ANDROGENS AND CORONARY ARTERY DISEASE

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ABSTRACT

This chapter reviews data that examine the relationship between androgens and coronary artery disease (CAD) in men. Androgens can exert both beneficial and deleterious actions on a myriad of factors implicated in the pathogenic mechanisms of atherosclerosis and CAD.

Androgen/androgen receptor (AR) can modulate arterial disease and vascular function via genomic (AR) or non-genomic mechanisms in animal models and in vitro experimental studies. The diversity and complexity of the actions of testosterone (and its metabolites E2 and DHT) and DHEA on the vasculature reflect the multiple cellular targets in the vessel wall, differences between species, gender, concomitant disease and, most importantly, level/dosage of testosterone exposure.

At present, it is not possible to determine the net effect of androgens on CAD pathogenesis and clinical outcomes. While observational clinical studies showed a consistent association between low testosterone and CAD (risk factors, events and mortality), and some experimental studies may suggest positive effects of androgens on CAD risk factors, body composition and individual vascular mechanisms, it is hazardous to suggest that manipulation of the androgenic milieu will result in clinical benefits in a complex multifactorial condition such as CAD. This ongoing uncertainty also underlines recent concerns regarding the possibility of adverse cardiovascular side effects in androgen treatment of endocrine and non-endocrine conditions, hampering efforts to exploit the potential therapeutic benefits of testosterone for men in the treatment of osteoporosis, sarcopaenia, chronic debilitating disease and obesity-related hypoandrogenism in the ageing male population. Large-scale prospective randomised placebo-controlled trials of sufficient size and duration are urgently needed to assess not only the benefits in terms of meaningful clinical benefits and patient-important outcomes but also to document the risks of serious adverse events in testosterone treatment. In the meantime, for patients with established pathological hypogonadism, there are no substantive data to suggest that physiological testosterone therapy is associated with increased cardiovascular risk and their management should not deviate from current recommended practice. For complete coverage of this and related topics, please visit www.endotext.org.

INTRODUCTION

Coronary artery disease (CAD) is one of the leading causes of mortality in men and women, being 5th in the rank order of disabilities in 1990. CAD is predicted to become the leading global cause of disease burden by 2020 (1). Current trends confirm the above prediction. In a report from the American College of Cardiology in 2012, CAD was estimated to result in 17.3 million deaths worldwide each year (2). According to an estimate by the World Health Organisation, there will be 20 million deaths due to CAD globally in 2015. The age-adjusted morbidity and mortality rates from CAD are 2.5- to 4.5-fold higher in men than in women, the sex-specific gap narrowing after the menopause (3). The lifetime risk of CAD at the age of 50 years is 1 in 2 for men and 1 in 2.5 for women (4). This male preponderance is remarkably consistent across 52 countries with hugely divergent rates of CAD mortality and lifestyles (5). The universality of this disparity makes it likely that there is an intrinsic sexual dimorphism in susceptibility to CAD that may involve genetic, hormonal, lifestyle or ageing factors. Sex hormones can influence a multitude of factors implicated in the pathogenesis of atherosclerosis and coronary artery disease - the traditional view being that androgens are harmful and estrogens beneficial but the oestrogen hypothesis was seriously challenged in light of the results from the Women's Health Initiative (6,7). Indeed, the sex-specific disparity in CAD may involve diverse mechanisms ranging from *in utero* sex hormone imprinting, gender-specific behaviour, distribution of visceral body fat to vascular and myocardial structural/functional adaptation to ageing, pressure overload and disease (8). As the therapeutic applications for androgen administration widen to 'non-classical' indications (9) including male contraception, physiological ageing, chronic debilitating conditions and hormone replacement in postmenopausal women (10), it becomes increasingly pertinent to consider whether natural or induced changes in levels of testosterone or dehydroepiandrosterone (DHEA) will impact on the risks of coronary artery disease in men and women. This question is one of the major safety issues for androgen therapy. This chapter synthesises data from PubMed publications on a variety of disciplines into a global assessment of the relationship between androgens and CAD in men. The role of androgens in CV diseases in women is dealt with in the Female Reproduction section.

Testosterone and Coronary artery Disease

Observational Clinical Studies

It is important to emphasize the limitations of observational studies on the associations between serum levels of endogenous androgens and CAD. The CAD endpoints are extremely variable (mortality, morbidity such as myocardial infarction and angina, angiography, ultrasound, arterial calcification, post-mortem findings and unspecified 'cardiac events'). Study groups are heterogeneous in terms of age, number of subjects and selection criteria. Most CAD patients will be on medications and have modified their lifestyle. In some studies, selection of poorly-matched controls may have introduced biases. The time interval from myocardial infarction (MI) to blood sampling varied from 3 months to many years and was not always standardised for diurnal variation of hormone levels. Not all studies adjusted for confounding factors such as smoking, blood pressure, obesity, diabetes, and

KEY POINTS

Observational clinical studies have shown a consistent association between low endogenous testosterone and CAD (coronary artery disease), CV (cardiovascular) risk factors as well as CV mortality.

Low DHEAS levels also appear to be associated with CAD in observational clinical studies but interventional studies with DHEA have failed to show any significant cardiovascular benefits.

Although some experimental studies with TRT (testosterone replacement therapy) suggest beneficial effects of testosterone on CV risk factors, at present TRT cannot be recommended for the treatment of these conditions in the absence of pathological hypogonadism.

Apparently beneficial effects of TRT in patients with symptomatic CAD reported in preliminary clinical studies are unclear. TRT is contraindicated in heart failure.

Recently, retrospective observational studies have raised concerns regarding increased risk of CV adverse events with TRT in elderly men. Although many small prospective clinical trials have shown no significant increase in CV events, they are insufficiently powered to assess this risk.

Physicians should therefore be highly vigilant about the possibility of harm from TRT, particularly with higher doses in older men with pre-existing CAD or CV risk factors.

The risk of increased venous thromboembolism with TRT is extremely low and appears to be mainly in patients with underlying genetic or acquired hypercoagulable states.

Physiological TRT for patients with established pathological hypogonadism should not deviate from current recommended practice as there are no substantive data suggestive of increased CV (or prostate) disease risks.

The initiation or continuation of TRT in newly diagnosed or established patients with pathological hypogonadism who have developed a new cardiovascular event should delay or stop treatment for 3 to 6 months

Large-scale prospective randomised placebo-controlled trials of testosterone treatment for non-pathological 'hypogonadism' of sufficient size and duration are urgently required to assess not only the benefits (in terms of meaningful clinical benefits and patient-important outcomes) but also to document any risks of serious adverse events.

At present, it is not possible to determine the net effect of androgens on CAD pathogenesis or clinical outcomes.

The possible benefits versus potential risks and the current lack of definitive safety data should be carefully discussed with each individual patient before starting TRT.

dyslipidemia. Finally, chronic illnesses including CAD can lower serum levels of testosterone; such a potential bi-directional relationship makes interpretation and deduction of clinical implication difficult.

Endogenous testosterone and CAD

Cross-Sectional Studies

Of the cross-sectional studies (11-48) investigating the relationships between circulating testosterone and CAD in men, 20 of 42 showed no association while the remainder found lower levels of testosterone in cases compared to controls (Table 1a). Many of the studies did not have sufficient statistical power due to the small number of subjects investigated. Three larger studies (19,27) found a negative relationship between testosterone and ischaemic heart disease or abdominal aortic calcification. In one Korean study (49) which investigated 291 men (mean age 52.8 +/- 9.3 years) who were non obese, non-diabetic, normotensive and with no history of previous CAD, bioavailable testosterone was inversely associated with the coronary calcium score. A few studies (24,48,50,51) have also found an inverse correlation between testosterone and severity of CAD. As with all cross-sectional studies, the directionality between cause and consequence and the significance of the observed negative relationship is unclear.

Prospective Cohort or Case-Control Studies: Association between Testosterone and coronary artery disease / cardiovascular events

Eleven of eighteen (27,52-60) non-cross sectional studies showed no significant relationship or predictive value between testosterone and clinical or biochemical indicators of CAD (Table 1b).

In the six prospective cohort studies which followed, 1009 Californian men aged 40-79 over 12 years (53), 2512 men aged 45-59 in South Wales (Caerphilly) for 5 years (56), 890 Baltimore men aged 53.8±16 yr for up to 31 years (58), 2084 men aged 56±12 yr from Framingham, Massachusetts for 10 yrs (59), 1568 men from Tromso, Norway aged 59.6±10 yrs for 10 years (60) and 254 elderly men of Framingham cohort with mean age of 75.5 yrs for 10 years (61), there was no correlation between baseline testosterone levels and subsequent development of fatal or non-fatal CAD, stroke or heart failure after adjusting for relevant confounders.

In the Rotterdam study, 282 Dutch men were followed for 6.5 years (27) and this showed that low testosterone was associated with progression of abdominal aortic atherosclerosis detected by radiological calcification. The Japanese study which followed 171 middle aged men found that low testosterone was significantly associated with cardiovascular events (62). The Health in Men Study (HIMS) of Western Australia (63,64) showed that eutinising hormone (LH) levels had positive relationship with cardiovascular events and dihydrotestosterone had inverse relationship for ischaemic strokes. The 5 yr follow up study of 2416 community dwelling Swedish men of MrOS cohort (65) also demonstrated a significant inverse association between endogenous testosterone and major cardiovascular events.

In a recent study which followed 3650 men aged >65 years from the French Three-City cohort for 4 years, a J shaped association between plasma total testosterone and coronary artery disease risk was found (66). In another recent study which followed 1032 American community dwelling men with mean age of 76 years for 9 years, similar J or U shaped

association was found between serum dihydrotestosterone levels and incident coronary artery disease (67).

In the 4 case-control studies, baseline testosterone levels in cases of CAD and matched controls, from the Multiple Risk Factors Interventional Trial (52), Honolulu Heart Programme (54), Baltimore Longitudinal Study of Ageing (55) and the Helsinki Heart Study (57), did not predict CAD events during observation periods of 6-8, 19-20, 9.5 and 5 years, respectively.

Prospective Cohort or Case-Control Studies: Association between Testosterone and

Cardiovascular/all-cause Mortality

There have been a number of recent publications describing the association between serum testosterone levels and cardiovascular mortality in middle-aged and older men followed for 3 to 18 years (59,60,68-71) (Table 1c).

In the Caerphilly (68), MMAS (69), Tromso (60) and Framingham Heart Study (61) cohorts, there was no significant association between testosterone and cardiovascular mortality. Possible explanations for the lack of a relationship in these cohorts include small sample size of elderly men with co-morbidities (61), younger age of the men studied (59,68,69), a relatively small number of deaths (60,68,69) and higher testosterone levels with a mean of 16.1 nmol/L in men in the lowest quartile of serum testosterone (59,68).

A number of studies have demonstrated significant associations between testosterone levels and cardiovascular as well as all-cause mortality. In the Rancho-Bernardo cohort, after adjusting for health status, adiposity and other cardiovascular risk factors, men with testosterone levels in the lowest quartile (mean 7.1nmol/L) had a 40% increased risk of cardiovascular death when compared with men in the highest quartile (mean 15.1nmol/L) (70). The association remained significant after accounting for the possibility of reverse causality by excluding deaths that occurred within the first five years of follow-up. When all-cause mortality in this cohort was analysed as a function of deciles of total testosterone, no additional survival benefit was evident for men with a testosterone level above 10nmol/L. In the EPIC-Norfolk prospective population study (71) men with testosterone levels of 9.5nmol/L had an almost 50% increased risk of cardiovascular death, after adjusting for other risk factors, when compared to men with testosterone levels of 24.2nmol/L. Again the association remained evident after excluding deaths within the first two years of follow-up. The MrOS Swedish cohort with a mean follow up of 4.5 years showed that men with lowest quartile of testosterone and low estrogen had increased risk of all-cause mortality which almost doubled (72). The Study of Health in Pomerania (SHIP) which followed 1954 men for an average of 7.2 years found a significant inverse association between serum total testosterone and mortality from all causes including cardiovascular events (73). The NHANES III prospective cohort study found that low free testosterone and low bioavailable testosterone at baseline were independently associated with higher risks of cardiovascular and all-cause mortality during the first 9 year but not the subsequent 9 to 18 year follow-up period (74). A prospective study of 1687 Italian men with erectile dysfunction found that low testosterone was significantly associated with higher mortality from major adverse cardiovascular events. In a longitudinal study of 930 UK men who had coronary angiogram followed for 6.9 years, low total and bioavailable testosterone had significant negative impact on survival (75).The LURIC study which followed 2069 German men referred for coronary angiography for 7.7 years demonstrated that low testosterone and low vitamin D were significantly associated with all-cause mortality (76). The HIM Study (77), which followed 3637 elderly men for 5.1years, showed that low free testosterone, higher SHBG and higher LH levels were associated with cardiovascular mortality. The European Male Ageing study (EMAS) which followed 2599 European men for 4.3 years showed significant inverse

association between low total testosterone and all-cause mortality and cardiovascular mortality (78). In the Turku cohort of 187 non-diabetic men followed for 10 years, there was a significant inverse association between total testosterone and mortality (79). In a Veterans study which followed men >40 years of age for 4.3 years, comparison between men with testosterone <8.7 nmol/L with those with testosterone >12 nmol/L demonstrated increased mortality in men with low testosterone levels (80). In a prospective cohort study of 581 UK men with type 2 diabetes followed for 5.8 years, the risk of mortality increased in patients with baseline total testosterone <10.4 nmol/L; the risk of cardiovascular mortality significantly increased in those with total testosterone <8.4 nmol/L (81). The studies in the cohort of patients with chronic kidney disease on haemodialysis (82-85) also demonstrated significant negative association between testosterone and increased risk of mortality.

In the recent analysis of the Veterans study which followed 1032 men with mean age of 76 years for 9 years, J or U-shaped association was found between serum dihydrotestosterone levels and mortality (67). Further analysis of the HIM study from Western Australia also demonstrated J or U shaped association between dihydrotestosterone and cardiovascular and all-cause mortality (64,86).

Araujo et al conducted a systematic review and meta-analysis of 12 community-based studies. They concluded that there was a significantly increased risk of both cardiovascular and all-cause mortality in men with low baseline testosterone (87). Corona et al, in their meta-analysis, also found that low testosterone and high oestradiol independently predicted an increased risk of CAD and mortality (88). In general, these meta-analyses have limitations due to between-study heterogeneity.

In summary, only a minority of studies have shown no association between testosterone and CVD mortality. The majority of studies have demonstrated that CAD and cardiovascular deaths are more common in men with low testosterone. Recently two studies have raised the possibility that high levels of endogenous androgens may also increase the risk of CAD and mortality (J or U shaped association). Although the cause and effect nature of the relationship remains to be determined, it is likely that low testosterone is a biomarker for poor cardiovascular health.

Klinefelter Syndrome

Klinefelter syndrome, characterized by small testes, azoospermia, gynaecomastia and a variable degree of hypogonadism with a 47,XXY karyotype has a prevalence of 1 in 500 to 640 men (89). It is estimated that more than 50% of cases are not diagnosed making Klinefelter syndrome potentially the largest single unrecognized (and thus untreated) cause of androgen deficiency in young men (90). Other clinical manifestations also occur in many systems aside from infertility and androgen deficiency (91) with increased mortality due to a variety of causes (92,93) resulting in a reduced life expectancy of 2.1 years (92). An increased IHD risk has been documented both before and after the diagnosis of Klinefelter syndrome (94). However, while circulatory system mortality was increased by 30-40% (92,93), the excess mortality was due to deaths from pulmonary embolism and peripheral vascular disease and IHD mortality was actually reduced (HR 0.7 [0.50-0.90]) (92,93). The relative contributions of the chromosomal abnormality itself, direct cardiovascular effects of testosterone, indirect effects of androgen deficiency including adiposity and diabetes mellitus, and lifestyle factors, such as smoking, are not known. These studies are also unable to account for the role of testosterone replacement therapy. A recent study (95) which investigated the nature of cardiovascular abnormalities in Klinefelter syndrome, demonstrated numerous abnormalities including left ventricular diastolic dysfunction, impaired cardiopulmonary performance, chronotropic incompetence, and increased intima-media thickness which were not reversed by testosterone replacement therapy. These

abnormalities which seem to be more common in Klinefelter's syndrome may represent the underlying pathophysiology for cardiovascular mortality (95).

Interventional Clinical Studies

The potential consequence of decreasing endogenous or increasing circulating levels of testosterone on CAD is discussed under various clinical interventional scenarios.

Endogenous Androgen Deprivation

Castration

A frequently cited but misquoted study (96) compared the life expectancy of institutionalised mentally retarded castrated with intact white males; castrated males lived an average of 13.6 years longer than intact controls. However, the excess mortality in intact inmates was due to infections with no difference in cardiovascular disease mortality between the two groups. The authors correctly concluded that post-pubertal castration did not decrease the frequency of deaths due to cardiovascular disease.

Cross Gender Hormone Therapy

Cross-gender anti-androgen treatment in 816 male-to-female orchidectomised transexuals aged 18-86 years (97) by administration of ethinylestradiol 100µg/day and cyproterone acetate 100mg/day for 7734 patient-years was not associated with any significant difference in arterial cardiovascular mortality or morbidity compared to the general male population despite a 20-fold increase in venous thromboembolic complications. A long term follow up study of 966 male-to-female transsexuals on different high dose estrogen regimens and cyproterone acetate 100 mg / day, followed up for a median of 18.5 years, found a higher mortality rate due to CAD in the individuals aged between 40 and 64 years (98). Another study which followed 100 transsexuals for an average of 10 years also found increased CAD risk in male-to-female transsexuals (99). A case control study which compared 214 male-to-female transsexuals to age and gender matched controls with an average follow up period of 7.4 years found a higher prevalence of CAD in transwomen (100). In the above studies estrogen treatment was also associated with CAD risk. In a recent study, transdermal estrogen preparations as compared to oral estrogen preparations have been shown to reduce cardiovascular risk in male to female transsexuals (101).

Androgen Deprivation Therapy in Prostate Cancer

Androgen deprivation therapy (ADT) is the mainstay of treatment for metastatic prostate cancer and is increasingly used in locally advanced disease. The resultant profound hypoandrogenism is associated with an adverse cardio-metabolic profile characterized by accumulation of body fat (102), reduction in insulin sensitivity (103), increase in the incidence of diabetes (104) and worsening of the control of diabetes among diabetic men (105). More recently it has been appreciated that the risk of cardiovascular disease (106-110) is also significantly increased, and this, in addition to diabetes, is the leading cause of non-cancer deaths in prostate cancer survivors.

An observational study of 73,196 men aged 66 years and older followed for 10 years found that GnRH agonist use was associated with a 16% increase in the risk of coronary heart disease and sudden cardiac death; the incident diabetes risk was increased by 44% (106). The increase in risk was evident after only 4 months of ADT and remained elevated for the

duration of treatment. In a further study of 5077 men stratified according to co-morbidities, established coronary artery disease, and the presence of cardiovascular risk factors, the excess risk was confined to those men with a history of myocardial infarction or heart failure. After 5 years, these men had a 2-fold increase in all-cause mortality with no increase seen in men with either no co-morbidities or a single cardiovascular risk factor (111).

A meta-analysis from six observational studies of ADT reporting either CAD or cardiovascular mortality as outcome, with a total of 129,802 patients on ADT and 165,605 controls showed that the incidence of CAD events was 10% higher in ADT group. Cardiovascular mortality was also significantly higher in the ADT group (112). Another meta-analysis which included 126,898 patients in four cohort studies and 10,760 patients in nine randomised control trials showed a significant increase in cardiovascular morbidity associated with acute myocardial infarction and nonfatal CV events (113).

Androgen Excess from Anabolic Steroid Abuse

It should be emphasised that pathological data from men abusing anabolic-androgenic steroids (AAS) in doses several orders of magnitude higher than those prescribed in clinical practice **should not be extrapolated** to the legitimate therapeutic use of approved testosterone preparations or indeed to androgen physiology.

AAS abuse, previously only prevalent in athletes and body builders (114,115), is said to have increased significantly in recent years, especially amongst adolescents (116,117). Case reports of cardiovascular events in young male body builders abusing pharmacological doses of multiple anabolic agents include arrhythmia, hypertension, acute myocardial infarction, cardiomyopathy and stroke (118). Postmortem examinations in 2 young body builders using AAS who died of acute myocardial infarction did not reveal any lesions in the coronary arteries (119). It is not possible to draw firm scientific conclusions about the cardiac toxicity or atherogenicity of AAS from these sporadic case reports, when the baseline denominator information on prevalence and extent of exposure is shrouded in uncertainty and secrecy. Nevertheless, it has been conjectured that dose-dependent androgen– induced vasospasm, endothelial dysfunction, platelet aggregation, activation of the coagulation cascade, atherogenic lipid profiles (decreased HDL–cholesterol and increased LDL-cholesterol), and direct myocardial injury leading to abnormal left ventricular function (including diastolic dysfunction), cardiac hypertrophy and electrical remodelling are relevant mechanisms precipitating sudden cardiac deaths in young power athletes and body builders (118,120).

Exogenous Testosterone Treatment in Ageing Men

Observational studies

A retrospective cohort study of men in the Veterans Affairs system with low testosterone who had CAD diagnosed by coronary angiography reported that men who received testosterone therapy had increased risk of cardiovascular events compared to those who did not (121). A second retrospective study which collected data from a large healthcare database in California, following an initial testosterone therapy prescription (n=55,593) reported increased risk of nonfatal MI in the first 90 days following the initial prescription as compared to one year prior initial prescription (122). In the most recent multi-cohort retrospective study including commercially-insured and Medicare populations in the United States and general practitioner's records from the United Kingdom (n= 544115), safety of various dosage forms of testosterone preparations were compared. It showed that men who were prescribed injectable preparation of testosterone had a higher risk of cardiovascular events as

compared to gel preparation (HR 1.26; 95% CI 1.18-1.35) (123). Careful analysis of these studies shows several flaws which limit the conclusions that can be drawn about the relationship between CAD and testosterone therapy in these populations.

Another retrospective observational study which analyzed data from 1031 male veterans showed that testosterone therapy in middle-aged hypogonadal men reduced mortality compared to those who did not receive testosterone therapy (124). The most comprehensive long term retrospective audit evaluating the safety of testosterone therapy in 401 hypogonadal men with a mean follow up of 5.41 years did not find an increased risk of major adverse cardiovascular events compared to population with similar co-morbidities not on testosterone therapy (125). In a recently published observational study which analysed data of population-based cohort of men >66 years of age receiving intramuscular testosterone from a Medicare claims database, testosterone therapy was not associated with an increased risk of myocardial infarction (126).

In the most recent retrospective observational analysis of community based healthcare system in Wisconsin which included 7245 hypogonadal men with mean age of 54 years, cardiovascular event rate was 5.5 % in men who received testosterone therapy compared to 6.7% in the untreated arm at 3 year follow up. After adjustment for the baseline differences between treated and untreated patients, the difference in the cardiovascular event rate was not statistically significant (127).

Placebo controlled trials

A trial of testosterone therapy was terminated early as a result of adverse cardiovascular events in the Testosterone in Older Men with Mobility Limitations (TOM) trial, designed to determine the effects of testosterone for 6 months on leg strength and physical function in older men (128). Of the 209 men (mean age 74 years) randomized, 23 in the testosterone group compared to 5 in the placebo group experienced a cardiovascular-related adverse event. These men had a high rate of pre-existing cardiovascular disease (53%) and cardiovascular risk factors (hypertension 85%, diabetes 24%, hyperlipidaemia 63%, obesity 45%) at baseline and were treated with a high dose (100mg/day) of testosterone gel to achieve a serum testosterone of 17.4-34.7mmol/l (500-1000ng/dL). Men exposed to testosterone levels in the highest quartile were at greatest risk (hazard ratio of 2.4). The trial was not designed to assess cardiovascular risk and the authors urged caution in interpreting the results because of the small number of events and limitations with respect to ascertainment of the adverse events. Furthermore, a very similar trial in frail elderly (>65 yr) men (129) using a more conventional dose (50mg/day) of testosterone gel daily showed no increase in CV events in the testosterone treated group during and after treatment.

Meta-analyses

Xu et al conducted a systematic review and meta-analysis of placebo-controlled randomized trials of testosterone therapy among men lasting 12+ weeks reporting CV-related events through the end of 2012. The 27 trials included 2,994 mainly older men with low testosterone and/or chronic diseases, who experienced 180 CV-related events; 33 CV-related deaths were identified. They concluded that CV related events varied with the source of funding showing increased risk in the trials not funded by pharmaceutical industry. However, the TOM trial which was the only one of the 27 studies to report a statistically significant increase in the CV events had a major and disproportionate impact on the analysis. In addition to this, there were several methodological limitations to this study which influenced the results of the meta-analysis (130).

A meta-analysis of 19 randomized clinical trials conducted by Calof et al to determine the risks of adverse events associated with TRT (testosterone replacement therapy) in men ≥ 45

years of age with low or low-normal testosterone levels, found no significant differences in the rates of atrial fibrillation/arrhythmia, MI, chest pain/ischemia, coronary procedures or vascular events/cerebrovascular accidents between the testosterone and the placebo/non-intervention groups (131).

Haddad et al (132) conducted a systematic review and meta-analysis of 30 placebo controlled randomized trials which included 808 men in the testosterone replacement group and 834 men in the placebo group. It assessed the effect of testosterone use on CV events in men with different degrees of androgen deficiency. No significant differences were found among men receiving TRT compared with the control groups. Testosterone use in men with low testosterone levels led to changes in blood pressure and in all lipid fractions (total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides). Results were similar in patients with low-normal to normal testosterone levels (132).

Another meta-analysis conducted by Fernández-Balsells et al, was unable to discern any effect of testosterone on rates of death, myocardial infarction, revascularization procedures or cardiac arrhythmias in men with low/low-normal testosterone levels treated with testosterone for at least 3 months (133). However such risk estimates are imprecise because of the small numbers of subjects, short duration of treatment and limited number of events. None of the included studies were powered for actual cardiovascular events; additionally, several of the studies included men with significant co-morbidities (133).

Ruige et al (2013) conducted a meta-analysis of 10 RCTs to evaluate the effect of testosterone treatment on CV risk. No statistically significant difference between placebo and testosterone treatment was found for testosterone treatment and CV-risk (134).

Corona et al (2014) conducted a systematic review and meta-analysis on 75 placebo-controlled randomised clinical trials with 3016 patients in the testosterone supplementation groups and 2448 patients in the untreated group. Mean duration of the treatment was 34 weeks. This meta-analysis did not show a significant association between testosterone supplementation and major adverse CV events (135).

In the most recent meta-analysis involving 29 heterogenous studies with 122,899 men, it was shown that testosterone therapy did not increase the risk of cardiovascular events significantly (RR 1.168, CI 0.794 – 1.718; P 0.431) (136).

In summary, three retrospective studies (121-123), one placebo controlled trial, the TOM trial (128), and one meta-analysis which was largely based on the TOM trial, have reported increased risk of cardiovascular events with testosterone therapy. The men in these studies were older and had significant co-morbidities including CAD and CV risk factors. Reassuringly, a large number of studies, including several meta-analyses, have shown no significant increase in the risk of cardiovascular events with testosterone therapy. Nevertheless, the unexpected finding of the TOM trial (128) and the reports of Vigen (121), Finkle (122) and Layton (123) should alert physicians and clinical investigators to the possibility of harm from testosterone treatment, particularly with higher doses, in older (>65 yr) men with pre-existing cardiovascular disease and/or risks factors.

The US Food and Drug Authority (FDA) has recently instigated product labeling changes to include warnings regarding the potential increased risk of cardiovascular events with testosterone replacement therapy and strongly recommended further studies to investigate this possible association, while recognising that current evidence was inconclusive (137).

The Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines Agency (EMA) concluded that there is no consistent evidence to support an increased risk of

heart problems associated with testosterone therapy in hypogonadal men. Nevertheless, PRAC recommended that product information of all testosterone-containing medicines in the European Union should be updated to include warnings against the use of these products in men suffering from severe heart, liver or kidney problems (138).

Exogenous Testosterone Treatment in Men with CAD and Heart Failure

Uncontrolled studies from the 1940s, largely of historical interests only, suggested that testosterone may improve symptomatic CAD in men. More recent data are considered below.

Bolus intravenous or intracoronary injections of pharmacological doses of testosterone acutely improved myocardial ischaemia or induced coronary artery dilatation in a small number of men with CAD (139-141). Whether this acute pharmacological action of testosterone can be translated into a therapeutic effect remains to be determined.

In a randomised placebo-controlled double-blind study, testosterone cypionate 200mg i.m. weekly for 8 weeks decreased ST segment depression during exercise testing in 25 men with positive tests (142). In a placebo-controlled crossover (but not double-blinded) study in 62 elderly men with CAD, oral testosterone undecanoate for 4 weeks improved subjective symptom scores and resting ECG (143). Transdermal testosterone patch 5mg daily for 12 weeks increased the time to 1-mm ST segment depression in men with symptomatic CAD (144) and oral testosterone selectively improved myocardial perfusion to areas supplied by unobstructed coronary arteries in men with coronary heart disease and baseline testosterone levels of 9nmol/L (145).

These preliminary data suggest ECG changes can improve after (maximum of 12 weeks) short-term testosterone supplement in CAD patients with low testosterone levels. A small study of 13 men with testosterone levels <12nmol/L and angina found that the benefits of intramuscular testosterone on myocardial ischaemia (as determined by ST segment depression) were maintained at 12-months (146). In a double blind randomized placebo controlled trial 87 diabetic male subjects (mean age: 74+/-7 years) with proven CAD were randomized to a 12 week treatment with either testosterone undecanoate or placebo, testosterone therapy significantly reduced the number of anginal attacks/weeks of 34% ($p<0.05$); the silent ischemic episodes of 26% ($p<0.05$), and the total ischemic burden of 21% ($p<0.05$) on ambulatory ECG monitoring (147).

It is unclear whether the apparently beneficial effects of testosterone in CAD are due to direct coronary artery dilatation or due to its effects on skeletal muscle resulting in non-specific improvements in exercise performance or due to its effects on the brain, modulating the central pain pathway. It is also unclear whether testosterone therapy can provide real symptomatic and functional benefits in men with CAD.

Currently, **heart failure** is a labeled contra-indication for testosterone treatment. However, there are recent limited data exploring the use of testosterone in patients with cardiac failure who frequently have low circulating testosterone levels. Cardiac output was acutely increased after two doses of buccal testosterone in men with heart failure (148), and 12 weeks of intramuscular therapy improved peak oxygen consumption in elderly men with stable chronic heart failure (149). Over 12-months of follow-up, transdermal testosterone improved functional capacity in men with moderately severe heart failure when compared to placebo (150). In a double blind randomized controlled feasibility study of testosterone

therapy during exercise rehabilitation in male patients with chronic heart failure who had low testosterone, testosterone therapy improved indices like shuttle walk test, hand grip strength, leg strength, echocardiographic measures and body mass (151).

Toma et al conducted a meta-analysis of 4 randomised controlled trials which showed that testosterone therapy was associated with a significant improvement in exercise capacity compared with placebo. The mean increase in the 6-minute walk test, incremental shuttle walk test, and peak oxygen consumption between the testosterone and placebo groups was 54.0 m (95% CI, 43.0–65.0 m), 46.7 m (95% CI, 12.6–80.9 m), and 2.70 mL/kg per min (95% CI, 2.68–2.72 mL/kg per min), respectively. Testosterone therapy was associated with a significant increase in exercise capacity as measured by units of pooled SDs (net effect, 0.52 SD; 95% CI, 0.10–0.94 SD). They did not find any significant adverse cardiovascular events (152).

These preliminary findings require validation in rigorously controlled clinical trials and do not alter the current established indications and contra-indications for testosterone therapy, especially in light of recent concerns regarding CV safety (vide supra).

In summary, the continuing uncertainty regarding CV adverse effects of testosterone treatment requires urgent investigation. Large-scale prospective randomised placebo-controlled trials of sufficient size and duration are urgently needed to assess not only the benefits in terms of meaningful clinical benefits and patient-important outcomes but also to document the risks of serious adverse events in testosterone treatment. In the meantime, physicians should be mindful of the possibility of potential (serious) harm from testosterone treatment particularly in older men with pre-existing CV disease or risk factors. The balance of potential risk versus possible benefits and the current lack of definitive safety data should be carefully discussed with each individual patient before starting treatment.

Dehydroepiandrosterone (DHEA) and Coronay artery disease (CAD)

DHEA and its sulphate, DHEAS, are weak but highly abundant adrenal androgens which show a progressive age-related decline from the third decade onwards (153,154) . There is a body of opinion suggesting that DHEA supplementation may be beneficial to the elderly in a variety of physiological functions including the prevention of cardiovascular disease (155,156). It has been implied that, against an androgenic milieu in men, DHEA acts as a pro-hormone for conversion to metabolites with predominantly oestrogenic effects and potentially anti-atherogenic actions (157,158).

Observational Clinical Studies

DHEA and CAD

Many observational studies in men have attempted to demonstrate a correlation between serum DHEAS levels and different CAD endpoints including the extent of atherosclerosis assessed by autopsy, coronary angiography, carotid vessel thickness/pulse wave, aortic calcification and clinical disease states including angina, myocardial infarction and mortality (Table 2). These have shown inverse (36,39,41,159-164) (mostly cross-sectional), null (24,27,39,43,161,164-170) or positive (30,57) relationships between DHEAS levels and CAD (Table 2).

Of the nested case control or prospective cohort studies, 10 of 21 studies (57,159,161,163) showed association between DHEAS levels and cardiac events (Table 2). In the Helsinki Heart Study of middle-aged dyslipidaemic men, higher DHEAS levels were associated with an increased risk of CAD (57). In the Honolulu Heart Study of 6000 men of Japanese descent followed for 18 years (161), low DHEA was associated with fatal but not non-fatal CAD. In the Rancho Bernardo cohort study, a preliminary report of 242 men also showed a negative relationship between DHEAS and CAD mortality (159). However, in the full analysis of the same study on 942 men over 19 years (164), there was only a modest negative relationship between DHEAS and those that survived their cardiac events, but none with CAD mortality. In the Swedish MrOS cohort, low DHEA and DHEAS were inversely associated with cardiovascular and all-cause mortality (171). Further analysis of the same cohort showed that low DHEA and DHEAS predict an increased risk of CAD (172). In the Vietnam Experience study involving 4255 US Vietnam – era army veterans followed up for 15 years, DHEAS was inversely associated with cardiovascular and all-cause mortality (173). In the Cardiovascular Health Study - All Stars study, which followed 989 elderly men and women for 9 years, DHEAS was negatively associated with CAD (174). In the MMAS prospective cohort study which followed 1709 men aged 40 – 70 years for 9 years, low baseline DHEA and DHEAS predicted incident CAD (175). In the Rotterdam study which had 1180 participants who were followed up for a mean of 12.3 years, low baseline DHEAS was shown to be an important indicator of future development of atrial fibrillation (176). In a study with 963 men and 1161 women of age 65-76 years and followed up for 7.4 years, there was no association of serum DHEAS with age adjusted all cause and CVD-mortalities (167). In the Framingham Heart Study which followed 1928 men for 10 years, the association of DHEAS with incident cardiovascular disease was not statistically significant (59).

In summary, one cross-sectional study and one nested prospective case-control study of a small number of men, have shown positive associations of DHEAS with CAD and CV mortality. Few cross-sectional studies (24,27,38,43) and several prospective studies (55,59,161,164-170) including two large population-based studies (59,167) have not shown any association of DHEAS with CAD or CV mortality. A majority of the cross-sectional studies (36,39,41,160,162,163), and of prospective studies which included large number of men (164,171-176), have shown a negative association of DHEAS with CAD and CV mortality. Overall, it appears that DHEAS is negatively associated with CAD. DHEAS levels also appear to be associated with increased mortality from all causes of death in men over the age of 50 (161,164,166), giving rise to the notion that this is a non-specific marker of poor health and lack of adaptive capacity to acute illnesses or a secondary phenomenon consequent upon various diseases of ageing such as malignancies and heart failure (153,169,177).

Interventional Studies with DHEAS

In a mixed population of Addison's disease and secondary hypoadrenalism (n=40), DHEA supplementation for 12 weeks normalized DHEAS and androstenedione (178). Apart from minor reductions of LDL-cholesterol, there were no other metabolic or vascular changes seen at the end of the study period (178). Administration of DHEA (25 mg od for 4 weeks) to 12 eugonadal, hypercholesterolaemic men (mean age 54 years) improved brachial artery flow-mediated dilation (bFMD) and serum PAI-1 concentration when compared to a matched group treated with placebo (179). The only RCT which has investigated the effects of 50 mg of DHEA administration over 1 year in both men and women (n=280, age range: 60-79 years) found that there were no significant benefits in men. Women above the age of 70

years, benefitted by increased bone density but aortic pulse wave velocity (aPWV) remained unchanged in either gender (180).

Taken together, data from observational and interventional studies in men do not support the hypothesis that DHEAS 'deficiency' is a risk factor for CAD fatalities or that DHEA may confer an anti-atherogenic action in men. The interventional studies showed there were no discernible effects on vascular function in either short or long term in either gender. DHEA may be a non-specific marker for ill health in general.

Androgens and arterial disease - Animal studies

Androgen/androgen receptor (AR) can modulate arterial disease and vascular function (*vide infra*) via genomic (AR) or non-genomic mechanisms. The diversity and complexity of the actions of testosterone (and its metabolites E2 and DHT) on the vasculature reflect the multiple cellular targets in the vessel wall, differences between species, gender, concomitant disease and, most importantly, level/dosage of testosterone exposure.

Animal Models

The correlation between low circulating testosterone and atherosclerosis is one of the most important pieces of clinical evidence suggesting a potential cardiovascular benefit of androgen replacement therapy (ART) to hypogonadal men (181-183). However, the outcomes of recent clinical studies of androgen replacement therapy are controversial and contradictory, demonstrating an excess, null effect or reduction of cardiovascular events in apparently hypogonadal men using ART (121,122,128). Srininvas Shankar *et al* (184) and Shores *et al* (124) also showed no effect or apparent beneficial effect of testosterone replacement in hypogonadal or elderly men, respectively. In preclinical research, a number of animal models (predominantly in pigs, rabbits, rats or mice) have been developed to investigate different aspects of vascular pathology. Exploiting the power of these models to address the key mechanisms underpinning androgens and cardiovascular health is critical for interpretation of the complicated and inconsistent data generated from clinical and epidemiological studies. The models available can be broadly separated into investigations of atherosclerosis (using genetically-altered animals with or without modified diets) and of neointimal proliferation of the vascular wall (the fibroproliferative response to acute vascular injury).

Models of Neointimal Lesion Formation

Clinically, the acute formation of a fibroproliferative neointima in the blood vessel is most commonly seen after percutaneous vascular interventions (e.g. balloon angioplasty, stenting). It is attributed to the dramatic acute injury (stretching, removal of the endothelium) to the vessel wall, which produces an acute inflammatory and fibro-proliferative response characterized by infiltration and proliferation of smooth muscle-like cells in the sub-endothelial space (185-188). Uncontrolled neointimal thickening, with narrowing of the lumen, may restrict blood flow and thus induce ischemia in the downstream tissue. Animal models of neointimal proliferation exploit this acute response by using a number of different techniques to induce lesion formation. These can be divided into techniques that remove the endothelium and those that do not.

Induction of Neointimal Lesions using Intraluminal Injury

Neointima formation is induced by inserting an injuring device (e.g. balloon, wire, stent (185,186)) into a suitable artery to denude the endothelium and over-expand the medial layer. In mice, there is evidence that the neointimal lesion is formed predominantly from circulating bone marrow-derived progenitor cells (188). This is a good model in which to observe the vascular response to acute injury and study the mechanism of vascular inflammation and neointimal cell proliferation in the absence of conventional cardiovascular risk factors.

Induction of Neointimal Lesions using Non-denuding Injury

External, non-denuding injury can be induced by ligating the target artery or by placing a silastic cuff around the vessel. The mechanisms underlying lesion formation are not wholly clear but may involve disturbed blood flow and altered shear stress leading to endothelial dysfunction. Neointimal lesion formation occurs mainly through migration and proliferation of media-derived mural smooth muscle cells (187). In general, ligation injury causes less direct vascular damage than wire injury, producing smaller neointimal lesions.

Models of Atherosclerosis

Atherosclerosis can be induced using a “western-type” diet (high fat and/or high cholesterol diet) in experimental animals. The rabbit is a popular species in which to study atherosclerosis due to its high sensitivity to dietary cholesterol (189,190). With the establishment of transgenic mouse lines (e.g. apolipoprotein E deficient ($apoE^{-/-}$) and low density lipoprotein receptor knockout ($LDLR^{-/-}$), rodent models of diet-induced atherosclerosis are now widely used (190). Atherosclerotic lesions present a more complex pathology than injury-induced neointimal lesions, including leucocyte infiltration, foam cell formation, lipid deposition, vascular wall calcification and plaque erosion. As a consequence, characterization and quantification of atherosclerotic lesions may vary considerably depending on the artery selected for analysis and the technique (e.g. *en face* staining, histology, lipid extraction) used. This can complicate comparison between different investigations.

Animal Models for Androgen Deficiency

Orchidectomy (Odx) is used to abrogate endogenous testosterone production as a model to study the impact of hypogonadism. In recent years, transgenic mouse models have allowed us to manipulate the androgen receptor (AR) at global and cell-specific levels (191-193). Testicular feminized mice (Tfm), which have a non-functional AR (192), and global AR knockout mice (ARKO) (193), are both models of complete androgen insensitivity. These mice have cryptorchid testes and produce little testosterone. The circulating testosterone level in these mice is less than 10% of that in wild type mice (192,193). Testosterone replacement is often applied to these animals to investigate testosterone -mediated AR-independent effects (193).

Androgen and Vascular Disease

Androgen and Atherosclerosis

The influence of androgens on the development of atherosclerosis has been investigated in a number of animal models (Table 3) (189,194-201). Driven by the clinical question of whether pharmacological androgen treatment is protective against atherosclerosis, the "castration plus androgen replacement therapy" model is widely used in preclinical studies. Androgens, including testosterone, dihydrotestosterone (DHT) and dehydroepiandrosterone (DHEA), have been consistently reported to suppress atherosclerosis in male animals in the past decades (195,199-201). This association is robust, when considering the wide range of techniques employed to quantify the atheroma, which include measuring: cholesterol content in homogenized arteries, plaque size based on histology, and lipid accumulation using *en face* oil-red staining. Evidence suggests that exogenous testosterone exerts a dose-dependent inhibition on plaque development, even when extended to supra-physiological concentrations. Noticeably, the anti-atherosclerotic effect of androgens was only observed in males (194,196). Pharmacological testosterone for males has been demonstrated to exacerbate atherosclerosis in castrated females in multiple species including: monkeys, rabbits and mice (194,196,197). Given these observations, the following discussion will focus on the role of androgen in male cardiovascular health.

Pharmacological tools have been widely employed to investigate whether the anti-atherosclerotic effect of testosterone is mediated by AR-dependent signalling or by aromatase-dependent conversion of testosterone into estradiol. Li *et al.* reported that the AR antagonist flutamide reversed testosterone-mediated inhibition of atherosclerotic plaque formation in castrated rabbits, suggesting AR activation is required for inhibition of atherosclerosis (199). Nathan *et al*. showed that the aromatase inhibitor anastrazole counteracted the anti-atherosclerotic effect of testosterone in mice, demonstrating that testosterone-derived oestrogen also contributes to inhibition of atherosclerosis (198). Studies using transgenic mice with global AR insensitivity confirmed that AR activation is necessary for the protective effect of testosterone against atherosclerosis, but the finding that testosterone supplementation in AR deficient mice still reduced atherosclerotic plaque supports the existence of AR-independent mechanisms also (192,193).

In contrast to atherosclerosis, neointimal lesion formation induced by acute mechanical injury, in the absence of cardiovascular risk factors, is mainly driven by infiltration and proliferation of smooth muscle-like cells (202). The evidence for androgens altering neointimal hyperplasia is limited and controversial. An inverse relationship between serum testosterone and neointima formation following angioplasty was reported in a male swine model. However, the inhibitory effect of testosterone was only observed in moderate injury but not in severe injury (203). Conversely, in a rat model, neither Odx nor Odx plus testosterone replacement altered neointima formation (204). More recently, we have demonstrated that endogenous but not exogenous testosterone reduced total neointimal volume (measured in 3-dimensions using optical projection tomography) but not maximum cross-sectional area (by conventional histology) (191). These data suggest that endogenous testosterone only exerts moderate suppression of neointimal cell recruitment/proliferation and could be overwhelmed by severe vascular injury. Further investigation using vascular smooth muscle and endothelial cell-specific AR knockout mice revealed that testosterone - mediated inhibition of neointimal lesion formation is not dependent on vascular AR expression, which suggests that non-classical mechanisms (eg AR in non-vascular cells or AR-independent inhibition of lesion development) are involved in modulation of neotinimal proliferation.

Cell-specific Effects of Androgen

It is difficult to determine the mechanism of androgen-mediated alteration in vascular remodeling using *in vivo* models. *In vitro* studies have shown that nitric oxide (NO) production is increased in endothelial cells upon stimulation with testosterone (205,206). NO is well known for its ability to suppress leukocyte adhesion and inhibit smooth muscle proliferation (207). Thus testosterone may promote vascular health via activation of endothelial NO production. Conversely, testosterone is reported to stimulate proliferation and migration in cultured smooth muscle cells (205,208), which is not consistent with its *in vivo* benefit in reducing vascular restenosis. Questions remain as to whether these counteracting mechanisms occur *in vivo* and what effect they have on regulation of vascular homeostasis. The mouse femoral artery ligation model preserves an intact endothelium and induces a mild injury, and is thus a useful tool to study the subtle balance between endothelial cells and smooth muscle cells. Using this model, endothelium selective AR deletion caused an increase in neointimal lesion formation, suggesting a protective role for endothelial AR. This effect was not observed, however, in mice with deletion of AR from both endothelium and smooth muscle, suggesting additional AR-mediated regulatory interactions between these cells (191).

In addition to vascular cells, we identified a very small population of perivascular cells expressing abundant AR in healthy arteries and the number of these AR-positive cells increased following vascular injury (191). These unidentified cells may regulate perivascular inflammation and the subsequent neointimal lesion development. Recently, Huang *et al.* bred $LDLR^{-/-}$ mice with endothelial cell, smooth muscle cell or macrophage/monocyte-specific AR knockout to investigate the role of cell-specific AR in atherosclerosis (209). Their results suggest that AR in monocytes/macrophages, rather than in endothelial and smooth muscle cells, regulates atherogenesis. This study supports the concept that AR expressed in non-vascular cells play a critical role in regulation of vascular disease.

Metabolic effects of androgen

In addition to the direct effects on vasculature, testosterone plays an important role in regulating cardiovascular risk factors such as blood lipid and glucose metabolism in male animals. Both endogenous and exogenous testosterone have been reported to reduce LDL and VLDL cholesterol as well as atherosclerotic plaques (193,200). The disturbed blood lipid homeostasis may be attributed to altered lipid metabolism in the liver where low testosterone levels induce hepatic steatosis with dysregulation of lipid assembly and secretion (210,211). Furthermore, orchidectomised male mice also demonstrated higher fasting glucose and insulin levels (211). By using adipocyte-specific ARKO mice, McInnes *et al.* demonstrated that androgen/AR signalling in adipose tissue is important in maintenance of glucose homeostasis (212). The impact of androgens on cardiovascular risk factors will be further discussed in section 5.

DHEA and atherosclerosis

DHEA (3-15nM) and its sulphated form, DHEAS (3-10μM), are the most abundant steroid hormones in the human circulation. DHEA can be converted to androgen and oestrogen at a similar rate (213,214). In animal studies, supplementation of DHEA (0.3-0.5% w/w in diet) consistently decreases atherosclerosis in both sexes with/without castration (Table 3) (195,215-218). Hayashi *et al*. (217) demonstrated that the anti-atherogenic effects of DHEA in ovariectomised female rabbits can be partially (50%) blocked by the aromatase inhibitor fadrozole, suggesting a role for conversion of DHEA to oestrogen. Other studies suggest that DHEA exerts its effects via ER/AR independent pathways (219,220). Together with the fact that DHEA is a very weak agonist for AR/ER and no specific DHEA receptor has been

identified, the mechanism through which DHEA protects against cardiovascular disease is still elusive. Savineau *et al.* recently reviewed the cellular and molecular mechanisms of DHEA in cardiovascular diseases as well as its metabolic interaction with other endocrine hormones (213).

Effects of androgens on cardiovascular risk factors

The net effect of androgens on cardiovascular risk is difficult to assess for at least six reasons.

- The risk factors for cardiovascular disease are numerous and ever increasing. Testosterone can influence several risk factors simultaneously, some of which at first sight appear beneficial, (e.g. lowering lipoprotein(a) (Lp(a)), insulin, fibrinogen, and plasminogen activator type 1 (PAI-1)), while others are considered adverse (e.g. suppressing HDL-C).
- Endogenous testosterone appears to have opposite effects on cardiovascular risk factors to that of exogenously administered testosterone.
- The associations between serum concentrations of endogenous testosterone and cardiovascular risk factors are confounded by the complex interactions between endogenous androgens, body fat distribution, and insulin sensitivity.
- A causal relationship between some of the aforementioned changes in risk factors and atherosclerosis has not been proven. Of particular importance is the example where exogenous testosterone -induced suppression of HDL-C may not necessarily be accompanied by changes in cardiovascular risk.
- Testosterone can exert its metabolic effects directly or via its metabolites E2 and dihydrotestosterone. The effects of testosterone and E2, in particular, can be either be additive (e.g. on Lp(a)) or counter-regulatory (for example on HDL-C).
- Polymorphisms in the genes encoding the androgen receptor, sex hormone binding globulin (SHBG) and 5αreductase regulate genomic effects and bioavailability of testosterone and dihydrotestosterone, respectively. Thus, at any given serum concentration of testosterone, the metabolic effects at individual target tissue sites are pleiotrophic and complex.

Associations of Endogenous Testosterone with Cardiovascular Risk Factors

Several population-based studies have found statistically significant correlations between plasma levels of testosterone and various risk factors:

Blood pressure and arterial stiffness

Several population based studies have shown an inverse relationship between testosterone and blood pressure (53,221-228). A similar inverse relationship has also been demonstrated between testosterone and arterial stiffness. In a retrospective, cross-sectional study of older men, free serum testosterone was negatively correlated with vascular stiffness (229). The Baltimore Longitudinal Study of Ageing, in a subpopulation of 206 men, showed serum testosterone levels to be an independent negative predictor for developing arterial stiffness (230). Studies demonstrating the development of arterial stiffness within three months of starting androgen deprivation therapy in patients with prostate cancer further support this inverse relationship (231,232).

Obesity and body fat distribution

Population-based studies have shown that endogenous testosterone is consistently and inversely associated with BMI. In the EMAS study which included 3200 European men, mean total testosterone and free testosterone were significantly lower in overweight (TT –2.32 nmol/L & FT -17.60 pmol/L) and obese (TT –5.09 nmol/L & FT -53.72 pmol/L) compared with the non-obese reference group across all ages (233). The Swedish MrOS study, which included an analysis of 2416 men, showed a statistically significant decrease in BMI with increasing quartiles of total testosterone (65). The Hypogonadism in Males (HIM) study compared 836 hypogonadal men with 1326 eugonadal men. The mean BMI for hypogonadal men was found to be 31.5 compared with 28.5 for eugonadal men (234). The percentage of body fat evaluated in a study of 57 men aged between 70 and 80 years was also found to have negative correlation with testosterone levels (235).

Several cross-sectional (236,237) and longitudinal studies (238,239) have demonstrated that abdominal adiposity is inversely associated with testosterone levels. Studies which have used CT or MRI scans to measure abdominal fat have confirmed significant negative correlations between testosterone concentrations and visceral fat (240-243).

Dyslipidaemia and Inflammation

Observational studies have shown that low endogenous testosterone is associated with high total cholesterol, high LDL and low HDL (244).
The association between levels of endogenous testosterone and the markers of inflammation from epidemiological studies shows conflicting results. Some studies (70,245-249) have shown significant negative associations between testosterone and markers of inflammation while others have failed to demonstrate any association (250-253).

Metabolic syndrome

It is increasingly appreciated that low serum testosterone in men is associated with metabolic syndrome (MetS). Several cross-sectional and longitudinal studies have shown negative association of testosterone with MetS.

Corona et al (2011) demonstrated in a meta-analysis of 20 studies, that patients with MetS showed significantly lower testosterone compared to healthy individuals. Lower baseline testosterone was demonstrated among patients with incident MetS compared to controls in longitudinal studies (254). Brand et al (2011), in another meta-analysis of 52 observational studies comprising 22,043 men and 7839 women, showed that endogenous TT and FT levels were lower in men with MetS but higher in women with MetS. In both sexes, higher SHBG levels were associated with a reduced risk (255). Recently in a further meta-analysis of observational studies, Brand et al (2014) showed an inverse relationship between TT, FT and SHBG with the MetS (256). The magnitude of associations was largest in non-overweight men and varied across individual MetS components with stronger associations observed with hypertriglyceridaemia, abdominal obesity and hyperglycaemia. The associations were weakest for hypertension (256).

Type 2 Diabetes Mellitus (T2DM)

A large body of evidence has emerged in the recent years showing a consistent association between low testosterone and T2DM.
In a meta-analysis (257) of prospective studies, there was a difference in testosterone of −2.48 nmol /L (95% CI −4.04 to −0.93, *P* = 0.02) between men with and without T2DM. Men with higher testosterone levels (range, 15.6- 21.0 nmol/L) had a 42% lower risk of type

2 diabetes (RR, 0.58; 95% CI, 0.39 to 0.87) (258). Longitudinal studies of the cohorts of Tromso (259), MRIFT (260), Gothenburg (261), Rancho Bernardo (262), Koppio (263), MMAS (264,265) and Cardiovascular Health Study (266) all reported inverse relationships between baseline testosterone and future development of T2DM. A further meta-analysis by Corona et.al showed a significant inverse association between testosterone and T2DM in both cross-sectional and longitudinal studies analyses (267).

The relationship between androgens, body fat distribution, and insulin sensitivity, of which the latter two are also involved in the regulation of HDL and triglyceride metabolism, is complex (268,269). It is not clear whether androgens regulate adipose tissue and insulin sensitivity or whether, vice versa, adipocytes and insulin regulate testosterone levels; probably a bi-directional relationship exists. Morbidly obese and insulin resistant men frequently have low serum levels of testosterone which increase upon weight loss (270,271). E2 levels show the opposite changes to testosterone with obesity and weight loss. It has therefore been suggested that obesity may cause hypotestosteronemia by increased aromatisation of testosterone to E2 in the adipose tissue but direct evidence for this is lacking. Supporting a role of insulin in the determination of testosterone levels in men, hyperinsulinemic-euglycemic clamp studies in young healthy men documented that increasing insulin resistance was associated with a decrease in Leydig cell testosterone secretion (272). In support of the notion that testosterone is the dominant driving factor in the bi-directional relationship, however, quantitative CT analysis of hypogonadal men (mean age 52 years) has shown that they have a greater subcutaneous fat area and a trend towards an increased visceral fat area when compared to age-matched eugonadal men (273).
In summary, low testosterone is associated with all the components of metabolic syndrome – central adiposity, hypertension, dyslipidaemia, insulin resistance and type 2 diabetes. It is therefore important to measure serum testosterone in these men who have symptoms suggestive of testosterone deficiency.

Role of the Androgen Receptor (AR)

As androgen action is inversely proportional to the number of CAG repeats in exon 1 of the AR gene (274,275), it has been suggested that there may be an association between CAG repeat length and cardiovascular disease. Shorter CAG repeat length has been associated with both negative predictors of CV risk including lower levels of HDL-cholesterol (276,277) and reduced flow-mediated vasodilatation (276) and presumed beneficial changes such as lower body fat and plasma insulin in healthy men (278) and reduced body fat in men with Type 2 Diabetes (279) or CAD (280) . A shorter CAG repeat length was correlated with more severe CAD in men aged 36-86 years of age (280) but not with CAD or MI in middle-aged men (277). However the above preliminary results have not been replicated in larger population studies. AR CAG repeat numbers did not show any cross-sectional associations with serum lipid levels in over 3000 European men (281) nor predicted incident heart disease (or changes in HDL-cholesterol, LDL-cholesterol or BMI) in American men followed for 15 years (282).

Effects of Exogenous Testosterone and DHEA on Cardiovascular Risk Factors

The effects of exogenous androgen on cardiovascular risk factors varies with the dose, route, duration and type of treatment, as well as the age, gender and conditions of the recipients (for review see (283)). The most consistent findings were decreases in fat mass (and increase in lean mass) plasma levels of HDL cholesterol, lipoprotein (a) (Lp(a)) and fibrinogen, which are accompanied by much less prominent declines of LDL cholesterol and triglycerides .

Blood pressure

Animal studies suggest that androgens may promote retention of sodium and water and consequently elevate blood pressure (284). The mechanism for this is thought to be up-regulation of the renin-angiotensin system in the proximal tubules (284-286). The increased risk of developing hypertension with androgens has been confirmed in men who abuse anabolic steroids (120,287,288). Randomised, placebo controlled interventional studies with testosterone replacement therapy have not shown significant increase in blood pressure. **However, considering the possibility of elevation of blood pressure by androgens, it is important to monitor blood pressure during testosterone replacement therapy.**

Visceral Fat Mass

Improvement in body composition with reduction in fat mass has been a very consistent finding in almost all the testosterone replacement studies. Placebo controlled trials of testosterone replacement therapy in ageing men have shown decreases in total fat mass ranging from 1- 4.5 kg in studies of 3 – 36 months duration (289). A Malaysian study which used intramuscular testosterone undecanoate for 48 months showed significant fat loss (290). A recent non randomized Italian study which investigated intramuscular testosterone undecanoate in aging men for 5 years showed a reduction in waist circumference by 9.6 +/- 3.8 cm and weight reduced by 15 +/- 2.8 kg (291). Data on the effects of exogenous testosterone specifically on visceral fat are limited. Intra-abdominal visceral fat loss was seen (on MRI) in non-obese ageing men treated for 12 months with transdermal testosterone (292) and 6 months with intramuscular testosterone (293). Similar results have been reported in obese men (294,295). A recent study investigated testosterone replacement and withdrawal in severely obese men and showed favourable changes in the body composition with testosterone replacement (296). Studies using intramuscular and transdermal testosterone in diabetic patients showed significant improvements in body composition (297-299). A recent meta-analysis of the management of late onset hypogonadism also confirmed that testosterone therapy resulted in significant fat loss (300). These data indicate that in men, the dominant action in the bi-directional relationship is that testosterone reduces fat mass especially in the abdomen and improves insulin action. In agreement, androgens activate the expression of beta adrenergic receptors, adenylate cyclase, protein kinase A and hormone sensitive lipase in adipocytes (301). As a result, testosterone stimulates lipolysis and thereby reduces fat storage in adipocytes.

Testosterone replacement therapy has favourable effects on visceral adiposity in men with obesity having low testosterone. The benefits are possibly greater in men who are more obese with lower testosterone. An Australian study which compared TRT and lifestyle modification with lifestyle modification alone has shown that TRT offered added benefits on visceral fat loss and cardiovascular risk but long term benefits versus risk profile needs to be established in further interventional studies. With the currently available data, TRT cannot be recommended to be used as a general weight loss therapy in obese men who do not have hypogonadism.

Lipids and Lipoproteins

HDL-Cholesterol

In the majority of studies, substitution of testosterone in hypogonadal men and men with low/low-normal testosterone levels has decreased HDL-C levels (133,283). In *normal young* men, supraphysiological doses of testosterone or androgen-like anabolic steroids decreased

HDL-C by 20% or more. Conversely, surgical castration or medical castration by GnRH analogues increased HDL-C (283). In *hypogonadal* patients or in *elderly* men, substitution of testosterone led to minor or no decrease in HDL-C. Meta-analyses have suggested that suppression of HDL-C is directly correlated with the dose of testosterone but inversely related to the age and duration of treatment (302-304). Transdermal application of testosterone or dihydrotestosterone exerted less effect on HDL-C than oral and intramuscular administration.

Since low HDL-C is an important CAD risk factor and HDL exerts several potentially anti-atherogenic actions, lowering of HDL-C by testosterone treatment may potentially increase cardiovascular risks. However, the epidemiological association of low HDL-C with CAD has not been proven to represent a causal relationship. Instead, low HDL-C often coincides with other components of the metabolic syndrome, and may therefore merely be a surrogate marker for other co-existing pro-atherogenic condition(s). Moreover, in transgenic animal models, only increases of HDL-C induced by apoA-I overproduction but not by inhibition of HDL catabolism were consistently found to prevent atherosclerosis (305). Therefore, the *mechanism* of HDL modification or turnover rather than *levels* of HDL-C per se, appear to determine the (anti)-atherogenicity of HDL modification (Figure 1).

Two genes involved in the catabolism of HDL are up-regulated by testosterone, namely scavenger receptor B1 (SR-B1) and hepatic lipase (HL). SR-B1 mediates the selective uptake of HDL lipids into hepatocytes and steroidogenic cells, including Sertoli and Leydig cells, as well as cholesterol efflux from peripheral cells including macrophages. Testosterone up-regulates SR-B1 in the human hepatocyte cell line HepG2 and in macrophages and thereby stimulates hepatic selective cholesterol uptake and peripheral cholesterol efflux, respectively (306). HL hydrolyses phospholipids on the surface of HDL thereby facilitating the selective uptake of HDL lipids by SR-B1. The activity of HL in post-heparin plasma is increased after administration of exogenous testosterone (307) and slightly decreased by suppression of testosterone after GnRH antagonist treatment (308). Increasing both SR-B1 and HL activities are consistent with the HDL lowering effect of testosterone. Interestingly, in transgenic mice, overexpression of SR-BI or HL caused a dramatic fall in HDL-C but inhibited rather than enhanced atherosclerosis (305). This again demonstrates the fallacy of extrapolating the HDL-C lowering effect of testosterone to increased cardiovascular risk.

Any effects of exogenous DHEA on cardiovascular risk factors appear to be marginal. In men aged 60 – 84 yr, DHEA 100mg daily for 3 months decreased total and HDL-cholesterol (309) but this was not confirmed in a larger study (310).

Lipoprotein (a)

Results of many case-control studies and most prospective population studies demonstrate that lipoprotein(a) (Lp(a)) levels higher than 30 mg/dL (75 nmol/L) are an independent risk factor for coronary, cerebrovascular, and peripheral atherosclerotic vessel diseases, especially if it coincides with other cardiovascular risk factors (311). Although Lp(a) levels are predominantly genetically determined, administration of testosterone to men consistently and significantly decreased serum levels of Lp(a) by 25% to 59% (283,312). Conversely Lp(a) levels were increased by 40% to 60% in men in whom endogenous testosterone was suppressed by treatment with the GnRH analogues (283,313,314). The Lp(a) lowering effect of testosterone is independent of E2, which also reduces Lp(a) levels. It is not known how testosterone regulates Lp(a). It is also not known whether the decrease in circulating Lp(a) induced by testosterone will reduce cardiovascular risk but it illustrates the complex multi-faceted actions of testosterone on lipid metabolism, the interpretation of which is prone to over-simplification.

LDL and total cholesterol

Testosterone therapy has been shown to significantly reduce total cholesterol and LDL cholesterol in hypogonadal men (315-318). This effect is observed even in eugonadal men

(319-321). Physiological testosterone replacement in hypogonadal men with CAD has been shown to reduce serum total cholesterol levels despite these patients already being treated with statins (322). Meta-analysis of clinical trials has also confirmed that testosterone therapy significantly lowers total cholesterol & LDL cholesterol and this effect is also seen in men already treated with statins (323).

Lipid Constituents

Unregulated uptake of oxidatively modified lipoproteins via type A scavenger receptors leads to the intracellular accumulation of cholesteryl esters in macrophages and to foam cell formation (305). Testosterone increases the oxidation of LDL by placental macrophages in vitro (306). In a study which used monocyte-derived- macrophages obtained from healthy male and female donors, it was found that DHT dose-dependently stimulates the uptake of acetylated LDL by scavenger receptor type A and, hence, the intracellular cholesteryl ester accumulation in macrophages. In addition to the higher expression of the androgen receptor in male donors, this effect was only seen in macrophages of male but not female donors. The stimulatory effect of DHT was blocked by the androgen receptor antagonist hydroxyflutamide (324).
Non-hepatic and non-steroidogenic cells cannot metabolize cholesterol and, therefore, can only dispose of excess cholesterol by secretion. Cholesterol efflux from cells is hence central to the regulation of the cellular cholesterol homeostasis. Non-specific and passive (i.e. aqueous diffusion) as well as specific and active processes (i.e. receptor-mediated) are involved. To date, two plasma membrane proteins are known to facilitate cholesterol efflux. Interaction of the scavenger receptor B1 with mature lipid-containing HDL is thought to facilitate cholesterol efflux by re-organizing the distribution of cholesterol within bilayer plasma membrane (Figure 1)**.** The ATP binding cassette transporter A1 mediates phospholipid and cholesterol efflux to extracellular lipid-free apolipoproteins by translocating these lipids from intracellular compartments to the plasma membrane and/or by forming a pore within the plasma membrane, through which the lipids are secreted (145). We have found that testosterone up-regulates the expression of the scavenger receptor B1 in human monocyte-derived macrophages thereby stimulating HDL-induced cholesterol efflux. No effect of testosterone was seen on the expression of the ATP binding cassette transporter A1 (231,325,326).
In a cross-sectional study of 65 men (mean age: 60 $\pm$ 8 years), serum testosterone had strong inverse correlation with anti-oxidised LDL-cholesterol antibody (r=-0.346, p=0.0047). The relationship persisted after adjusting for other common CVD risk factors. This would suggest either a direct role for testosterone on oxidised LDL-cholesterol metabolism or an immune response to oxidised LDL-cholesterol (232). Testosterone replacement in hypogonadal men with CAD resulted in a lower HDL-cholesterol and apolipoprotein A1 when compared to placebo (230).

Macrophage Functions

Circulating monocytes migrate into the vascular wall and differentiate into macrophages. They bind to modified lipoproteins which have permeated the vascular endothelium. The uptake of modified (e.g. by oxidation) lipoproteins by macrophages leads to the formation of large foam cells. These, together with T-lymphocytes, release inflammatory mediators which stimulate the proliferation and migration of smooth muscle cells. Human monocyte-derived macrophages express the androgen receptor in a gender-specific manner. Macrophages from men exhibit a fourfold higher expression of the androgen receptor than macrophages from women (327). There is also evidence that testosterone regulates macrophage function by non-genomic effects via a G-protein-coupled, agonist-sequestrable plasma membrane receptor which initiates calcium- and 1,4,5-triose-phosphate-signaling (328).

In summary, testosterone therapy reduces total cholesterol, LDL cholesterol and Lp(a) which can be beneficial to reduce cardiovascular risk. It also reduces HDL cholesterol. Although low HDL cholesterol is associated with increased cardiovascular risk, it cannot be assumed that testosterone induced reduction in HDL cholesterol is pro-atherogenic as it may reflect accelerated reverse cholesterol transport instead. The effect of testosterone therapy on macrophages could raise the possibility of increasing cardiovascular risk by excess production of pro-inflammatory cytokines. However, the data from clinical studies investigating this aspect have been inconclusive. Overall, testosterone therapy does not appear to increase pro-atherogenic lipid profile.

Insulin Resistance and Diabetes

Young, lean men did not demonstrate any change in insulin sensitivity across a wide range of serum testosterone levels in a dose-response study despite a dose-related reduction in fat mass (329). Centrally obese middle-aged men receiving testosterone showed an improvement in insulin sensitivity (by hyperinsulinaemic / euglycaemic clamp studies) and a lowering of serum insulin levels (294). In ageing men hCG administered for 3 months did not affect insulin sensitivity (as measured by euglycaemic clamp) (330). It is unclear whether changes in serum testosterone regulate insulin sensitivity independent of their effect on fat mass (specifically visceral fat). Comparison of data sets is difficult as those middle-aged men showing improved insulin sensitivity had higher fat mass and greater waist circumference at baseline (163) than the ageing men treated with hCG (330). Whilst anabolic steroids (oxandrolone) demonstrate a significant reduction in abdominal fat (295) they have been associated with insulin resistance, possibly mediated through a direct hepatotoxic mechanism.

Several recent studies have demonstrated varying beneficial effects of testosterone replacement therapy on insulin resistance (149,291,297,298,331-341) and type 2 diabetes (291,297,298,332,333,342-345), and reduced fat mass (291,304,331,332,342-348).

Cai et al (2014) (349) reviewed five randomised controlled trials including 351 participants with a mean follow-up time of 6.5-months, assessing the metabolic effects of testosterone replacement therapy on hypogonadal men with type 2 diabetes mellitus. They showed that testosterone therapy reduced fasting plasma glucose, fasting insulin, HBA1C and triglyceride levels significantly.

A more recent meta-analysis by Grossman et al (2014) (350) of seven randomised controlled trials including 833 diabetic men with hypogonadism treated with testosterone for periods ranging between 3 to 12 months, demonstrated only modest improvements in insulin resistance. There was no improvement in glycaemic control with the mean difference in HBA1C of -0.15 (-0.39, 0.10, P 0.25)

In summary, although there are possible favourable effects of testosterone therapy on insulin sensitivity, there is no convincing evidence that testosterone treatment improves glycaemic control significantly. Therefore one concludes that currently testosterone therapy has no role in the management of hyperglycaemia in patients with T2DM.

The Haemostatic System

In agreement with an important role of thrombus formation in the pathogenesis of acute coronary events and stroke, prospective studies have identified various haemostatic

variables as cardiovascular risk factors (351). Among them factor VII, fibrinogen, the fibrinolysis inhibitor plasminogen activator inhibitor – 1 (PAI-1) and tissue plasminogen activator antigen (tPA) are major factors.

Effects of testosterone on coagulation cascade and fibrinolytic pathway

Testosterone has been associated with various pro-thrombotic factors of both extrinsic and intrinsic pathways of the coagulation cascade. In a cross-sectional study, low testosterone has been associated with higher levels of factor VII and fibrinogen (352). Longitudinal case control studies from Norway in elderly men have shown that low testosterone is associated with lower plasma levels of the inhibitor protein, tissue factor pathway inhibitor (TFPI), which promotes tissue factor induced coagulation (353). In an interventional follow up study, elderly hypogonadal men who had testosterone replacement therapy for one year, showed no difference in levels of TFPI, activated factor VII or in coagulation time despite normalization of testosterone levels (354). Inappropriate timing of laboratory assays with testosterone administration, endothelial changes due to aging and differences between short term and long term effects of testosterone have been suggested as possible causes for this in a further analysis (355).

The fibrinolytic pathway is initiated by plasmin, stimulated by tissue plasminogen activator (tPA) and inhibited by plasminogen activator inhibitor–1 (PAI-1) and plasminogen activator inhibitor–2 (PAI–2). Cross sectional studies have shown that lower endogenous testosterone levels were associated with higher levels of PAI-I in subjects with coronary artery disease, suggesting increased cardiovascular risk from reduced fibrinolysis (24). This negative association between endogenous testosterone levels and PAI-1 has also been shown in obese patients and those with greater central obesity compared with healthy controls (356). In another study similar inverse association between testosterone and PAI-1 has been demonstrated in men with newly diagnosed hyperlipidemia (357). Cell culture studies using human umbilical vein endothelial cells have demonstrated that physiologic dosing of testosterone stimulated tissue plasminogen activator (tPA) and decreased PAI-1 production (358).

Administration of supraphysiological dosages of testosterone to 32 healthy men in a trial of male contraception, led to a sustained decrease of fibrinogen of 15 to 20% over 52 weeks of treatment (359). In this study the doubling of serum testosterone levels initially also led to significant decreases of PAI-1, protein S, and protein C as well as to increases of anti-thrombin and beta-thromboglobulin. Likewise PAI-1 was decreased in men who received the anabolic androgen, stanozolol. However, 12-months of a more ‘physiological’ transdermal testosterone did not alter PAI-1 or fibrinogen levels in men with chronic stable angina (360). Suppression of testosterone in patients with benign prostate hypertrophy by GnRH analogues exerted no significant effects on plasma fibrinogen levels (361). In agreement with the lowering effects of testosterone on PAI-1, testosterone inhibited the secretion of PAI-1 from bovine aortic endothelial cells in vitro.

Taken together the data indicate that testosterone may lower fibrinogen and PAI-1, although the magnitude of effect is likely to vary according to the type and route of the administered androgen. However, the evaluation of risk factors associated with cardiovascular events in the older men of the TOM trial showed that men who had cardiovascular events had significantly higher free testosterone, higher fibrinogen levels and significantly lower PAI-1 levels as compared to those who did not have cardiovascular events (362). This is suggestive of the possibility that these factors could also be contributing to the cardiovascular events in older men.

Effects of testosterone on platelets

In the formation of thrombus, platelets play an important role through adhesion to endothelium, aggregation and stimulation of coagulation through release of metabolites like

thromboxane A2 (TX-A2). These metabolites further promote platelet activity. High dosages of androgens were found to decrease cyclooxygenase activity and thereby increase platelet aggregability (363). In vitro studies in animal models suggest that testosterone deficiency is associated with decreased platelet aggregation and TX-A2 receptor density and that testosterone therapy improves both platelet activity and TX-A2 receptor density (357). In a randomized blinded placebo controlled study of healthy young men, intramuscular testosterone therapy at supraphysiological doses, was shown to increase platelet aggregation activity and thromboxane A2 receptor density (358). Surgical or medical castration in prostate cancer patients showed reduced platelet TX-A_2 receptor density but not affinity. This is associated with reduced platelet aggregation but not sensitivity to thromboxane mimetic (364). In a study using a human megakaryocytic cell line, administration of testosterone in culture resulted in increased gene expression of P2Y12, a receptor on the platelet surface which plays an important role in platelet aggregation, suggesting another pro-thrombotic mechanism (365). In animal studies with rat aortic endothelial cells, testosterone administration in vitro resulted in increased nitric oxide production and reduced platelet aggregation (366). Similarly in human umbilical vein endothelial cells, testosterone exposure at physiologic concentrations in cell culture resulted in increased nitric oxide production and nitric oxide synthase activity, which stopped with exposure to supraphysiologic concentrations of testosterone (367).

Testosterone therapy may have pro-thrombotic effect by increasing TX-A2 receptor density and promoting P2Y12 receptor expression. It can also have anti-thrombotic effect by increasing nitric oxide production. The dominant effect of TRT on platelet function needs to be evaluated in further interventional studies.

Testosterone therapy and venous thromboembolism

A recent series of studies (368-372) have described thromboembolic events in 42 patients (38 men and 4 women) who were on androgen replacement therapy. Of the 38 men, 24 were on transdermal testosterone gel, 13 were on intramuscular injections and 1 patient was on nandralone patch. Among 4 women, 2 were on transdermal testosterone patch and the remaining 2 were on testosterone – estrogen pellet treatment. Apart from 1 patient who had amaurosis fugax and 1 patient who had spinal cord infarction, all others had venous thromboembolism. 27 patients had deep venous thrombosis / pulmonary embolism, 12 patients had osteonecrosis which is thought to be due to thrombosis of the efferent veins of the bone leading to increased intracortical pressure and reduced arterial flow and 1 patient had central retinal vein thrombosis. The median time for thrombotic events after starting testosterone therapy in this series was 5 months. The most recent large retrospective cohort and nested case-control analysis which included 102,650 patients treated with TRT and 102,650 untreated patients with idiopathic VTE, it was found that there was no significant increase in the risk of VTE in patients on testosterone therapy (373). In another recent large multi-cohort retrospective analysis including 544,155 men on various forms of testosterone therapy, the authors did not show an increased risk of VTE (HR 0.92 95% CI 0.76-1.11) (123). **The possibility of increased risk of VTE in hypogonadal men on TRT was raised by few studies. Two large population based studies have reassuringly shown no increase in the risk of VTE in men on TRT. Overall, the risk of VTE appears to be increased in patients on TRT with underlying genetic hypercoagulable risk.**

However, considering significantly increased androgen use in men over 40 years of age and the few studies showing increased risk of venous thromboembolism, the United States Food and Drug Administration (FDA) is now requiring a change to drug labelling of all testosterone products by the manufacturers to provide a more general warning regarding venous blood clots and to ensure this risk is described consistently in the labelling of all approved testosterone products.

(http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm402054.htm)

Effects of androgens on VASCULAR function

Blood vessels are specialised tissues lined by a single layered endothelium, with a middle muscular layer comprising vascular smooth muscle cells (VSMC), and a fibrous coat – the adventitia. The cellular components are held together by a connective tissue framework and extracellular matrix.

Effect of Androgens on Vasomotor Function

The acute effect of androgen on vascular dilation is well-documented both in clinical and pre-clinical studies. A high dose of testosterone (>$1x10^{-5}$M) is able to induce nearly full dilation in isolated arteries. Preservation of testosterone-induced vasodilation in arteries lacking androgen receptor (AR) suggests that this response is independent of AR activation (191,374). Interestingly, similar vasodilation could also be achieved using corticosteroids, oestrogen or cholesterol at similar concentrations (>$1x10^{-5}$M), suggesting this may be a common feature of steroids acting directly on the cell membrane (375-377). Given the very high concentration required to produce this response, testosterone-induced acute vasodilation is very unlikely to have any physiological relevance.

There is evidence that AR-activation by physiological levels of testosterone modulates endothelial function via nitric oxide synthase-dependent mechanisms. Penile erection is an archetypal physiological function controlled by nitric oxide (NO) dependent vascular dilation. It has been demonstrated that circulating androgen levels correlate with nitric oxide synthase expression in the penile vasculature and with erectile function (378,379). Similar regulation has also been observed in vasculature of other organs. In cultured male human aortic endothelial cells, activation of AR stimulates eNOS phosphorylation and NO production within 15 minutes (206). Conversely, normalising circulating testosterone level in hypogonadal men reduces the concentrations of asymmetric dimethyl arginine (ADMA; an endogenous inhibitor of endothelial NOS) in plasma (380). However, the AR-dependent regulation of vasomotor function is more complex. First, acetylcholine-induced endothelial NO production and vasodilation in isolated arteries are not affected either in Tfm mice (381) or in mice with vascular-specific AR ablation (191). This suggests that the AR-NO signalling pathway is not involved in acute regulation of agonist-induced NO production and vasodilation. Second, AR-dependent signalling, including NO production, modulates agonist-induced vasoconstriction. Vasopressin induced a greater vasoconstriction in female mice than in males. This gender difference could be abolished by either using AR deficient mice or by pharmacologically blocking NOS (381). Ablation of AR in the smooth muscle but not in endothelial cells compromises phenylephrine (alpha-adrenergic agonist)-induced vasoconstriction (191). As androgen increases alpha-adrenergic receptor expression and activity in prostate smooth muscle and cardiac muscle (382,383), the same mechanism may also apply to vascular smooth muscle cells. In addition, rats treated with testosterone have increased Thromboxane-A_2 (TX-A_2) receptor density in aortic smooth muscle cells with a concomitant increase in response to TX-A_2 mimetics (384-386). This modulation of TX-A_2 receptor density in VSMC is mediated by DHT (387) and is more prominent in aortic VSMCs from male than from female rats (388). Testosterone has also been shown to directly influence vasoconstriction via regulating Neuropeptide Y1 receptor density (389), while estradiol has a vasodilatory property by increasing PGI_2 production (390) and decreasing cytosolic calcium (391). Each of these may be potential mechanisms responsible for gender differences in vascular function.

In summary, AR may regulate vasomotor function via diverse and opposing mechanisms. Indeed, clinical studies also replicate the complex profile of androgen/AR action on vascular function brachial artery flow-mediated dilation (FMD) which is commonly used to evaluate endothelial function (392). A bolus infusion of 2.3mg testosterone resulted in an increase (100-fold) in circulating testosterone concentration within one hour (7.7 versus 770 nM), and significantly increased bFMD. However, a vasodilatory effect was not observed with low dose testosterone (up to 24 nM) in the same study (393). Given the very high concentration required to produce this response, testosterone-induced acute brachial artery vasodilation is unlikely to have any physiological relevance. However, circulating testosterone has been reported to be negatively associated with FMD in the general population (aged 25 to 85 years) (394) as well as in prostate cancer patients receiving long-term androgen deprivation therapy (395). Surprisingly, androgen replacement therapy of hypogonadal men did not improve, but reduced, FMD (396,397). Furthermore, abuse of anabolic steroids among body-builders impaired vasomotor reactivity without affecting endothelial function (398). These findings highlight the complex nature of androgen action on vascular function as well as possible detrimental effects of un-physiological testosterone pharmacokinetics associated with exogenous testosterone treatment (399).

Effect of Androgens on the Vascular Framework

VSMCs are known to produce proteoglycans (PG) and glycosaminoglycan (GAG), which are important constituents of the extra-cellular matrix. Sex steroids have no effects *ex vivo* on GAG (400) or PG (401) synthesis by aortic VSMCs. In aortic VSMCs, testosterone leads to preservation of collagen deposition, reduction of elastin/collagen ratio, reduction of fibrillin-1 deposition and increased Matrix metalloproteinase-3 expression (402). These changes are potential mechanisms to explain the increased aortic stiffness observed in males compared with females. The gender difference in aortic stiffness might be associated with the male preponderance of vascular diseases. However, in a recent study using transgenic mice lacking vascular AR, no difference in arterial compliance was found between the transgenic and wild type littermates, indicating vascular AR has little impact on arterial structural development (191). Mechanism(s) underlying the gender difference in aortic stiffness require further investigation.

OESTROGEN AND CAD IN MEN

Endogenous Oestrogen

An increasing body of evidence indicates that important physiological actions of testosterone in men are mediated by the oestrogen receptors (ERs) following conversion to oestradiol by site-specific aromatases in target tissues (403,404). The existence of two nuclear ER subtypes α and β as well as a membrane-bound ER encoded by the same transcript as the ERα attest to the potential for many different biological estrogen effects. More recently an orphan G-protein coupled receptor 30 (GPR30, now called GPER) was reported to be a new type of membrane-bound ER, mediating the non-genomic effects of estradiol (405,406). ERs and aromatase are detectable in coronary arteries of monkey and man (407-409). The extra-glandular production of oestrogens (with circulating androgens as the immediate precursor substrate) may therefore play a role in male cardiovascular physiology and pathophysiology. The importance of locally-produced oestrogens from aromatisation of testosterone in males for cardiovascular health is highlighted by human and transgenic

mouse models of aromatase deficiency and oestrogen resistance. In aromatase-deficient men with undetectable circulating oestradiol and oestrone and high testosterone, raised BMI with central adiposity, metabolic syndrome, non-alcoholic fatty liver disease and dyslipidaemia with elevated total cholesterol, LDL-cholesterol, triglyceride and decreased HDL-cholesterol were reported (410,411). These metabolic abnormalities were correctable by low dose oestrogen replacement. Insulin resistance, acanthosis nigricans, low HDL-cholesterol, and impaired glucose tolerance were apparent in a 28 year old male with a null mutation in ER gene causing oestrogen resistance (412), with calcium deposition in a coronary artery indicating the presence of premature atherosclerosis (413). Flow-mediated endothelium-dependent NO-activated vasodilatation in the brachial artery (membrane ER-mediated) in response to hyperaemia was absent consistent with marked endothelial dysfunction (414).

Finkelstein et al. (404) studied the effects of varying doses of transdermal testosterone with and without concomitant aromatase inhibitor after suppressing endogenous gonadal steroids with goserelin acetate in healthy men. They demonstrated that decreases in lean mass, thigh-muscle area, and leg-press strength were attributable to reduced testosterone levels, whereas oestrogen deficiency, independent of testosterone, accounted for increases in the body fat. The increase in intra-abdominal fat, in particular, with aromatase inhibition may be taken to imply excess risks of cardiovascular disease with long-term oestrogen deficiency (404). In a further analysis, they also demonstrated that both serum HDL and leptin levels showed a marked inverse association with testosterone dose in both cohorts ($p<0.001$ for all). This relationship was not altered by suppressing estrogen production, indicating that testosterone alone regulates these measures. In contrast, fasting glucose, HOMA-IR, and intramuscular fat increased similarly in all men in the testosterone with aromatase inhibitor group, regardless of testosterone dose, and were significantly higher than in the group with testosterone without aromatase inhibitor ($P<0.05$ for all), indicating that estradiol primarily regulates these measures. Changes in blood pressure, LDL, and body weight were not significantly associated with either testosterone or estradiol levels. (415)

These clinical findings suggest that endogenous oestrogens play an important role in maintaining normal carbohydrate and lipid metabolism as well as endothelial function in men. They are compatible with data from transgenic knockout models confirming that ERα is important in preventing adipocyte hypertrophy, obesity, insulin resistance and hypercholesterolaemia (416-418) and maintaining basal NO release from vascular endothelium (414) in male animals. ERβ in vascular smooth muscle may also regulate vascular reactivity to oestradiol (419). In addition, many in vitro studies have demonstrated the direct actions of oestradiol in vasodilatation, inhibition of vascular smooth muscle cell proliferation/migration, inhibition of cytokine activation and expression of cell adhesion molecules in the vascular inflammatory response, and inhibition of platelet aggregation/adhesion (see Section 6 and for review see (420-423)).

Despite indirect evidence supporting possible atheroprotective effects of oestrogen in females, the mechanism by which oestrogen protects arteries is controversial. Villablanca *et al.* reported that estradiol prevents atherosclerosis independent of ERα (424) whilst stimulation of ERβ attenuates inflammation and atherogenesis (424). In contrast, data from Fontaine (425) and Billon-Galés (426) both support that activation of ERα protects against atherosclerosis, but disagree about which ERα subunit mediates this effect. In studies using male animals, the atheroprotective effect of oestrogen demonstrated apparent gender dimorphism; male animals did not benefit from oestrogen supplementation (194,427). Indeed, a transgenic animal study demonstrated that the presence of ERα in male arteries increases their susceptibility to early atherosclerosis (428). Interestingly, in global AR deficient Tfm mice, testosterone-derived estradiol seems to inhibit atherosclerosis via ERα-dependent mechanisms (192). Further investigation is needed to clarify whether this is due to the altered ER expression in these feminized males, or whether the pharmacological dose

of supplementation overrides the beneficial effect of oestrogen at a physiological level.

Reports of associations between endogenous oestrogen levels and cardiovascular disease in men are conflicting. Epidemiological studies have linked higher endogenous serum oestradiol levels with coronary heart disease (in men in the Framingham cohort) (31,429), and increased carotid artery intima-media thickness (430) and peripheral arterial disease (with men in the MrOS cohort) (431), and yet low oestradiol levels predicted all-cause mortality (independent of testosterone) in the MrOS cohort (72) and increased cardiovascular disease in the subgroup of older men in the Framingham cohort (59). There was no association with cardiovascular morbidity or mortality in the MrFIT (52), Rancho-Bernardo (53), Honolulu Heart Program (54), BLSA (55), Caerphilly (56), MMAS (41) or Tromso (60) cohorts. Interestingly, in a study of men with heart failure, both the highest and lowest quartiles of oestradiol predicted increased mortality (432). The relationships between oestradiol and the cardiovascular system in men are complex, and despite accounting for potential confounders such as lipids, glucose and insulin, the observational nature of these studies has inherent limitations in determining the cause and effect nature of the relationship. Specifically the effects of oestrogen on SHBG, and the contribution of serum testosterone and body fat, particularly visceral fat, are not well understood (433). Furthermore, inaccurate measurement of the low levels of oestradiol in men using immunoassay methods in the vast majority of the above studies (434) probably contributed to these conflicting findings. Recent studies which have used mass spectrometry measurements of oestradiol have not shown any significant association of oestradiol with cardiovascular disease. In a large population based study of 2143 Australian men, oestradiol measured by mass spectrometry was not associated with metabolic syndrome score (435). In men of the HIM study cohort, oestradiol measured by mass spectrometry was not associated with symptoms of intermittent claudication (436), did not predict the risk of myocardial infarction or stroke (64) and also did not predict all- cause mortality (86).

In summary the relationships between oestradiol and the cardiovascular system in men are complex, and despite accounting for potential confounders such as lipids, glucose and insulin, the observational nature of these studies has inherent limitations in determining the cause and effect nature of the relationship. Specifically the effects of oestrogen on SHBG, and the contribution of serum testosterone and body fat, particularly visceral fat, are not well understood. Furthermore, in the vast majority of the above studies, inaccuracies in the measurement by immunoassay of low levels of oestradiol in men probably contributed to some of the conflicting findings.

Exogenous Oestrogen

The therapeutic role of exogenous oestrogens has been studied in cardiovascular disease and prostate cancer. Huggins and Hodges first described the use of synthetic oestrogens to achieve androgen deprivation in 1941 (437,438) but subsequently the use of oral diethylstilboestrol was found to result in an increased number of cardiovascular deaths, primarily due to myocardial infarction (439). Although it was hypothesized that the toxicity of the oral oestrogens was due to their hepatic metabolism, and subsequent induction of a hypercoagulable state, the use of the parenterally administered compound, polyestradiol phosphate (PEP), also led to an increase in cardiovascular deaths in men with non-metastatic disease (440) and non-fatal cardiac events in men with metastatic disease (441). High doses of oral conjugated equine oestrogens (2.5mg) resulted in excess deaths when administered to men post myocardial infarction in the “Coronary Drug Project” in the 1960’s (442).

CONCLUSIONS AND THERAPEUTIC IMPLICATIONS

Current evidence indicates that the sex difference in CAD cannot be explained on the basis of ambient testosterone exposure. Androgens can exert both beneficial and deleterious actions on a myriad of factors implicated in the pathogenic mechanisms of atherosclerosis and CAD. At present, it is not possible to determine the net effect of testosterone on CAD.

The clinical implications of this ongoing uncertainty are;

1) The concern for the possibility of cardiovascular side effects in androgen treatment of endocrine and non-endocrine conditions

and

2) Whether testosterone may be used for the prevention or even treatment of CAD.

Efforts to exploit the therapeutic benefits of testosterone for men in the treatment of osteoporosis, sarcopaenia, chronic debilitating disease and age-related hypoandrogenism in the ageing male population should take heed of recent concerns regarding the possibility of increased cardiovascular event risks (121-123,128).

Some clinicians argue that androgen replacement in the elderly male may have the potential to prevent CAD. It is hazardous to extrapolate from cross-sectional observational data examining cardiovascular risk factors, or in vitro data studying isolated mechanisms with pharmacological doses of androgens, to predict that manipulation of the sex steroid milieu will result in clinical benefits in a complex multifactorial condition such as CAD. The lessons from estrogen HRT in postmenopausal women are especially salutary. Despite the overwhelmingly positive but indirect evidence on risk factors and disease incidence, randomised controlled interventional studies recently have not confirmed estrogens to be effective in primary or secondary prevention of CAD in women (443-445). Analogously, if HRT for ageing men were ever to become an acceptable therapeutic entity, the requirement for randomised controlled clinical interventional trials to assess the effects of androgens on CAD disease (and other) endpoints is obvious and endorsed by the FDA. In the absence of such information on testosterone and DHEA currently, priority must be given to established modes of intervention, which have been proven to be effective in prevention or treatment of CAD (e.g. weight reduction, smoking cessation, exercise, aspirin, statins/fibrates, anti-hypertensives, and vasodilators).

For men with established pathological hypogonadism, however, there are no substantive data to suggest that physiological testosterone therapy is associated with increased cardiovascular risk and their management should not deviate from current recommended practice. The issue of initiating or continuing TRT in newly diagnosed or established hypogonadal men in association with a major cardiovascular event remains contentious. Considering the concerns regarding the possibility of increased cardiovascular risk, expert opinion is to delay or stop TRT for 3 to 6 months

Finally, it is now well recognized that ADT in the treatment of metastatic prostate cancer increases cardiovascular risk. These men should have their cardiovascular risk factors identified and treated accordingly.

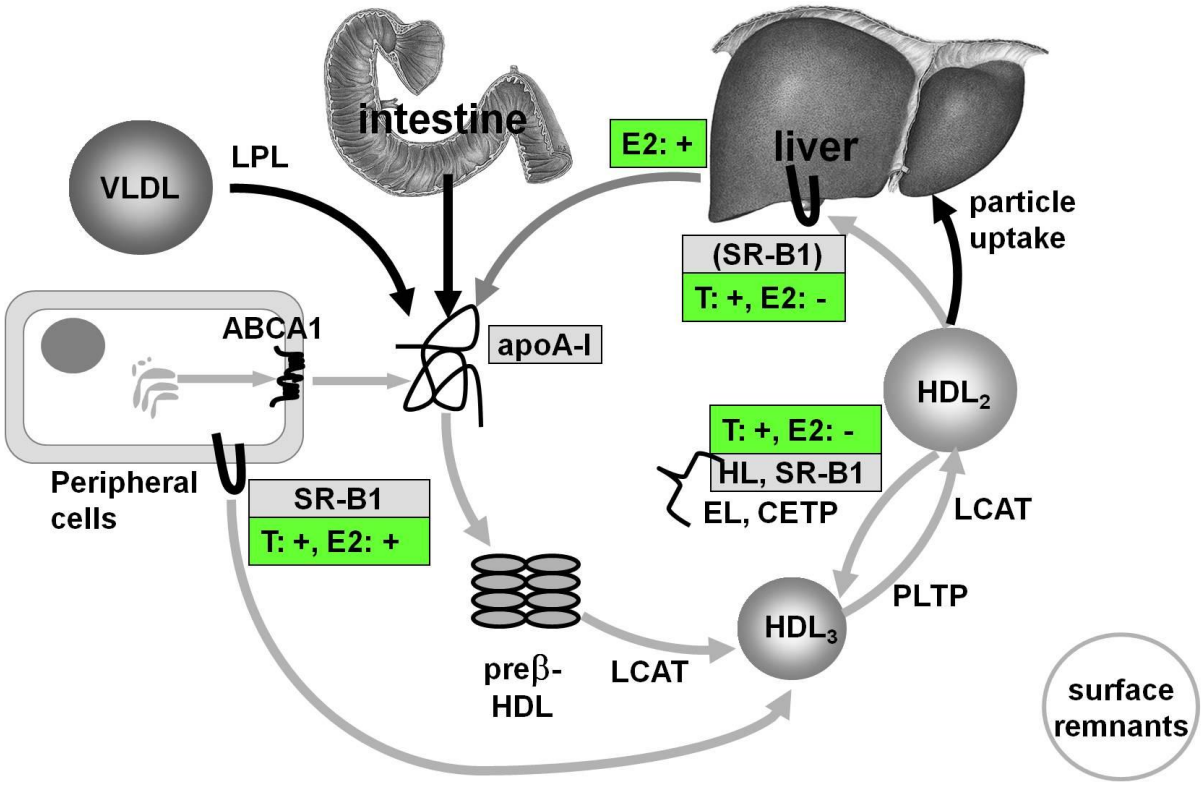

Figure 1 Pathways of HDL metabolism and regulation by testosterone and oestradiol

Mature HDL_3 and HDL_2 are generated from lipid-free apoA-I or lipid-poor pre β-HDL as the precursors. These precursors are produced as nascent HDL by the liver or intestine or are released from lipolyzed VLDL and chlyomicrons, or by interconversion of HDL_3 and HDL_2. ABCA1-mediated lipid efflux from cells is important for initial lipidation; LCAT-mediated esterification of cholesterol generates spherical particles which continue to grow upon ongoing cholesterol esterification, and PLTP-mediated particle fusion and surface remnant transfer. These mature HDL particles also continue to accept cellular cholesterol by processes which are facilitated by the scavenger receptor BI (SR-BI) and LCAT. Larger HDL_2 are converted into smaller HDL_3 upon CETP-mediated export of cholesteryl esters from HDL onto apoB-containing lipoproteins, SR-B1-mediated selective uptake of cholesteryl esters into liver and steroidogenic organs, and HL- and EL-mediated hydrolysis of phospholipids. HDL lipids are catabolized either separately from HDL proteins, i.e. by selective uptake or via CETP-transfer, or together with HDL proteins, ie. via uptake through as yet unknown HDL receptors or apoE receptors. Both the conversion of HDL_2 into HDL_3 and the PLTP-mediated conversion of HDL_3 into HDL_2 liberate lipid-free or poorly lipidated apoA-I, which is either re-used for the formation of mature HDL or is filtrated into the kidney. Grey arrows represent lipid transfer processes, black arrows represent protein transfer processes. The hepatic expression and activity of both HL and SR-B1 was shown to be up-regulated by testosterone and down-regulated by oestradiol. In addition oestradiol up-regulates the hepatic expression and secretion of apoA-I. These actions of testosterone and oestradiol are in good agreement with their lowering and increasing effect on HDL cholesterol, respectively. In addition both testosterone and oestradiol stimulate SR-BI expression in macrophages and thereby cholesterol efflux from these cells onto lipidated HDL.

Table 1a Relationships between circulating testosterone levels and coronary artery disease in men

Study (reference)[a]	n	Hormone	Endpoint	Relationship OR
Mendoza 1983 (11)	52	T	MI, angio	Negative
Barth 1983 (12)	20	T	CAD, angio	Negative
Hromadova 1985 (13)	67	T	Coronary angio	Negative
Breier 1985 (14)	139	T	CAD, angio	Negative
Aksut 1986 (15)	54	T	MI, angina	Negative
Sewdarsen 1986 (16)	56	T, free T	MI	Negative
Chute 1987 (17)	146	T, free T	CAD, angio	Negative
Hämäläinen 1987 (18)	57	T, free T	CHD, angio	Negative
Lichtenstein 1987 (19)	2512	T	IHD	Negative
Swartz 1987 (20)	71	T	MI	Negative
Sewdarsen 1988 (21)	20	T	MI, angio	Negative
Sewdarsen 1990 (22)	224	T	MI	Negative
Rice 1993 (23)	272	T, free T	MI	Negative
Phillips et al 1994 (24)	55	T, free T	CAD, Angio	Negative
Zhao 1998 (25)	201	T	CAD	Negative
English 2000 (26)	90	T, free T, bio T	CAD, angio	Negative
Hak 2002 (27)	504	T, free T	Aortic calcification	Negative 0.4 (0.1-1.0)
Luria 1982 (28)	50	T	MI	Null
Labropoulos 1982 (29)	144	T	MI	Null
Zumoff 1982 (30)	117	T	MI, CAD	Null

Phillips 1983 (31)	122	T	CHD	Null
Heller 1983 (32)	295	T	CHD	Null
Small 1985 (33)	100	T	IHD	Null
Franzen 1986 (34)	92	T	MI	Null
Baumann 1988 (35)	58	T	Atherosclerosis	Null
Slowinska-Srzednicka 1989 (36)	108	T	MI, Angio	Null
Cengiz 1991 (37)	55	T	MI, angina	Null
Hauner et al 1991 (38)	274	T	CAD, angio	Null
Mitchell et al 1994 (39)	98	T, free T	MI	Null
Marquez-Vidal 1995 (40)	116	T	MI	Null
Feldman et al 1998 (41)	1709	T, free T	Heart disease	Null 0.8
Kabakci 1999 (42)	337	T, free T	CAD, angio	Null
Schuler-Luttmann 2000 (43)	189	T, free T index	CAD, angio	Null
Turhan 2007 (44)	101	T, free T	CAD, angio	Negative
Fallah 2009 (45)	502	T, free T	CAD, angio	Null
Gu 2007 (46)	128	T, free T	CAD, angio	Negative
He 2007 (47)	414	T	CAD, angio	Null
Rosano 2007 (48)	119,	T, bioT	CAD, angio	Negative
Dobrzycki et al 2003 (50)	96	T, free T	CAD, angio	Negative
Hu et al 2011(446)	87	T	CAD, angio	Negative
Li et al 2012 (51)	803	T	CAD, angio	Negative
Park et al 2012 (49)	291	T, free T, bio T	Coronary calcium score, angio	Negative

Footnotes:

[a] note that all studies were cross-sectional

T: total testosterone

free T: unbound testosterone measured by equilibrium dialysis or analogue assay

free T index: unbound testosterone derived from total testosterone and SHBG

bioT: bioavailable (non-SHBG bound) testosterone

*adjusted for cardiovascular risk factors

CAD: coronary artery disease

CHD: coronary heart disease

IHD: ischaemic heart disease

MI: myocardial infarction

Angio: coronary angiography

OR: odds ratio (95% confidence intervals)

Negative relationship indicates lower T levels in patients with CAD compared to controls and a null relationship indicates no difference between cases and controls.

Table 1b Prospective cohort or nested case-control studies

Study (reference)	n (cases, controls)	Type of study, duration of follow	Hormone	Endpoint	Relationship OR
Cauley 1987 (52)	163, 163	Nested case-control 6-8 yr	T, free T	MI	Null
Barrett-Connor 1988 (53)	1009	Prospective cohort 12 yr	T	IHD	Null
Phillips 1988 (54)	96, 96	Nested case-control 19-20 yr	T	MI	Null
Contoreggi et al 1990 (55)	46, 124	Nested case-control 9.5 yr	T	CAD	Null
Yarnell 1993 (56)	2512	Prospective cohort 5 yr	T	CHD	Null
Hautanen et al 1994 (57)	62, 97	Nested case-control 5 yr	T	Cardiac endpoints	Null
Harman 2001 (58)	890	Prospective cohort 31 yr	T, free T index	CAD	Null
Hak 2002 (27)	282	Prospective cohort 6.5 yr	T, free T	Aortic calcification	Negative
Arnlov 2006 (59)	2084	Prospective cohort 10 yr	T	CVD	Null
Vikan 2009 (60)	1568	Prospective cohort 10 yr	T, free T	MI	Null

Akishita et al 2010 (62)	171	Prospective cohort 6.5 yr	T	CV events	Negative
Corona et al 2010 (447)	1687	Prospective 4.3 yrs	T	MI	Negative
Hyde et al 2011 (63)	3637	Prospective cohort 5.1 yr	T	CV events	Negative
Ohlson et al 2011(65)	2416	Prospective cohort 5 yr	T	CV events	Negative
Haring et al 2013 (61)	254	Prospective cohort 5 yr	T	CV events	Null
Soisson et al 2013 (66)	3650	Prospective cohort 4 yr	T, Bio T	CAD	J Shaped
Yeap et al 2014 (64)	3690	Prospective cohort 6.6 yr	T, DHT	MI	Null
Shores et al 2014 (67)	1032	Prospective cohort 9 yrs	T, free T, DHT	CVD	Negative

Table 1c Relationships between circulating testosterone levels and cardiovascular / all-cause mortality in men

Study (ref)	Cohort / Country	No.	Age (yrs)	Follow-up (yrs)	HR CV Mortality	HR All-cause Mortality	Summary
Smith 2005 (68)	Caerphilly	2512	52	16.5	Not significant	Not significant	No association
Araujo 2007 (69)	MMAS	1686	55	15.4	RR: Not significant	RR:Not significant	No association
Vikan 2009 (60)	Tromso	1568	60	10.0	TT – Not significant	FT HR 1.24 (1.01, 1.53)	Low FT is associated with higher risk of all cause mortality
Haring 2013 (61)	Framingham heart study	254	75.5	10	Not significant	Not significant	No association
Corona 2010 (147)	Italy	1687		4.3	TT 7.1 (1.8, 28.6)		Low TT associated with higher CV mortality
Carrero 2011 (82)	Sweden (ESRD)	260	59	3	TT 1.9 (1.0, 3.9)		Low testosterone is associated with higher risk of CV mortality
Hyde 2012 (77)	Australia	3637		5.1	FT 1.71 (1.12, 2.62)		Low testosterone is associated with higher risk of CV mortality
Khaw 2007	EPIC-Norfolk	2314	67	7.0	TT 2.29 (1.60,		Low TT is associated

(71)					3.26)		with higher CV and all-cause mortality
Yeap 2014 (86)	HIMS	3690		6.7			
Laughlin 2008 (70)	Rancho-Bernardo	794	74	11.8	TT 1.38 (1.02, 1.85) BT 1.36 (1.04, 1.79)	TT 1.44 BT 1.50	Low TT and BT are associated with higher CV and all-cause mortality
Carrero 2009 (83)	Sweden (Hemodialusis)	126	63	3.5	TT 3.19 (1.49 , 6.83)	TT 2.03 (1.24, 3.31)	Low TT is associated with higher CV and all-cause mortality
Menke 2010 (74)	NHANES III	1114	40	18	FT 1.53 (1.05, 2.23) BT 1.63 (1.12, 2.37)	FT 1.43 (1.09, 1.87) BT 1.52 (1.15, 2.02)	Decrease in FT and BT is associated with increased risk of all-cause and CV mortality during the first 9 years of follow up
Malkin 2010 (75)	UK	930 (CAD) / 148 (No CAD)	60.7 / 55.7	6.9	BT 2.2 (1.2, 3.9) TT 2.5 (1.2 to 5.3)	BT 2.27 (1.45, 3.60) TT 1.86 (1.1 to 3.2)	Low TT and BT are associated with higher CV and all-cause mortality
Haring 2010 (73)	SHIP	1954	58	7.2	TT 2.56 (1.15, 6.52)	TT 2.24 (1.41, 3.57)	Low TT is associated with higher CV and all-cause mortality

Haring 2011 (84)	SHIP (CKD pts)	1822	51	9.9	TT 2.01 (1.21, 3.34)	TT 1.40 (1.02, 1.92)	Low TT is associated with higher CV and all-cause mortality
Kyriazis 2011 (85)	Greece	111	65	3	TT 2.92 (1.08, 7.87)	TT 2.53 (1.22, 5.25)	Low TT is associated with higher CV and all-cause mortality
Lerchbaum 2012 (76)	LURIC	2069	54 - 74	7.7	FT 1.77 (1.23, 2.55)	FT 2.11 (1.60, 2.79)	Low FT is associated with higher CV and all-cause mortality
Shores 2006 (80)	Veterans	858	61	4.3		TT 1.88 (1.34, 2.63)	Low TT is associated with higher all-cause mortality
Lehtonen 2008 (79)	Turku	187	71	10		TT 0.95 (0.91,1.00)	Low TT is associated with higher all-cause mortality
Tivesten 2009 (72)	MrOS	3014	75	4.5		TT 1.65 (1.29, 2.12)	Low TT is associated with higher all-cause mortality
Muraleedharan 20134 (81)	UK	581	59.5	5.8		TT 2.3(1.3, 3.9)	Low TT is associated with higher CV and all-cause mortality
Yeap 2014 (86)	Australia	1920	70 - 89	6.7		T: quartile [Q] Q2:Q1=0.82 (0.69,	U shaped association between TT and all-cause

						0.98); Q3:Q1=0.78 (0.65, 0.94); Q4:Q1=0.86 (0.72,1.04)	mortality
Pye 2014 (78)	EMAS	2599		4.3		TT 2.3 (1.2, 4.2)	Low T is associated with higher risk of all-cause mortality
Shores 2014 (69)(67)	USA	1032	76.5	9		DHT (<25ng/dl) HR 1.48 (1.06, 2.08) DHT (>75 ng/dl) HR 1.42 (0.99, 2.04)	U shaped association. Low and high DHT associated with higher all cause mortality but wide confidence intervals at higher levels reflect uncertainty

Table 2 Relationships between circulating DHEA and DHEAS levels and coronary artery disease in men

Study (ref)	n (age, yrs)	Study type	Hormone	Endpoint	Relationship OR
Zumoff 1982 (30)	38, 79 (21-85)	Cross	DHEA, DHEAS	CAD, angio	Positive
Slowinska-Srzednicka 1989 (36)	108 (26-40)	Cross	DHEAS	MI, angio	Negative
Herrington 1990 (160)	101 (<50)	Cross	DHEA, DHEAS	CAD, angio	Negative
Ishihara 1992 (162)	69 (15-83)	Cross	DHEA, DHEAS	Aortic calcific, pulse wave	Negative
Mitchell 1994 (39)	98 (<56)	Cross	DHEAS	MI	Negative
Herrington 1995 (163)	206 & 61 (none)	Cross	DHEA, DHEAS	Angio, graft vasculopathy	Negative
Feldman 1998 (41)	1709 (40-70)	Cross	DHEAS	Heart disease	Negative 0.6 (0.5-0.8)
Hauner 1991 (38)	274 (30-74)	Cross	DHEAS	CAD, angio	Null
Phillips 1994 (24)	55 (39-89)	Cross	DHEAS	Angio	Null
Schuler-Luttmann 2000 (43)	189 (<70)	Cross al	DHEAS	CAD, angio	Null#
Hak 2002 (27)	504 (55-89)	Cross	DHEAS	Abdominal aortic calcification	Null 0.9 (0.3-2.2)
Barrett-Connor 1986 ((159)	242 (50-79)	Prosp cohort 12 yr	DHEAS	CAD mortality	Negative 0.6
Contoreggi 1990 (55)	46, 124 (41-92)	Nested case-control 9.5	DHEAS	CAD	Null

		yr			
Lacroix 1992 (161)	238, 476 (48-71)	Nested case-control 18 yr	DHEAS	MI, autopsy	Negative[ψ] 0.5 (0.2-1.1)
Lacroix 1992 (161)	238, 476 (48-71)	Nested case-control 18 yr	DHEAS	CAD, MI	Null[ω]
Newcomer 1994 (165)	157, 169 (40-84)	Nested Case-control 28 mth	DHEAS	MI	Null 1.0 (0.4-2.6)
Barrett-Connor 1995 (164)	942 (65.2)	Prosp cohort 19 yr	DHEAS	CAD deaths	Null
Barrett-Connor 1995 (164)	942 (65.2)	Prosp cohort 19 yr	DHEAS	CAD survivors	Negative 0.9
Berr 1996 (166)	266 (66->80)	Prosp cohort 4 yr	DHEAS	Cardiovascular deaths	Null*
Jansson 1998 (168)	42, 53 (<70)	Nested Case-control (survivors) 1yr	DHEAS	Reinfarction & CAD deaths	Null
Tilvis 1999 (169)	571(75-85)	Prosp cohort 5 yr	DHEAS	CVD deaths	Null
Kiechl 2000 (170)	371(40-79)	Prosp cohort 5 yr	DHEAS	CVD, CIMT	Null 1.1 (0.9-1.4)
Trevedi 2001 (167)	963 (65-7)	Prosp cohort 7.4 yr	DHEAS	CVD mortality	Null 0.6 (0.3-1.3)
Hak 2002 (27)	287 (55-89)	Prosp cohort 6.5 yr	DHEAS	Abdominal aortic calcification	Null
Hautanen 1994	62, 97	Nested Case-	DHEAS	MI, Cardiac	Positive 2.0

(57)	(48)	control 5 yr		deaths	(1.0-4.9)
Ohlsson 2010 (171)	2644, (69-81)	Prosp cohort 4.5 yrs	DHEA DHEA,S	CVD deaths	Negative
Tivesten 2014 (172)	2416, (69-81)	Prosp cohort 5 yrs	DHEA DHEA,S	CHD	Negative
Krijthe 2014 (176)	1180	Prosp 12.3yrs	DHEA	Atrial fibrillation	Negative
Philips 2010 (173)	4255,	Prosp cohort 15yrs	DHEAS	CVD deaths	Negative
Sanders 2010 (174)	989, 85.2 yrs	Long cohort 9 yrs	DHEA DHEAS	CVD	Negative
Feldman 2001(175)	1167, (40 – 69)	Prosp cohort 9 yrs	DHEA DHEAS	CVD	Negative
Arnlov 2006 (59)	1928, 55 yrs	Prosp cohort, 10yrs	DHEAS	CVD	Null

Cross: cross-sectional study
Prosp: prospective study
Long: longitudinal study
*Negative trend statistically insignificant
negative only upon univariate analysis, null upon multivariate analysis
□□population sample rather than patients
□ fatal cases
□□non-fatal cases
CAD: coronary artery disease
OR: odds ratio (95% confidence intervals)
CVD: cardiovascular disease
CIMT: Carotid intima-media thickness ultrasound
MI: myocardial infarction
Angio: coronary angiography
Negative relationship indicates lower DHEA(S) levels in patients with CAD compared to controls, positive relationship indicates higher DHEA(S) levels in CAD and a null relationship indicates no difference between cases and controls.

Table 3 Relationship between androgens and atherosclerosis in animals fed on atherogenic cholesterol-enriched diets

Study (ref)	Model	n	Dura-tion	Horm-one	Circ.T	Targeted vessel	Measure-ment	Effect
Larsen 1993 (189)	Male odx rabbits	36	17wks	T	Odx: 1.1-2.5nM Odx +T: 50-100nM	Abdominal aorta	Cholesterol content	↓
Bruck 1997 (194)	Male odx rabbits Female ovx rabbits	32	12wks	T	Odx: N.D. Odx +T: 26.5nM	Aortic arch	Plaque area	↓ in male ↑ in female
Alexander-sen 1999 (195)	Male odx rabbits	100	30wks	DHEA T	Sham: 1.28nM Odx: 0.68nM Odx +DHEA: 1.89nM Odx +Oral T: 2.5nM Odx +Inj T: 37.8nM	Thoracic aorta	Cholesterol content	DHEA: mod↓ Oral T: mod↓ Inj T: sig↓
Adams 1995 (196)	Female ovx monkeys	64	24 mths	T		Coronary artery	Plaque area	↑*
Li 2008 (199)	Male odx rabbit	30	12wks	T	Sham: ~20nM Odx: ~2nM Odx +T: ~18nM Odx +T+flut: ~18nM	Aortic arch Thoracic aorta Abdominal aorta	Plaque area Fibrous cap	↓ plaque size ↑ fibrous cap

Qiu 2010 (201)	Male odx rabbit	39	8wks	DHT		Whole aorta	Lipid-containing area	↓
Hatch 2012 (200)	Male LDLR−/− mice#	5-11 /group	17wks	T		Aortic arch	Plaque area	↓
Elhage 1997 (197)	Male odx apoE-/- mice§ Female apoE-/- ovx mice§	70 70	8wks	T & E2	Odx +T: 5.9nM Ovx +T: 4.5nM	Aortic valve	Lipid-containing area	Castrate: null T and E2: ↓ in both sexes
Nathan 2001 (198)	Male LDLR-/- mice#	6-11	8wks	T & E2, aromatase inhibitor		Aortic sinus and the ascending aorta	Lipid-containing area	Castrate: ↑ T & E2: ↓ but reversed by arom inhibitor
Arad 1989 (215)	Male rabbits	15	8wks	DHEA		Whole aorta	Lipid-containing area	↓
Eich 1993 (216)	Male rabbits hetero-topic cardiac transplants	48	5wks	DHEA		Coronary artery from grafted heart	Cross-sectional plaque area	↓
Hayashi 2000 (217)	Female ovx rabbits	48	10wks	DHEA		Descending thoracic aorta	Plaque area	↓
Yamakawa 2009 (218)	Male apoE-/-	24	12wks	DHEA		Aortic root	Plaque area	↓

Ovx: ovariectomised

Odx: orchidectomised

T- testosterone

E2: oestradiol

DHEA: dehydroepiandroterone

Circ: circulating

↓: decrease

↑: increase

Mod: moderate

Sig: significant

Flut: flutamide

Arom: aromatase

§ apoE-/- mice: apoE knockout mice

#LDLR-/-mice: LDL-receptor knockout mice

Cetrorelix: GnRH antagonist

* T reversed atherosclerosis-related impairment of endothelium-dependent vasodilatation response i.e. functional benefit

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