardiovascular; testosterone; testosterone replacement

INTRODUCTION

Evidence from basic science and clinical studies has demonstrated that testosterone does affect the cardiovascular system in health and disease. The effects of testosterone may be different between normal physiological conditions compared with pathophysiological states. Testosterone has been shown to influence vascular reactivity with differences between vascular beds and tissue-specific systemic blood supply.[1] Furthermore, testosterone can affect peripheral vascular resistance, cardiac electrophysiology, and cardiac output.[2–4] Several epidemiological studies have shown an association of low testosterone with cardiovascular disease and conditions such as metabolic syndrome and type 2 diabetes, which have increased cardiovascular risk.[5] Whether or not low testosterone is merely a consequence of the underlying disease process or promotes the atherosclerotic progression is currently not fully understood. The major question that needs to be understood is whether or not testosterone ameliorates and/or stabilizes the atherosclerotic plaque. It is important to recognize that the effects of testosterone will depend on normalization of levels for an individual as benefits may not occur if there is undertreatment or overtreatment as is well recognized for many hormones, for example, thyroxine

[1]Robert Hague Centre for Diabetes and Endocrinology, Barnsley Hospital NHS Foundation Trust, Barnsley, UK; [2]Academic Unit of Diabetes, Endocrinology and Metabolism, Department of Oncology and Metabolism, University of Sheffield Medical School, Sheffield, UK; [3]Biomedical Research Centre, Sheffield Hallam University, Sheffield, UK.
Correspondence: Prof. TH Jones (hugh.jones@nhs.net or t.h.jones@sheffield.ac.uk)
Received: 07 November 2017; Accepted: 12 January 2018

replacement in hypothyroidism. This review will examine and discuss the current state of knowledge of the physiological mechanisms from randomized clinical trials (RCTs) to try and provide a considered analysis of current knowledge.

Prior to embarking on reviewing the available evidence, it is important to understand the biological parameters that contribute to normal androgenization. The actions of testosterone are mainly mediated by androgen receptor (AR) which binds to specific androgen response elements in the promoter regions of target genes. However, it has become increasingly evident that testosterone in common with other steroid hormones also has rapid-onset nongenomic actions, possibly AR-independent genomic mechanisms and effects mediated by its conversion to other active hormones – estradiol and dihydrotestosterone.

The AR CAG repeat polymorphism, which ranges from 9 to 35 repeats, is associated with receptor sensitivity, with the lower number of repeats having the greatest sensitivity.[6] The importance of AR sensitivity is reflected in significant impact on the phenotype. Men with greater receptor sensitivity usually have low normal serum testosterone levels whereas those with reduced AR sensitivity have levels in the upper normal range. The circulating levels of testosterone in turn are controlled by feedback on the hypothalamic–pituitary axis. The implications of this will be discussed further in the manuscript. Total testosterone in the circulation comprises free, albumin-bound, and sex hormone binding globulin (SHBG)-bound testosterone. The biologically available active hormone component is thought to be the sum of the free and albumin-bound testosterone (bioavailable testosterone) as testosterone bound to SHBG is tightly bound and dissociates slowly and is therefore considered to be relatively inactive.

ANGINA AND CARDIAC ISCHEMIA

RCTs of testosterone treatment in men with cardiac ischemia

A case series (published in 1946) that combined several case reports found that testosterone therapy improved or abolished symptomatic angina chest pain in the majority of patients treated.[7] However, the first randomized placebo-controlled trial using testosterone therapy in men with proven exercise-induced cardiac ischemia was reported in 1977.[8] Postexercise ST segment depression was assessed as the sum of measurements taken from electrocardiogram (ECG) leads II, V4–V6 at baseline and 2, 4, and 6 min of exercise. The sum of ST depression was reduced by 31.7% after 4 weeks' therapy with testosterone cypionate (200 mg intramuscular injection [i.m.] weekly) and by 51.2% after 8 weeks. At 1 month, a placebo-controlled crossover study with oral testosterone reported a 77.4% reduction in angina symptoms, 68.4% improvement in cardiac ischemia on a standard ECG, and 75% on an ECG Holter recording.[9]

In a definitive RCT, men with chronic stable angina already treated with two or more anti-angina medications and unselected for testosterone deficiency (mean total testosterone active group 13.55 ± 0.78 nmol l^{-1}, placebo 12.38 ± 0.72 nmol l^{-1}, $P = 0.3$) were treated with a daily 5 mg testosterone patch or placebo to achieve total testosterone (TT) in mid-normal physiological range (18.57 ± 1.6 nmol l^{-1}) which demonstrated cardiac improvements.[10] No significant change in estradiol was observed. Testosterone therapy led to a significant prolongation in exercise-induced time to 1 mm ST segment depression after 1 month, which was further improved after 3 months. The lower the baseline serum testosterone, the greater the response to testosterone therapy in reduction in exercise-induced cardiac ischemia. This finding was supported by a subsequent RCT that recruited men with angina and low testosterone (mean baseline TT 4.2 ± 0.5 nmol l^{-1}) replaced to normal physiological levels.[11] The beneficial effect of testosterone on cardiac ischemia persists for at least 12 months with no evidence of tachyphylaxis (baseline TT 9.9 ± 2.2 nmol l^{-1} replaced to the normal range by depot testosterone undecanoate, which increased by TT 9.2 + 8.5 nmol l^{-1}).[12] Supportive evidence has been provided by more recent RCT of elderly men with type 2 diabetes and proven CVD ($n = 85$) using oral testosterone undecanoate over 12 weeks. There was a reduction in the frequency of angina episodes and a decrease in the number of daily ischemic episodes and total ischemic burden using an ambulatory ECG recording.[13] A smaller 2-month RCT with crossover design ($n = 25$) again using oral testosterone undecanoate did not detect any increase in overall myocardial perfusion using magnetic resonance imaging (MRI) but did report that coronary arteries without stenosis had increased vascular perfusion.[14]

The potential mechanisms by which chronic testosterone therapy improves cardiac ischemia include enhancement of coronary blood flow, increased red cell hemoglobin concentration, and enhanced myocardial function. Two studies have reported that administration of intravenous testosterone just prior to exercise treadmill testing demonstrated an acute effect on reduction in cardiac ischemia.[15–17] Administration of testosterone (including physiological concentrations) directly into unobstructed (<50% stenosis) coronary arteries at angiography, in men with proven coronary artery disease, which had been preconstricted with acetylcholine, resulted in a rapid (within 2–3 min) vasodilation and increase in coronary blood flow.[18] This finding provides key evidence for an acute vasodilator action of testosterone on the coronary vasculature that excludes the chronic effects that may occur in prolonged treatment, but pharmacological doses of testosterone were used.

Mechanisms of action of testosterone effects on vasoreactivity

The clinical trials demonstrate that testosterone has acute and chronic responses on exercise-induced cardiac ischemia. The rapid-onset effect on vasodilatation within a few minutes implies a nongenomic action. The finding that the benefit on exercise-induced ischemia improves with time over 3 months suggests that testosterone has additional actions which could be mediated through genomic as well as nongenomic mechanisms. Furthermore, the effect of testosterone on exercise-induced cardiac ischemia inversely correlates with baseline testosterone. This suggests that coronary arteries are more sensitive to testosterone in men with greater degree of testosterone deficiency.

Laboratory studies on isolated vessels using wire and perfusion myography have shown that testosterone has a direct rapid effect on rat, rabbit, and porcine preconstricted isolated coronary arteries.[1,19,20] Testosterone has also been found to dilate arteries from systemic arteries (radial, iliac, subcutaneous gluteal, mammary, and prostatic) and other vascular beds (pulmonary and mesenteric).[1] This effect is independent of the vascular endothelium and is a direct effect on the vascular smooth muscle membrane.[1] High-dose concentrations are necessary to induce the vasoactive effects of testosterone in isolated vessels. Although this is a limitation on whether or not the vasoactive effect is physiological and/or pharmacological, it is well recognized that established vasoactive agents *in vivo* such as noradrenaline, acetylcholine, and chromokalin (a potassium channel opener) all require supraphysiological concentrations *in vitro*.[21] Moreover, these effects are supported by evidence that direct actions of testosterone on ion channels occur at physiological concentrations.[22,23]

Studies have shown that testosterone acts as a calcium channel blocker and also stimulates potassium channel opening in vascular smooth muscle.[1,24] Testosterone in single-cell patch clamping studies inactivates L-calcium channels in rat vascular smooth muscle cells

and human embryonic renal cells (HEK293) transfected with the α1C-subunit of the L-calcium channel that forms the channel pore.[22,23,25] Clinically used L-calcium channel blockers (*e.g.*, nifedipine, amlodipine) bind to a specific site on α1C-subunit to mediate their inhibitory effect. HEK293 cells, which have been transfected with a deactivating point mutation within the nifedipine-binding site, lose the ability to respond to testosterone.[25] This work clearly demonstrates that testosterone is an L-calcium channel blocker, which mediates in part the vasodilator action. Furthermore, testosterone has been found to inhibit receptor-operated calcium and intracellular store-operated calcium channels that result in reduced cytosolic calcium concentration and smooth muscle relaxation.[1,24]

There are also experimental data that testosterone has a potassium channel opening effect that would induce arterial vasodilation.[1,24] Testosterone stimulation of potassium channel activation has been reported in animal coronary arteries as well as other systemic and mesenteric vessels. The effect of testosterone on vascular reactivity is complex and may differ between arteries. These differences in effect may have actions that influence arterial tone during certain physiological situations.

A human study examined the effect of testosterone therapy on vascular reactivity in isolated human subcutaneous arteries.[21] This study compared the effect of chronic testosterone therapy on isolated subcutaneous resistance gluteal arteries taken before and 3 months after high-dose testosterone treatment in men with chronic heart failure. The effect of testosterone on isolated gluteal vessels before testosterone therapy was also compared with gluteal vessels from a group of healthy eugonadal men. Testosterone induced significant vasorelaxation in constricted gluteal vessels from men with CHF with low serum testosterone concentrations compared to healthy controls and men with CHF and normal serum testosterone concentrations. After 3 months' testosterone replacement in the hypogonadal subgroup, the vascular reactivity to testosterone in the isolated vessels became equivalent to the response observed in healthy and eugonadal men with heart failure. Importantly after testosterone therapy, the vasoconstrictor effect of noradrenaline increased and reduced the vasodilator response to acetylcholine and sodium nitroprusside (which breaks down to nitric oxide in the circulation). This effect physiologically would divert blood flow toward the vital organs, an important survival effect in chronic heart failure.

Testosterone is converted by aromatase to estradiol, which is a known vasoactive hormone. Estradiol mediates its vasoactive effects through stimulation of nitric oxide production. There is evidence from some studies that testosterone can stimulate the expression of nitric oxide synthase, which in turn would increase the capacity for greater nitric oxide release. However, it is not known whether this is a direct effect of testosterone or an indirect effect via estrogen.[1] Testosterone-stimulated nitric oxide is known to promote cyclic guanosine monophosphate (cGMP) release. cGMP is important in the vasorelaxation of vascular smooth muscle in the induction and maintenance of the penile erection.[26] This effect might be tissue specific, but effects of testosterone induction of tissue nitric oxide might have a role in other tissues.

CHRONIC HEART FAILURE

RCTs of testosterone treatment in men with CHF

Chronic heart failure (CHF) is a common clinical condition associated with high morbidity and mortality rates and a 5-year survival that is worse than many common cancers.[27] Low serum testosterone concentrations are common in men with CHF and might contribute to the degree of cardiac dysfunction and cachexia. The acute and chronic effects of testosterone therapy in CHF have been investigated by RCTs.

The effect of acute testosterone administration has been studied in one RCT in men ($n = 12$) with moderate-to-severe left ventricular dysfunction with a mean total testosterone level of 14.1 nmol l^{-1} (normal range: 7.5–37 nmol l^{-1}).[2] Patients were admitted to hospital for 2 days receiving placebo or testosterone (60 mg buccal tablet) and hemodynamic measurements were assessed by balloon flotation catheter inserted into the pulmonary circulation and cardiac output was measured by thermo-dilution technique. Pulmonary capillary wedge pressure, pulmonary artery pressure, systemic vascular resistance as well as cardiac output were measured over 8 h. The two key findings of this study are that testosterone reduced the peripheral vascular resistance and improved the cardiac output. These findings support a rapid-onset effect of testosterone on reducing peripheral vascular tone. The absence of an acute improvement in pulmonary wedge pressure suggests that the reduction in peripheral vascular resistance is the major contributor to improved left ventricular function. Direct effects of testosterone on cardiac myocytes such as improved vascular blood flow and glucose utilization may also be involved.

RCTs in men with CHF have used low doses of testosterone to avoid overtreatment as high dosages of anabolic-androgenic steroids can cause myocardial stiffening and hypertrophy.[28] The first RCT used intramuscular combined testosterone esters (Sustanon®) 100 mg every 2 weeks for 12 weeks in men ($n = 20$) with moderate heart failure (ventricular ejection fraction 35%).[29] This study was the first to report an improvement in functional exercise capacity that was associated with a beneficial effect on symptoms assessed by the Minnesota Living with Heart Failure questionnaire.[29]

In a larger ($n = 76$) and greater duration (12-month) study, men with chronic heart failure with a mean ventricular ejection fraction of 32.5% were randomized to testosterone 5 mg patch or placebo.[30] Functional exercise capacity, as assessed using the incremental shuttle walk test (which correlates with VO_{2max}), significantly improved from baseline in the testosterone-treated patients but declined in the placebo group. Serial echocardiography demonstrated a significant increase in left ventricular length with testosterone treatment that correlates with the efficiency of cardiac output. In cardiac failure, the heart becomes more globular with reduced left ventricular length and efficacy (Starling's law of the heart). This study also showed that there was a trend to reduction in left ventricular mass. Importantly, mean blood pressure was maintained in the treated patients but declined in the placebo group. The worsening of blood pressure and of ventricular ejection fraction indicated worsening heart failure in the placebo-treated group. [30] Supporting information from this study that testosterone has a beneficial effect on cardiac function was evidence that there was a significant improvement in New York Heart Association Class score of heart failure in 33% of patients. Furthermore, a greater increase in serum testosterone level correlated with a greater benefit on functional exercise capacity. Hand grip strength also improved in the dominant hand, a finding suggesting that improved overall skeletal muscle strength might contribute to the observed improved exercise capacity. There was no excess of serious adverse events in the testosterone-treated patients, and it is important to recognize that the beneficial effects in this trial occurred with a small increase in total testosterone of only 6 nmol l^{-1}.

The beneficial effects of testosterone in CHF were confirmed by a 12-week study of seventy men with CHF (NYHA II or II with a left ventricular ejection fraction of <40%).[31] The patients received either intramuscular depot testosterone undecanoate or placebo. The testosterone-treated group had significant improvements compared to placebo in the 6-min walk test and VO_{2max}. This trial also found that testosterone therapy improved the vagal nerve-mediated arterial baroreceptor cardiac reflex sensitivity (BRS), a reflex which is known to be suppressed in CHF and is associated with a poor prognosis. In another RCT (n = 50) with CHF, testosterone therapy over 12 weeks resulted in significant improvement in diastolic function, distance traveled in the 6-min walk test, and quality-of-life scores.[32]

Mechanisms of action of testosterone effects in CHF

Chronic heart failure is associated with adaptive changes in vascular tone of the circulation, increasing peripheral vasoconstriction. This response is mediated initially by acute changes in systemic neurovascular hormones, mainly noradrenaline and adrenaline, which persist in chronic heart failure and cause adverse effects on cardiac function in the long term.[33] CHF is also associated with a decrease in baroreceptor sensitivity.[33] Arterial baroreceptors, which are mainly situated in the carotid sinuses and aortic arch and are supplied by the vagus nerve, are important stretch sensors of arterial wall pressure changes. The most common cause of cardiac failure and impaired left ventricular dysfunction is coronary artery disease with resultant cardiac ischemia leading to diminished cardiac muscle contractility and weakness. Systemic effects of cardiac failure include fatigue, lethargy, and breathlessness but also a chronic inflammatory state, insulin resistance, and an anabolic–catabolic imbalance, eventually leading to cardiac cachexia. Loss of muscle mass aggravates the symptoms of CHF leading to increasing weakness and decreasing exercise capacity.

Low testosterone concentrations are common in men with CHF and are associated with an increased mortality. Testosterone deficiency might contribute to the morbidity of CHF because testosterone deficiency is associated with reduced cardiac function, loss of lean muscle mass and strength, chronic anemia, and insulin resistance.[34,35] Testosterone treatment therefore might improve the symptoms of CHF through several potential mechanisms (summarized in **Figure 1**). First, the acute effect of testosterone in reducing peripheral vascular resistance leads to an increased cardiac output.[2] It is not clear, however, that this effect is maintained over longer time periods. Second, testosterone-induced coronary vasodilation may improve blood flow to ischemic areas of the cardiac muscle, enhancing contraction. Third, it is known from *in vitro* studies that testosterone is an arterial and venous dilator which could potentially *in vivo* reduce pulmonary vessel pressure, reducing the preload pressure on the right side of the heart.[1] This however was not observed in the study when testosterone was acutely administered to men with chronic heart failure in terms of a reduction in pulmonary capillary wedge pressure.[2]

Testosterone replacement in men with CHF enhances carotid sinus baroreceptor sensitivity.[31] There is evidence that this action of testosterone may be mediated at the level of the brain stem as androgen receptor blockade inhibits baroreceptor responses.[36] Furthermore, androgen receptors have been detected in neurons in the brain stem which are known to be involved in blood pressure control.[37] This study also detected reduction in the gradient of ventilation to carbon dioxide output (VE/VCO_2 slope) and an increase in peak VO_2 and peak workload on testosterone therapy. The VE/VCO_2 slope reflects the increase in ventilation in response to CO_2 production and correlates with ventilator drive. The greater the VE/VO_2 slope, there is an increased risk of a major cardiac event. This specific effect on improved ventilation provides convincing evidence that this is a direct action of testosterone, which would contribute to the benefit on functional exercise capacity observed in all the RCTs described above.

Chronic anemia is common in men with CHF and an increase in hematocrit may improve the symptoms of CHF. Testosterone replacement can result in small rises in hemoglobin and hematocrit which have been detected in one study but not in the others.[30] Brain natriuretic peptide is a marker of the degree of heart failure. A trend toward lowering brain natriuretic peptide levels was found in one study, but this may have been underpowered to show a significant change.[29]

CHF is associated with reduced muscle mass and atrophy, which affects a greater proportion of type II muscle fibres.[38,39] It has been demonstrated in men with CHF that decreasing serum testosterone levels correlate with worsening functional exercise capacity.[35] Exercise training in men with CHF can improve muscle structure and function and the degree of ventilation.[40] The trials of testosterone therapy have shown an increase in dominant but not nondominant hand grip strength but no evidence of muscle bulk as assessed by CT scanning of mid-thigh and mid-calf after 3 months and 12 months.[28,29] Maximal voluntary contraction of the quadriceps and an increase in peak torque significantly increased in another trial.[31] This evidence overall shows that there may be a beneficial effect on muscle function, which is likely to be a contributory component to the reported improvement in functional exercise capacity.

CHF induces a state of chronic inflammation and insulin resistance that play a role in cardiac cachexia and could be important factors that impair skeletal muscle function and contribute to muscle atrophy.[41–43] Tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) are elevated in men with CHF.[41] These specific cytokines have all been shown to induce insulin resistance and have a major role in the effects of cachexia. Testosterone replacement therapy in men with cardiovascular disease suppresses circulating levels of TNF-α, which has also been implicated in CHF.[44] However, TNF-α was not suppressed over 3 months of testosterone treatment in men with CHF.[45]

There is some evidence that testosterone replacement therapy improves insulin sensitivity in states of insulin resistance in men with CHF and metabolic syndrome or type 2 diabetes.[31,46,47] The positive effect of testosterone on insulin resistance in CHF was demonstrated in a double-blind randomized placebo-controlled crossover trial over 3 months with patients randomized to testosterone (Sustanon® 250) or placebo for 4 weeks, and then a 4-week washout period followed by the opposite therapy.[47] Testosterone treatment significantly increased insulin sensitivity and reduced fasting glucose. There was no significant difference in glucose utilization or insulin release between treated and placebo groups in response to a standard glucose tolerance test.[47] This effect of testosterone on reducing insulin resistance was confirmed in a subsequent study, but fasting glucose was not reported.[31] Testosterone therapy also resulted in a small but significant reduction in body fat percentage.[31]

These findings collectively demonstrate that the effects of testosterone in men with CHF have multifactorial effects on

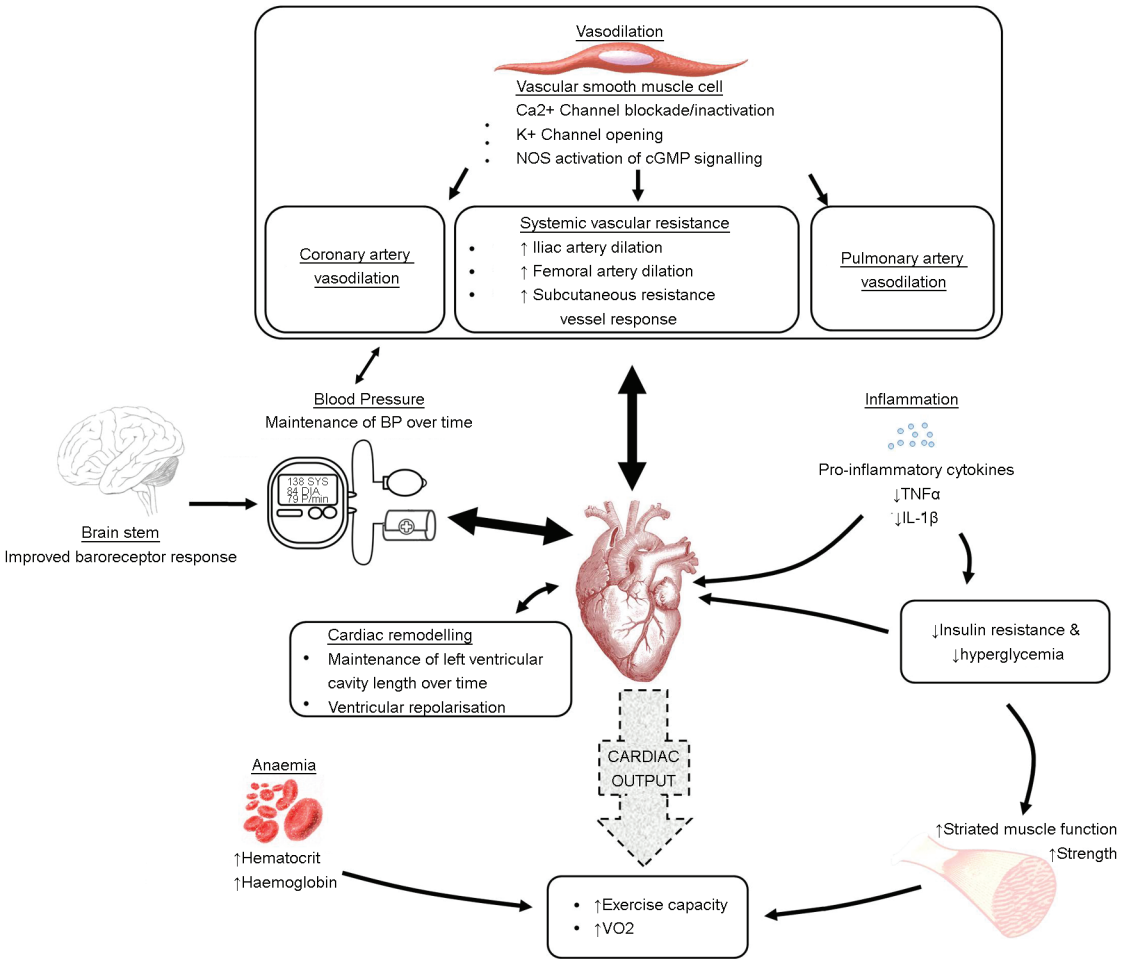

Figure 1: Putative beneficial effects of testosterone on heart failure. Evidence from multiple clinical, experimental, and mechanistic studies suggests potential beneficial testosterone actions on heart failure.

different systems and organs which all contribute independently to the clinical differences observed in improved cardiac and exercise function (**Table 1** and **Figure 1**).

CARDIAC ELECTROPHYSIOLOGY

RCTs of the effects of testosterone therapy on cardiac electrophysiology

The main effect of testosterone on the electrocardiogram is on the QT_c interval, which is a standardization value, which compensates for variability in the QT due to changes in heart rate. It is known that men have a shorter QT_c than women, a difference that is not present before puberty.[48] The Third National Health and Nutrition Examination Survey (NHANES) and the Multi-ethnic Study of Atherosclerosis reported that there was an inverse relationship between QT-interval duration and testosterone levels in men.[49] Evidence also demonstrates that men with hypogonadism, including men with obesity-related hypogonadism, have prolonged QT_c intervals.[50,51] The clinical importance of a prolonged QT_c is its association with an increased risk for ventricular tachycardia, ventricular fibrillation and torsades de points which can all cause sudden death.

Testosterone therapy at physiological dosages was reported to reduce QT dispersion (maximum QT – minimum QT interval) in a combined analysis of two RCTs of men with chronic stable angina and congestive cardiac failure, respectively.[3] A subsequent RCT of thirty men with chronic cardiac failure found that testosterone therapy also shortened the QT and QT_c intervals.[4]

Mechanisms of action of testosterone effects in cardiac electrophysiology

An animal study compared QT_c interval between orchidectomized testosterone-replaced and orchidectomized testosterone-nonreplaced mice.[52] Testosterone replacement significantly decreased ventricular repolarization by increasing the ultra-rapid potassium current, I (Kur), in isolated ventricular cardiac myocytes using whole cell voltage and current clamps. I (Kur), which is the major current involved in cardiac myocyte repolarization, is mediated by the Kv1.5 potassium channel. This study also demonstrated that testosterone increased protein expression of the Kv1.5 potassium channel. Another study found that testosterone correlated inversely with action potential duration and the risk of early after depolarizations in female rabbits.[53] A greater percentage of early after depolarizations is associated with an increased risk of arrhythmia.[54] These basic science experimental findings, although limited in number, support evidence from clinical trials for a potentially important role of testosterone as an anti-arrhythmic agent. It is also known that testosterone levels fall acutely after myocardial infarction and these studies raise the question as to whether or not low testosterone may increase the risk of post-myocardial infarction arrhythmias.[55]

Table 1: Summary of effects of testosterone therapy on cardiovascular parameters

Parameter	*Condition*	*Treatment effect*
Exercise-induced ischemia	Chronic stable angina	↑ Time to 1 mm ST depression
Myocardial perfusion	CAD	↑ Perfusion of coronary territories with no stenosis
Coronary artery blood flow	Coronary angiography	↑ Blood flow and artery diameter
Functional exercise capacity	CHF	↑ Distance (shuttle walk/6-min walk tests)
VO_{2max}	CHF	↑ VO_{2max}
BRS	CHF	↑ Carotid baroreceptor sensitivity
LV length	CHF	Maintained compared to ↓ placebo
Blood pressure	CHF	Maintained compared to ↓ placebo
NYHA	CHF	Improved class in 30%
Q-T interval	CAD, CHF	↓ QT, QT_c
Body composition	CAD, T2D	↓ Percentage fat mass, ↑ lean mass, ↓ WC
Cholesterol	CAD, T2D	↓ Total-C, ↓ LDL-C (some studies)
HDL-C	CAD, T2D	Different responses ↓, ↔, ↑ HDL-C
Triglycerides	CAD, T2D	No response
Insulin resistance	T2D, CHF	↓ Insulin resistance (majority confirmed by clamp studies)
HbA1c	T2D	↔ (unselected for poor control)
Fasting glucose	CHF, T2D	↓ CHF, ↔ or ↓ T2D
TNF-α	CAD, CHF	↓ CAD or ↔ CHF, CAD
IL-10	CAD	↑
tPA/PAI-1/ fibrinogen	CAD	↔

BRS: baroreceptor cardiac reflex sensitivity; LV: left ventricular; NYHA: New York Heart Association; HDL-C: high-density lipoprotein-cholesterol; HbA1c: glycated hemoglobin; TNF-α: tumor necrosis factor-α; IL-10: interleukin-10; tPA: tissue plasminogen activator; PAI-1: plasminogen activator inhibitor-1; CAD: coronary artery disease; CHF: chronic heart failure; T2D type 2 diabetes; WC: waist circumference; LDL-C: low-density lipoprotein-cholesterol; VO_{2max}: maximum oxygen consumption; ↑: increase; ↓: decrease; ↔: no significant change

EFFECTS OF TESTOSTERONE THERAPY ON CARDIOVASCULAR RISK AND ATHEROSCLEROSIS

Modifiable cardiovascular risk factors for myocardial infarction include diabetes (insulin resistance), abdominal obesity, hypertension, dyslipidemia, smoking, reduced physical activity, reduced daily fruit and vegetable consumption, regular alcohol consumption, and stress.[56] Testosterone deficiency in men is associated with insulin resistance (type 2 diabetes), abdominal obesity, hypertension, and dyslipidemia as well as lack of physical activity.[46,57] In most but not all studies, RCTs using testosterone replacement therapy have shown to improve insulin resistance, glycemic control, visceral adiposity, and cholesterol (**Table 1**).[58] Levels of HDL-cholesterol may fall, remain unchanged, or rise with testosterone replacement.

Insulin resistance and glycemic control

Insulin resistance is the major biochemical abnormality in men with metabolic syndrome or type 2 diabetes and is considered to be an intermediary cardiovascular risk factor that promotes hyperglycemia, dyslipidemia, hypertension, and endothelial dysfunction. In the presence of significant insulin resistance, testosterone replacement therapy increases insulin sensitivity. This effect is evident after 3 months' treatment and maintained for at least up to 12 months.[59–64] Initial trials measured insulin resistance using homeostatic mechanism of insulin resistance (HOMA-IR). A 12-month study showed that testosterone improved insulin sensitivity by 15%, an effect that is equivalent to that observed by metformin usage, a mainstay of management in type 2 diabetes.[62] One study did not show a change in HOMA-IR; however, the baseline mean HOMA-IR was in the normal insulin sensitivity range.[65] These findings have been subsequently confirmed using hyperinsulinemic euglycemic clamp studies which confirmed that testosterone therapy increased the glucose disposal rate as well as reducing HOMA-IR.[64]

Glycated hemoglobin (HbA1c) measurement is universally used in patients with diabetes for the assessment of glycemic control. The studies above have either shown a small but significant reduction in HbA1c or no effect. A meta-analysis of RCTs did not demonstrate an overall change in HbA1c but this has to be taken into context that no study exclusively had been performed on men with poor control.[66] In small studies including men with good control and not statistically powered for HbA1c improvement, it would be unlikely to observe a benefit. Clinical trials of new drugs for diabetes usually require large numbers with the study confined to men with HbA1c levels ≥6.5% or 7%, and the current trials may not have been of sufficient duration to detect a change and included men with good control. On this basis from RCTs, it is not known whether or not testosterone replacement has a positive effect on glycemic control. However, some studies have demonstrated that there is an improvement in HbA1c[59,62,63] and fasting glucose.[59,64]

Dyslipidemia

The response of serum lipid parameters to testosterone replacement in trials has been mixed. Specifically, total cholesterol and low-density lipoprotein-cholesterol (LDL-C) fall by between 5% and 14% from baseline in studies involving healthy men and men with CVD, metabolic syndrome, and type 2 diabetes.[11,13,44,59,62,63,67,68] However, some studies have shown no response at all.[67,68] The differences between formulations used, whether or not adequate testosterone levels were attained, age of population, statin therapy, and duration of the study may affect the result.

HDL-cholesterol also may rise, fall, or elicit no change in levels.[67–69] HDL-cholesterol after an initial decrease can return to baseline after prolonged replacement. One hypothesis is that testosterone first stimulates the reverse transport of excess cholesterol from peripheral tissues to the liver for excretion. This depletes the HDL-cholesterol pool before it recovers after stabilization of the body's cholesterol balance.

The majority of RCTs have not identified a significant change in triglycerides.[68] Lowering of fasting triglycerides has been evident in trials using the long-acting depot testosterone undecanoate (reference). Lipoprotein (a), a strong predictor for the future development of atherosclerosis, decreased in one trial of testosterone therapy by approximately 25%.[62] The clinical effect of this change is not known but potentially could be an important factor in reducing the development of CVD.

Body composition

RCTs of testosterone replacement therapy in hypogonadal men consistently report an increase in lean body mass and a decrease in percentage fat mass which is independent of the formulation of testosterone used in the study.[70] The majority of the studies also found a reduction in waist circumference, which correlates positively with

visceral adiposity.[70] Reductions in body weight may not be initially evident as the gain in lean mass balances out the fat loss which occurs.[60] In addition, the studies published are between 3 and 12 months and may not be of sufficient length to detect changes. Serum leptin, which positively correlates with fat mass, falls with testosterone replacement.[59,60] A registry study has demonstrated reduction in weight that continues for at least 8 years on testosterone replacement.[71]

Inflammation

Atherosclerosis is associated with a chronic inflammatory state that is reflected in the elevation of circulating cytokines. Some trials have recorded a suppression of TNF-α, whereas others have not.[11,44,45,60] Serum interleukin-1β and C-reactive protein decreased with testosterone in one study but this has not been confirmed in other trials. [60,64] Serum interleukin-10, an anti-inflammatory and anti-atherogenic cytokine, increases in response to testosterone therapy.[44] In one study of men with diabetes mellitus and low serum testosterone, testosterone replacement decreased serum adiponectin but did not cause significant changes in serum IL-β, IL-6, or hs-CRP.[72] In two subsequent RCTs of testosterone replacement, one in men with metabolic syndrome and the second in men with type 2 diabetes, suppressed TNF-α, IL-1β, and hs-CRP. [60,64] It is recognized that testosterone has immunomodulatory actions but it is not clear how this relates directly to atherosclerosis *in vivo* as this has not been investigated.[1]

Coagulation

Only two RCTs have studied the effect of testosterone on coagulation. These trials did not detect any effect on plasma fibrinogen, factor VII, plasminogen activator inhibitor-1 activity, or tissue plasminogen activator activity.[14,73]

Hepatic steatosis

Hepatic steatosis is associated with an increased risk of cardiovascular disease.[74] An 18-week RCT of testosterone undecanoate (depot intramuscular injection) against placebo in hypogonadal men ($n = 67$) with obesity and severe obstructive sleep apnea found a significant reduction in hepatic fat as assessed by computed tomography (CT) scanning.[75] This change was associated with increased insulin sensitivity and increased muscle mass. No changes in body composition with regard to fat deposits were identified; however, the study may not have been of sufficient duration.

ATHEROSCLEROSIS

There is no definitive RCTs of testosterone replacement therapy that report direct effects on atherosclerosis, plaque stability, or disease progression or amelioration. Carotid intimal media thickness (CIMT) is considered to be a surrogate marker of the degree of atherosclerosis. A small RCT over 12 months which evaluated the effect of testosterone on CIMT as well as angina reported a trend to an improvement in this measurement although this study was underpowered.[12] A second trial using testosterone undecanoate found that there was a significant reduction in CIMT in the treated group and a reduction in highly sensitive CRP over a 24-month period in men with metabolic syndrome and late-onset hypogonadism ($n = 50$, randomized at 4:1 on treatment to placebo).[61] An adequately powered RCT (TEAAM trial) that studied the effect of testosterone gel therapy against placebo over 3 years did not detect any changes in either CIMT or coronary artery calcium scores.[76] Participants ($n = 306$) were randomized of which 211 completed the study with all participants included in the primary analysis. The study group (mean age: 67.6 years) consisted of 15% with cardiovascular disease, 15% with diabetes, 42% with hypertension, and 27% with obesity. The study has some limitations, including that patients did not specifically have to have hypogonadism (testosterone deficiency) but just a serum testosterone <400 ng dl^{-1} (13.9 nmol l^{-1}). Statin use in the study was high and may be a confounding factor. There were no significant differences in cardiovascular events between the study groups, but the results of the study cannot be extrapolated to give any clinical guidance on the risk of future major adverse cardiac events (MACE) related to testosterone therapy.

A more recent large (n = 138 completers) 12-month RCT of hypogonadal men >65 years examined the effect of testosterone on coronary artery plaque volume.[77] Testosterone treatment was associated with a greater increase in noncalcified plaque volume. The clinical inference from this change is unclear. There was no change in the coronary artery calcium score between the groups. An exploratory analysis of plaque components found that testosterone therapy significantly increased the fibrous plaque volume compared to placebo. The changes in noncalcified coronary artery plaque volume were not associated with changes in the levels of total testosterone as a result of treatment. An increase in plaque size alone may not be associated with an increased risk of MACEs as the plaque content, stabilization, and risk of rupture might be more important to clinical outcomes. Testosterone in the short term could possibly promote a "healing response" within the plaque delivering a more stable plaque, as has been shown for statin therapy, and may result initially in an increase in plaque volume.

MECHANISMS OF ACTION OF TESTOSTERONE EFFECTS ON CARDIOVASCULAR RISK FACTORS AND ATHEROSCLEROSIS

Insulin resistance is associated with impairment of glucose utilization, which includes glucose uptake and metabolism by cells. The three major tissues that account for whole-body insulin resistance are muscle, liver, and fat. Seventy percentage of reduced insulin sensitivity is accounted for by striated muscle. The key glucose uptake transporters are the GLUT transporter family, with GLUT4 being the predominant transporter in muscle and adipose tissues. Insulin stimulates glucose uptake by increasing the translocation of GLUT4 transporters from the cytosol to the cell membrane. In the liver, the mechanism for insulin-stimulated uptake of glucose is less clear. Hepatocytes express the insulin-independent bidirectional GLUT2 transporter.

In a hyperinsulinemic euglycemic clamp RCT of testosterone replacement therapy, eugonadal hypogonadal men with type 2 diabetes had reduced baseline subcutaneous adipose tissue mRNA expression of the insulin receptor-β (IR-β), insulin receptor substrate-1 (IRS-1), Akt-2 (involved in IR-β downstream signal transduction), and GLUT4 and protein expression of IR-β and Akt when compared to eugonadal men with type 2 diabetes.[64] Testosterone replacement after 24 weeks significantly increased mRNA expression in hypogonadal men with type 2 diabetes of IR-β, IRS-1, Akt-2, and GLUT4.[62]

Testosterone has been shown to stimulate IRS-1, IRS-2, Akt, glucose uptake, and GLUT4 translocation in adipocytes and striated muscle.[78–82] Importantly, testosterone increases GLUT4-dependent glucose uptake in cardiomyocytes.[83] Testosterone has also been shown to increase the expression of the key regulatory enzymes of glycolysis, hexokinase (glucokinase in the liver), and phosphofructokinase, which impacts on the rate of this pathway.[78,82] Recent evidence has reported that testosterone produced a rapid nongenomic action promoting the activation of Akt, Erk, and mTOR, all factors involved in downstream insulin receptor stimulation.[84]

Testosterone in animal and cell culture studies has also been demonstrated to alter lipid metabolism.[78] Testosterone reduces *de novo* lipogenesis in adipose and liver tissue as well as regulating free fatty

acid and triglyceride uptake into subcutaneous fat depots.[78] Lipoprotein lipase (LPL) is an extracellular enzyme on the surface of adipocytes, which converts triglycerides into free fatty acids for uptake into the cell for conversion back into triglycerides for storage.[85] Testosterone inhibits LPL activity and therefore would reduce fatty acid uptake into tissues, but testosterone deficiency is associated with increased LPL resulting in enhanced uptake.[78,86,87] There may be differences between tissues in the testosterone-induced LPL activity, for example in mice, a low testosterone state is associated with an decreased LPL mRNA expression in subcutaneous but not in visceral fat.[82]

Testosterone deficiency is also associated with increased fat deposition in the liver, which in animal studies has led to the development of marked hepatic steatosis.[88] Testosterone has also been shown to suppress *de novo* lipogenesis in the liver and protect against hepatic steatosis in testosterone-deficient mice.[89] Testosterone replacement reduced mRNA and protein expression of acetyl CoA carboxylase and fatty acid synthase, the two regulatory enzymes of fatty acid synthesis.[89]

Glucose in the circulation that cannot be metabolized for energy production under normal physiological conditions is stored as glycogen in liver and muscle or converted to lipids that are stored in subcutaneous adipose tissues. When there is excess lipid that cannot be removed from the circulation, this leads to an "overspill" of lipid into visceral fat, liver, and then other tissues including arterial walls, promoting the development of atherosclerosis (**Figure 2**).[90] Testosterone deficiency as described above adversely affects carbohydrate and lipid metabolism, promoting fat accumulation in visceral adipose tissue, liver, and lipid streaks within the arterial walls. The increase in waist circumference as a measure of visceral adiposity has been observed in many clinical studies. A selective knockdown of the AR in adipocytes in mice causes visceral adiposity, insulin resistance, and hyperglycemia.[91] This study demonstrates a key role of testosterone action on adipocyte metabolism which would impact on the body's overall state of cardiometabolic function.

The changes in enzymes under conditions of low testosterone in subcutaneous fat suggest that there is an impairment of free fatty acid uptake and decreased expression of the GLUT4 transporter and reduced glucose uptake. Testosterone deficiency may result in a physiological state where the subcutaneous fat uptake, acting as a 'buffer' protecting against ectopic fat deposition is impaired, causing "overspill" of fat and deposition in visceral adipocytes, liver, and arterial walls, leading eventually to hepatic steatosis and atherosclerosis.[78,89]

The reason why testosterone replacement in some clinical trials produces a small fall in total and LDL-cholesterol is not fully clear. Testosterone replacement in deficient mice increased the expression of mRNA and protein of the major cholesterol cellular efflux transporter ABCA1, apo-E lipoprotein, and a master regulator of cholesterol metabolism liver X receptor in liver and in subcutaneous adipose tissue.[89] There is evidence that testosterone stimulates cholesterol efflux from the THP-1 monocyte-macrophage cell line, which is associated with translocation of the ABCA-1 transporter to the cell membrane.[92]

The effect on HDL-cholesterol is not well understood at present. In both healthy men and men with type 2 diabetes, HDL-cholesterol correlates positively with testosterone serum levels.[93,94] HDL-cholesterol is a cholesterol acceptor which transports cholesterol from peripheral tissues to the liver for excretion. In the initial stages of clearing excess cholesterol from the tissues, it has been hypothesized that HDL-cholesterol is used up and metabolized, producing an initial small fall in levels which then recovers after prolonged treatment.[95] This hypothesis, however, remains unproven.

Animal experiments have found that a state of testosterone deficiency promotes the development of lipid streak formation, the first stage of atherosclerotic plaque, in the aortae of animals fed with a cholesterol-rich diet.[96–99] This effect is associated with the development of marked hepatic steatosis.[89] The dominant cell type within the lipid streak has been identified as macrophages. Testosterone substitution has been shown to protect and ameliorate against the development of the early plaques.[96–99] The exact mechanism by which testosterone mediates these effects is not clear, but evidence suggests that both AR-dependent and AR-independent actions have a role.[98,100] Potential mechanisms are reduction of lipid uptake into the arterial wall and an anti-inflammatory effect directly at the level of the macrophage. Testosterone as described above suppresses serum pro-inflammatory cytokines and promotes anti-inflammatory actions. Testosterone specifically was found to inhibit the release of TNF-α, IL-1β, and IL6 from cultured monocytes from hypogonadal men with type 2 diabetes (T2D).[101]

CARDIOVASCULAR EVENTS IN RCTS

There have been no adequately powered studies of testosterone replacement therapy which have been performed to assess whether or not there is an increase, decrease, or no effect on the incidence of MACE. A recent meta-analysis of 39 RCTs and 10 observational studies did not identify any significant association of testosterone therapy with MACE events (myocardial infarction, stroke, and mortality).[102] No individual RCT where testosterone was replaced to the normal healthy range reported an increase in MACE. The duration of the trials ranged between 6 weeks and 3 years and included 5451 men (3230 received testosterone and 2221 placebo). There was no increased risk independently of myocardial infarction, stroke, or death.

No RCT which specifically studied men with known CVD and/or metabolic syndrome ± type 2 diabetes reported an increased risk of any cardiovascular event.[8,10–14,21,29–32,59–65,102] The TOM trial was an RCT (6 months' duration) which had a primary outcome of the effect of testosterone on muscle mass and strength in a frail cohort of elderly men with multiple comorbidities.[103] The initiation of testosterone gel used twice (100 mg) the recommended licensed dose (50mg od), with six individuals titrated to three times the dose (150 mg). Although the study was not powered for the assessment of cardiovascular events, it was considered that there was an excess of cardiovascular-related events in the treatment arm. These events were not well documented, included self-reported syncope, leg edema which is known to occur with over-replaced testosterone treatment, and tests such as exercise treadmill ECG which could have been abnormal prior to the study, not being performed in patients before entry into the trial. The CV-related events occurred mainly in those individuals on the higher testosterone doses. A similar 6-month RCT examining the effect of testosterone therapy on muscle strength in intermediate-frail and frail elderly men which used standard testosterone gel dose (50 mg od) reported no differences in CV events compared with placebo.[104]

The clinical importance for men to have testosterone levels within the mid-to-upper normal healthy range is emphasized in the following studies. Men with endogenous testosterone levels within the mid-to-higher normal healthy range have the lowest rate of MACE.[105] Evidence from a large epidemiological study supports the meta-analysis that normalization of testosterone within the mid-normal range reduces MACE compared to men on testosterone replacement who have been undertreated.[106]

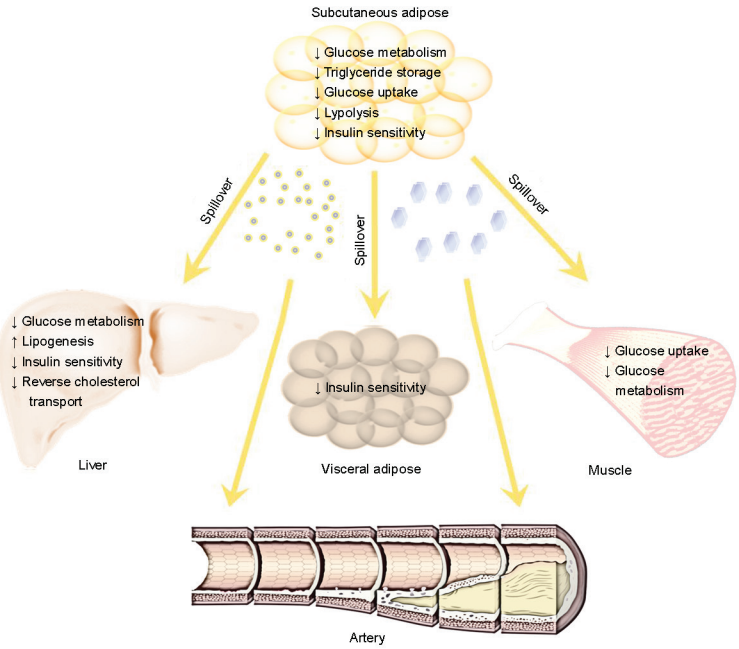

Figure 2: Potential detrimental metabolic actions of testosterone deficiency. In testosterone deficiency, excess deposition of fat resulting from poor lipid and glucose control and inadequate storage capacity in subcutaneous adipose depots may lead to "overspill" into visceral fat. Concurrently, testosterone deficiency and elevated circulating glucose and lipid cause further metabolic dysregulation in visceral adipose tissue, with ensuing metabolic consequences in liver and muscle, resulting in lipid accumulation. This ectopic lipid accumulation has pathological consequences when it is ultimately deposited in tissues such as the liver (hepatic steatosis) and the arterial wall (atherosclerosis).

CONCLUSIONS

RCTs confirm that testosterone does improve cardiac ischemia in men with chronic stable angina and functional exercise capacity and $VO_{2\,max}$ in men with chronic heart failure. These effects have been shown to persist for at least 1 year. These findings are supported by mechanistic studies, which have shown that testosterone is a coronary vasodilator as well as actions on other arteries within the body. These effects are acute as well as chronic with testosterone able to alter vascular responsiveness to established endogenous vasoactive agents including noradrenaline and acetylcholine. The effects of testosterone in chronic heart failure patients are probably a combination of effects on reducing peripheral vascular resistance, improved left ventricular function and effects on skeletal muscle strength, and general well-being. Testosterone may have anti-arrhythmic property with a documented effect on shortening the Q-T interval, but there are no studies which have been powered adequately to determine if this action translates into a clinically relevant effect. The beneficial effects on these three clinical states appear to be primarily mediated via an AR-independent action on L-calcium channel blocking and potassium channel opening.

RCTs have consistently reported that testosterone replacement therapy reduces fat mass and increases lean mass. The majorities of trials have reported a reduction in waist circumference, but do not usually become statistically significant in most studies until after 12 months' treatment. Long-term registry studies report that the effect of testosterone on waist circumference and BMI shows that benefits gradually accrue over several years. Testosterone improves insulin resistance, but an effect on glycemic control is not yet clear as no RCTs have specifically examined the effect of testosterone replacement in hypogonadal men with uncontrolled diabetes which would be a standard study required for any new treatment for this condition. Scientific studies have provided insight into the importance of testosterone in the regulation of carbohydrate and lipid metabolism, which provide support to a positive effect on metabolic pathways, which contribute to cardiovascular risk benefits. The effects of testosterone on reducing serum cholesterol are small, but the tissue-specific effects at the level of the plaque are unknown. Animal studies do provide evidence that testosterone can protect against the development of early stages of atherosclerosis, but it is not clear that this effect translates into humans. The RCTs which have examined the effect of testosterone replacement on CIMT and plaque calcium and volume have not demonstrated any convincing evidence for testosterone as having a beneficial or adverse effect on the plaque. The major limitation is not having a specific technique, which can assess plaque stability *in vivo* which can be applied in large RCTs. The knowledge that testosterone replacement in men with type 2 diabetes and hypogonadism improves mortality does signify that the hormone does have an important role in health. The questions remain as to whether the actions of testosterone are involved with cardiovascular health protection and/or stabilization/amelioration of established cardiovascular disease.

REFERENCES

1 Kelly DM, Jones TH. Testosterone: a vascular hormone in health and disease. *J Endocrinol* 2013; 217: R47–71.
2 Pugh PJ, Jones TH, Channer KS. Acute haemodynamic effects of testosterone in men with chronic heart failure. *Eur Heart J* 2003; 24: 909–15.
3 Malkin CJ, Morris PD, Pugh PJ, English KM, Channer KS. Effect of testosterone therapy on QT dispersion in men with heart failure. *Am J Cardiol* 2003; 92: 1241–3.
4 Schwartz JB, Volterrani M, Caminiti G, Marazzi G, Fini M, *et al*. Effects of testosterone on the Q-T interval in older men and older women with chronic heart failure. *Int J Androl* 2011; 34: e415–21.
5 Zarotsky V, Huang MY, Carman W, Morgentaler A, Singhal PK, *et al*. Systematic literature review of the risk factors, comorbidities and consequences of hypogonadism in men. *Andrology* 2014; 2: 819–34.
6 Zitzmann M, Nieschlag E. The CAG repeat polymorphism within the androgen receptor gene and maleness. *Int J Androl* 2003; 26: 76–83.
7 Lesser MA. Testosterone propionate therapy in one hundred cases of angina pectoris. *J Clin Endocrinol Metab* 1946; 6: 549–57.

8 Jaffe MD. Effect of testosterone cypionate on post exercise ST segment depression. *Eur Heart J* 1977; 39: 1217–22.
9 Wu SZ, Weng XZ. Therapeutic effects of an androgenic preparation on myocardial ischemia and cardiac function in 62 elderly male coronary heart disease patients. *Chin Med J (Engl)* 1993; 106: 415–8.
10 English KM, Steeds RP, Jones TH, Diver MJ, Channer KS. Low-dose transdermal testosterone therapy improves angina threshold in men with chronic stable angina: a randomized, double-blind, placebo-controlled study. *Circulation* 2000; 102: 1906–11.
11 Malkin CJ, Pugh PJ, Morris PD, Kerry KE, Jones RD, *et al*. Testosterone replacement in hypogonadal men with angina improves ischaemic threshold and quality of life. *Heart* 2004; 90: 871–6.
12 Mathur A, Malkin C, Saeed B, Muthusamy R, Jones TH, *et al*. Long-term benefits of testosterone replacement therapy on angina threshold and atheroma in men. *Eur J Endocrinol* 2009; 161: 443–9.
13 Cornoldi A, Caminiti G, Marazzi G, Vitale C, Patrizi R, *et al*. Effects of chronic testosterone administration on myocardial ischemia, lipid metabolism and insulin resistance in elderly male diabetic patients with coronary artery disease. *Int J Cardiol* 2010; 142: 50–5.
14 Webb CM, Elkington AG, Kraidly MM, Keenan N, Pennell DJ, *et al*. Effects of oral testosterone treatment on myocardial perfusion and vascular function in men with low plasma testosterone and coronary heart disease. *Am J Cardiol* 2008; 101: 618–24.
15 Webb CM, Adamson DL, de Zeigler D, Collins P. Effect of acute testosterone on myocardial ischemia in men with coronary artery disease. *Am J Cardiol* 1999; 83: 437–9.
16 Rosano GM, Leonardo F, Pagnotta P, Pelliccia F, Panina G, *et al*. Acute anti-ischemic effect of testosterone in men with coronary artery disease. *Circulation* 1999; 99: 1666–70.
17 Thompson PD, Ahlberg AW, Moyna NM, Duncan B, Ferraro-Borgida M, *et al*. Effect of intravenous testosterone on myocardial ischemia in men with coronary artery disease. *Am Heart J* 2002; 143: 249–56.
18 Webb CM, McNeill JG, Hayward CS, de Zeigler D, Collins P. Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. *Circulation* 1999; 100: 1690–6.
19 English KM, Jones RD, Jones TH, Morice AH, Channer KS. Aging reduces the responsiveness of coronary arteries from male Wistar rats to the vasodilatory action of testosterone. *Clin Sci* 2000; 99: 77–82.
20 English KM, Jones RD, Jones TH, Morice AH, Channer KS. Gender differences in the vasomotor effects of different steroid hormones in rat pulmonary and coronary arteries. *Horm Metab Res* 2001; 33: 645–52.
21 Malkin CJ, Jones RD, Jones TH, Channer KS. Effect of testosterone on *ex vivo* vascular reactivity in man. *Clin Sci (Lond)* 2006; 111: 265–74.
22 Hall J, Jones RD, Jones TH, Channer KS, Peers C. Selective inhibition of L-type Ca^{2+} channels in A7r5 cells by physiological levels of testosterone. *Endocrinol* 2006; 147: 2675–80.
23 Scragg JL, Jones RD, Channer KS, Jones TH, Peers C. Testosterone is a potent inhibitor of L-type Ca^{2+} channels. *Biochem Biophys Res Commun* 2004; 318: 503–6.
24 Jones RD, Pugh PJ, Jones TH, Channer KS. The vasodilatory action of testosterone: a potassium-channel opening or a calcium antagonistic action? *Br J Pharmacol* 2003; 138: 733–44.
25 Scragg JL, Dallas ML, Peers C. Molecular requirements for L-type Ca^{2+} channel blockade by testosterone. *Cell Calcium* 2007; 42: 11–5.
26 Traish AM, Goldstein I, Kim NN. Testosterone and erectile function: from basic research to a new clinical paradigm for managing men with androgen insufficiency and erectile dysfunction. *Eur Urol* 2007; 52: 54–70.
27 Askoxylakis V, Thieke C, Pleger ST, Most P, Tanner J, *et al*. Long-term survival of cancer patients compared to heart failure and stroke: a systematic review. *BMC Cancer* 2010; 10: 105.
28 Urhausen A, Albers T, Kindermann W. Are the cardiac effects of anabolic steroid abuse in strength athletes reversible. *Heart* 2004; 90: 496–501.
29 Pugh PJ, Jones RD, West JN, Jones TH, Channer KS. Testosterone treatment for men with chronic heart failure. *Heart* 2004; 90: 446–7.
30 Malkin CJ, Pugh PJ, West JN, van Beek EJ, Jones TH, *et al*. Testosterone therapy in men with moderate severity heart failure: a double-blind randomized placebo controlled trial. *Eur Heart J* 2006; 27: 57–64.
31 Caminiti G, Volterrani M, Iellamo F, Marazzi G, Massaro R, *et al*. Effect of long-acting testosterone treatment on functional exercise capacity, skeletal muscle performance, insulin resistance, and baroreflex sensitivity in elderly patients with chronic heart failure a double-blind, placebo-controlled, randomized study. *J Am Coll Cardiol* 2009; 54: 919–27.
32 Mirdamadi A, Garakyaraghi M, Pourmoghaddas A, Bahmani A, Mahmoudi H, *et al*. Beneficial effects of testosterone therapy on functional capacity, cardiovascular parameters, and quality of life in patients with congestive heart failure. *Biomed Res Int* 2014; 2014: 392432.
33 Malkin CJ, Channer KS, Jones TH. Testosterone and heart failure. *Curr Opin Endocrinol Diab Obes* 2010; 17: 262–8.
34 Wang XF, Wang JH, Li JY. Androgen deficiency in elderly men with systolic chronic heart failure. *J Geriatr Cardiol* 2010; 7: 138–42.
35 Jankowska EA, Filippatos G, Ponikowska B, Borodulin-Nadzieja L, Anker SD, *et al*. Reduction in circulating testosterone relates to exercise capacity in men with chronic heart failure. *J Card Fail* 2009; 5: 442–50.
36 Simerly RB, Chang C, Muramatsu M, Swanson LW. Distribution of androgen and estrogen mRNA-containing cells in the rat brain: an *in situ* hybridization study. *J Comp Neurol* 1990; 294: 76–95.
37 Ward GR, Abdel-Rahman AA. Orchiectomy or androgen receptor blockade attenuates baroreflex-mediated bradycardia in conscious rats. *BMC Pharmacol* 2006; 6: 2.
38 Coats AJ. Heart failure: what causes the symptoms of heart failure? *Heart* 2001; 86: 574–8.
39 Piepoli MF, Kaczmarek A, Francis DP, Davies LC, Rauchhaus M, *et al*. Reduced peripheral skeletal muscle mass and abnormal reflex physiology in chronic heart failure. *Circulation* 2006; 114: 126–34.
40 Piepoli M, Clark AL, Volterrani M, Adamopoulous S, Sleight P, *et al*. Contribution of muscle afferents to the hemodynamic, autonomic, and ventilatory responses to exercise in patients with chronic heart failure: effects of physical training. *Circulation* 1996; 93: 940–52.
41 Gullestad L, Ueland T, Vinge LE, Finsen A, Yndestad A, *et al*. Inflammatory cytokines in heart failure: mediators and markers. *Cardiology* 2012; 122: 23–5.
42 Suskin N, McKelvie RS, Burns RJ, Latini R, Pericak D, *et al*. Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. *Eur Heart J* 2000; 21: 1368–75.
43 Doehner W, Gathercole D, Cicoira M, Krack A, Coats AJ, *et al*. Reduced glucose transporter GLUT4 in skeletal muscle predicts insulin resistance in non-diabetic chronic heart failure patients independently of body composition. *Int J Cardiol* 2010; 138: 19–24.
44 Malkin CJ, Pugh PJ, Jones RD, Kapoor D, Channer KS, *et al*. The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men. *J Clin Endocrinol Metab* 2004; 89: 3313–8.
45 Pugh PJ, Jones RD, Malkin CJ, Hall J, Nettleship JE, *et al*. Physiologic testosterone therapy has no effect on serum levels of tumour necrosis factor-α in men with chronic heart failure. *Endocrine Res* 2005; 31: 271–83.
46 Rao PM, Kelly DM, Jones TH. Testosterone and insulin resistance in the metabolic syndrome and T2DM in men. *Nat Rev Endocrinol* 2013; 9: 479–93.
47 Malkin CJ, Channer KS, Jones TH. The effect of testosterone on insulin sensitivity in men with heart failure. *Eur J Heart Failure* 2007; 9: 44–50.
48 Nakagawa M, Ooie T, Ou B, Ichinose M, Takahashi N, *et al*. Gender differences in autonomic modulation of ventricular repolarization in humans. *J Cardiovasc Electrophysiol* 2005; 16: 278–84.
49 Zhang Y, Ouyang P, Post WS, Dalal D, Vaidya D, *et al*. Sex-steroid hormones and the electrocardiographic QT-interval duration: findings from the third national health and nutrition examination survey and the multi-ethnic study of atherosclerosis. *Am J Epidemiol* 2011; 174: 403–11.
50 Pecori Giraldi F, Toja PM, Filippini B, Michailidis J, Scacchi M, *et al*. Increased prevalence of prolonged QT duration in males with primary and secondary hypogonadism: a pilot study. *Int J Androl* 2010; 22: e132–8.
51 Pecori Giraldi F, Toja PM, Filippini B, Michailidis J, Scacchi M, *et al*. High prevalence of prolonged QT interval in obese hypogonadal males. *Obesity* 2011; 19: 2015–8.
52 Brouillette J, Rivard K, Lizotte E, Fiset C. Sex and strain differences in adult mouse cardiac repolarization: importance of androgens. *Cardiovasc Res* 2005; 65: 148–57.
53 Pham TV, Rosen MR. Sex, hormones and repolarization. *Cardiovasc Res* 2002; 53: 740–51.
54 Pham TV, Sosunov EA, Anyukhovsky EP, Danilo P Jr, Rosem MR. Testosterone diminishes the proarrhythmic effects of dofetilide in normal female rabbits. *Circulation* 2002; 106: 2132–6.
55 Pugh PJ, Channer KS, Parry H, Downes T, Jones TH. Bio-available testosterone levels fall acutely following myocardial infarction in men: association with fibrinolytic factors. *Endocr Res* 2002; 28: 161–73.
56 Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, *et al*. Effect of potentially modifiable risk factors associated with myocardial function in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004; 364: 937–52.
57 Jones TH. Effects of testosterone on type 2 diabetes and components of the metabolic syndrome. *J Diabetes* 2010; 2: 146–56.
58 Corona G, Monami M, Rastrelli G, Aversa A, Sforza A, *et al*. Type 2 diabetes mellitus and testosterone: a meta-analysis study. *Int J Androl* 2010; 34: 528–40.
59 Kapoor D, Goodwin E, Channer KS, Jones TH. Testosterone replacement therapy improves insulin resistance, glycaemic control, visceral adiposity and hypercholesterolaemia in hypogonadal men with type 2 diabetes. *Eur J Endocrinol* 2006; 154: 899–906.
60 Kalinchenko SY, Tishova YA, Mskhalaya GJ, Gooren LJ, Giltay EJ, *et al*. Effects of testosterone supplementation on markers of the metabolic syndrome and inflammation in hypogonadal men with the metabolic syndrome: the double-blinded placebo-controlled Moscow study. *Clin Endocrinol* 2010; 73: 602–12.
61 Aversa A, Bruzziches R, Francomano D, Rosano G, Isidori AM, *et al*. Effects of testosterone undecanoate on cardiovascular risk factors and atherosclerosis in middle-aged men with late-onset hypogonadism and metabolic syndrome: results

from a 24 month randomized, double-blind, placebo-controlled study. *J Sex Med* 2010; 7: 3495–503.
62 Jones TH, Arver S, Behre HM, Buvat J, Meuleman E, *et al*. Testosterone replacement in hypogonadal men with type 2 diabetes and/or metabolic syndrome (the TIMES2 study). *Diabetes Care* 2011; 34: 828–37.
63 Hackett G, Cole N, Bhartia M, Kennedy D, Raju J, *et al*. Testosterone replacement therapy improves metabolic parameters in hypogonadal men with type 2 diabetes but not in men with coexisting depression: the BLAST study. *J Sex Med* 2014; 11: 840–56.
64 Dhindsa S, Ghanim H, Batra M, Kuhadiya D, Abuaysheh S, *et al*. Insulin resistance and inflammation in hypogonadotrophic hypogonadism and their reduction after testosterone replacement in men with type 2 diabetes. *Diabetes Care* 2016; 39: 82–91.
65 Gianatti EJ, Dupuis P, Hoermann R, Strauss BJ, Wentworth JM, *et al*. Effect of testosterone treatment on glucose metabolism in men with type 2 diabetes: a randomized controlled trial. *Diabetes Care* 2014; 37: 2098–107.
66 Grossmann M, Hoermann R, Wittert G, Yeap BB. Effects of testosterone treatment on glucose metabolism and symptoms in men with type 2 diabetes and the metabolic syndrome: a systematic review and meta-analysis of randomized controlled clinical trials. *Clin Endocrinol (Oxf)* 2015; 83: 344–51.
67 Isidori AM, Gianetta E, Greco EA, Gianfrilli D, Bonfacio V, *et al*. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: a meta-analysis. *Clin Endocrinol (Oxf)* 2005; 63: 280–93.
68 Huo S, Scialli AR, McGarvey S, Hill E, Tugertimer B, *et al*. Treatment of men for 'low testosterone': a systematic review. *PloS One* 2016; 11: e0162480.
69 Jones TH, Saad F. The effect of testosterone on risk factors for, and the mediators of, the atherosclerotic process. *Atherosclerosis* 2009; 207: 318–27.
70 Kelly DM, Jones TH. Testosterone and obesity. *Obes Rev* 2015; 16: 581–606.
71 Traish AM, Haider A, Haider KS, Doros G, Saad F. Long-term testosterone therapy improves cardiometabolic function and reduces risk of cardiovascular disease in men with hypogonadism: a real-life observational registry study setting comparing treated and untreated (control) groups. *J Cardiovasc Pharmacol Ther* 2017; 22: 414–33.
72 Kapoor D, Clarke S, Stanworth R, Channer KS, Jones TH. The effect of testosterone replacement therapy on adipocytokines and C-reactive protein in hypogonadal men with type 2 diabetes. *Eur J Endorcinol* 2007; 156: 592–602.
73 Smith AM, English KM, Malkin CJ, Jones RD, Jones TH, *et al*. Testosterone does not adversely affect fibrinogen or tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) levels in 46 men with chronic stable angina. *Eur J Endocrinol* 2005; 152: 285–91.
74 Bieghs V, Rensen PC, Hofker MH, Shiri-Sverdlov R. NASH and atherosclerosis are two aspects of a shared disease: central role for macrophages. *Atherosclerosis* 2012; 220: 287–93.
75 Hoyos CM, Yee BJ, Phillips CL, Machan EA, Grunstein RR, *et al*. Body compositional and cardiometabolic effects of testosterone therapy in obese men with severe obstructive sleep apnoea: a randomised placebo-controlled trial. *Eur J Endocrinol* 2012; 167: 531–41.
76 Basaria S, Harman M, Travison MG, Hodis H, Tsitouras P, *et al*. Effect of testosterone administration for 3 years on subclinical atherosclerosis progression in older men with low or low-normal testosterone levels. a randomized clinical trial. *JAMA* 2015; 314: 570–81.
77 Budoff MJ, Ellenberg SS, Lewis CE, Mohler ER, Wenger NK, *et al*. Testosterone treatment and coronary artery plaque volume in older men with low testosterone. *JAMA* 2017; 317: 708–16.
78 Kelly DM, Jones TH. Testosterone: a metabolic hormone in health and disease. *J Endocrinol* 2013; 217: R25–45.
79 Chen X, Li X, Huang HY, Li X, Lin JF. [Effects of testosterone on insulin receptor substrate-1 and glucose transporter 4 expression in cells sensitive to insulin]. *Zhonghua Yi Xue Za Zhi* 2006; 86: 1474–77. [Article in Chinese].
80 Sato K, Iemitsu M, Aizawa K, Ajisaka R. Testosterone and DHEA activate the glucose metabolism-related signaling pathway in skeletal muscle. *Am J Physiol Endocrinol Metab* 2008; 294: E961–8.
81 Mitsuhashi K, Senmaru T, Fukuda T, Yamazaki M, Shinomiya K, *et al*. Testosterone stimulates glucose uptake and GLUT4 translocation through KKB/AMPK signalling in 3T3-L1 adipocytes. *Endocrine* 2016; 51: 174–84.
82 Kelly DM, Akhtar S, Sellers DJ, Muraleeharan V, Channer KS, *et al*. Testosterone differentially regulates targets of lipid and glucose metabolism in liver, muscle and adipose tissues of the testicular feminised mouse. *Endocrine* 2016; 54: 504–15.
83 Wilson C, Contreras-Ferrat AE, Venegas N, Osorio-Fuentealba C, Pavez M, *et al*. Testosterone increases GLUT4-dependent glucose uptake in cardiomyocytes. *J Cell Physiol* 2013; 228: 2399–407.
84 Antinozzi C, Marampon F, Corinaldesi C, Vicini E, Sgro P, *et al*. Testosterone insulin-like effects: an *in vitro* study on the short-term metabolic effects of testosterone in human skeletal muscle cells. *J Endocrinol Invest* 2017; 40: 1133–43.
85 Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *New Eng J Med* 1989; 320: 1060–8.
86 Rebuffe-Scrive M, Marin P, Bjorntorp P. Effect of testosterone on abdominal adipose tissue in men. *Int J Obes* 1991; 15: 791–5.
87 Marin P, Lonn L, Andersson B, Oden B, Olbe L, *et al*. Assimilation of triglycerides in subcutaneous and intraabdominal adipose tissues *in vivo* in men: effects of testosterone. *J Clin Endocrin Metab* 1996; 81: 1018–22.
88 Volzke H, Aumann N, Krebs A, Nauck M, Steveling A, *et al*. Hepatic steatosis is associated with low serum testosterone and high serum DHEAS levels in men. *Int J Androl* 2010; 33: 45–53.
89 Kelly DM, Nettleship JE, Akhtar S, Muraleedharan V, Sellers DJ, *et al*. Testosterone suppresses the expression of regulatory enzymes of fatty acid synthesis and protects against hepatic steatosis in cholesterol-fed androgen deficient mice. *Life Sci* 2014; 109: 95–103.
90 Yu YH, Ginsberg HN. Adipocyte signaling and lipid homeostasis: sequelae of insulin-resistant adipose tissue. *Circ Res* 2005; 96: 1042–52.
91 McInnes KJ, Smith LB, Hunger NI, Saunders PT, Andrew R, *et al*. Deletion of the androgen receptor in adipose tissue in male mice elevates retinol binding protein 4 and reveals independent effects on visceral fat mass and on glucose homeostasis. *Diabetes* 2012; 61: 1072–81.
92 Kilby E, Jones H. Testosterone stimulates cholesterol metabolism and efflux from human macrophages via liver X receptor. *Endocrine* 2013; 32: P173.
93 Van Pottelbergh I, Braeckman L, De Bacquer D, De Backer G, Kaufman JM. Differential contribution of testosterone and estradiol in the determination of cholesterol and lipoprotein profile in healthy middle-aged men. *Atherosclerosis* 2003; 166: 95–102.
94 Stanworth RD, Kapoor D, Channer KS, Jones TH. Dyslipidaemia is associated with testosterone, oestradiol and androgen receptor CAG repeat polymorphism in men with type 2 diabetes. *Clin Endocrinol (Oxf)* 2011; 74: 624–30.
95 Wu FC, von Eckardstein A. Androgens and coronary artery disease. *Endocrine Rev* 2003; 24: 183–217.
96 Bruck B, Brehme U, Gugel N, Hanke S, Finking G, *et al*. Gender-specific differences in the effects of testosterone and estrogen on the development of atherosclerosis in rabbits. *Arterio Thromb Vasc Biol* 1997; 17: 2192–9.
97 Alexandersen P, Haarbo J, Byrjalsen I, Lawaetz H, Christiansen C. Natural androgens inhibit male atherosclerosis: a study in castrated, cholesterol-fed rabbits. *Circ Res* 1999; 84: 813–9.
98 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: 2427–34.
99 Kelly DM, Sellers DJ, Woodroofe MN, Jones TH, Channer KS. Effect of testosterone on inflammatory markers in the development of early atherogenesis in the testicular-feminized mouse model. *Endocrine Res* 2013; 38; 125–38.
100 Bourghardt J, Wilhelmson AS, Alexanderson C, De Gendt K, Verhoeven G, *et al*. Androgen receptor-dependent and independent atheroprotection by testosterone in male mice. *Endocrinol* 2010; 151: 5428–37.
101 Corrales JJ, Almeida M, Burgo R, Mories MT, Miralles JM, *et al*. Androgen-replacement therapy depresses the *ex vivo* production of inflammatory cytokines by circulating antigen-presenting cells in aging type-2 diabetic men with partial androgen deficiency. *J Endocrinol* 2006; 189: 595–604.
102 Alexander CG, Iyer G, Lucas E, Lin D, Singh S. Cardiovascular risks of exogenous testosterone use among men: a systematic review and meta-analysis. *Am J Med* 2017; 130: 293–305.
103 Basaria S, Coviello AD, Travison TG, Storer TW, Farwell WR, *et al*. Adverse effects associated with testosterone administration. *New Engl J Med* 2010; 363: 109–22.
104 Srinvas-Shankar U, Roberts SA, Connolly MJ, O'Connell MD, Adams JE, *et al*. Effects of testosterone on muscle strength, physical function, body composition, and quality of life in intermediate-frail and frail elderly men: a randomized, double-blind, placebo-controlled study. *L Clin Endocrinol Metab* 2010; 95: 639–50.
105 Hyde Z, Norman PE, Flicker L, Hankey GJ, Almeida OP, *et al*. Low free testosterone predicts mortality from cardiovascular disease but not other causes: the health in men study. *J Clin Endocrinol Metab* 2012; 97: 179–89.
106 Sharma R, Oni OA, Gupta K, Chen G, Sharma M, *et al*. Normalization of testosterone level is associated with reduced incidence of myocardial infarction and mortality in men. *Eur Heart J* 2015; 36: 2706–15.