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REVIEW ARTICLE

Testosterone and Peripheral Arterial Disease

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Abstract: Testosterone levels in men begin declining in the early years of adulthood, with a 1-2% reduction/year. Low testosterone levels in men are associated with obesity, metabolic syndrome, diabetes mellitus, dyslipidaemia, hypertension and increased cardiovascular mortality. However, observational studies of testosterone levels in males and their relationship with peripheral arterial disease (PAD) have yielded mixed results; only some cohorts show a clear association with low free testosterone levels. This discrepancy may, in part, be due to methodological issues with estimating free testosterone but also to different effects of testosterone on the vessel wall and metabolism. While testosterone improves glycaemic control, has anti-obesity effects and induces vasodilation, it also stimulates platelet aggregation and increases the haematocrit. Androgen deprivation treatment for advanced prostate cancer may be associated with elevated cardiovascular risk, as is testosterone abuse for performance enhancement. On the other hand, judicious treatment of male hypogonadism or testosterone treatment of trans-men appears to be safe.

Keywords: Testosterone, cardiovascular disease, peripheral arterial disease, vascular function, male hypogonadism, androgen-deprivation, performance-enhancing drug, trans-men.

1. INTRODUCTION

The male sex hormone testosterone exerts important extragonadal effects that involve the cardiovascular (CV) system and may influence the progression of atherosclerosis [1, 2]. Due to the rapidly growing population of older men, functional testosterone insufficiency is becoming a common condition. Androgen deprivation is widely employed in treating prostate cancer [3]. On the other hand, testosterone administration to men, aiming to improve libido and well-being, is becoming increasingly common [3]. Data on the CV effects of endogenous and exogenous testosterone are heterogeneous and sometimes conflicting.

The purpose of this narrative review is to consider the available evidence regarding the association between serum testosterone levels and peripheral arterial disease (PAD). This article is part of a review series that considers the links between specific endocrine disorders and PAD [4].

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2. TESTOSTERONE AND VASCULAR FUNCTION

Androgen receptors are widely distributed in several tissues, including vascular endothelial and smooth muscle cells [2, 5]. In experimental animals, testosterone induces relaxation of many vascular beds, including coronary, mesenteric, iliac, renal, and femoral arteries – largely by mechanisms such as potassium channel opening and calcium channel antagonistic effects but also by modulating nitric oxide (NO) release [2, 6, 7]. Although aging itself progressively impairs vascular function and accelerates atherosclerosis, there is evidence that androgen deficiency in males plays an independent role [2].

In apparently healthy men, testosterone levels were inversely correlated with aortic stiffness, measured as carotid-femoral pulse wave velocity [8]. The effect was more pronounced in young men and in subjects with higher blood pressure [8]. In middle-aged men with few co-morbidities,

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low total testosterone concentration (<12 nmol/l) was associated with increased central augmentation index, a measure of arterial stiffness, and decreased reactive hyperaemia index, a measure of microvascular reactivity [9].

Low serum total and free testosterone levels were associated with impaired endothelial function, measured by flow-mediated dilation of the brachial artery in men aged 25 to 85 years [10]. Men with hypogonadal hypogonadism were found to have endothelial dysfunction at least in part related to increased asymmetric dimethylarginine (ADMA) levels, an endogenous inhibitor of NO synthase [11, 12], and their levels of ADMA decreased with testosterone replacement [11].

A meta-analysis of prospective and cross-sectional studies of testosterone replacement suggested that acute testosterone replacement was associated with an improvement of endothelial function measured by flow-mediated dilation of the brachial artery, while chronic treatment was associated with deterioration of endothelial function, but statistical significance was not reached for either effect [13].

Testosterone up-regulates the expression of thromboxane A2 receptors, which stimulates platelet aggregation [14], and increases haemoglobin and haematocrit [15], which may exert prothrombogenic effects [15, 16]. The main effects of testosterone on metabolism and vascular function are shown in Fig. (**1**).

3. EPIDEMIOLOGICAL DATA ON LEVELS OF TESTOSTERONE AND PAD

The most common cause of testosterone deficiency in men is functional hypogonadism, caused by a disorder of the hypothalamic-pituitary-testicular axis [17]. The incidence of functional hypogonadism is influenced by aging and comorbidities [2]. Testosterone levels in men begin declining in the early years of adulthood, with a 1-2% reduction/year leading to age-related hypogonadism, which can occur in middle age and is characteristic of older age [2]. As shown in prospective studies, low testosterone levels in men are associated with other factors besides age, such as obesity [18], metabolic syndrome [19], diabetes mellitus [20], dyslipidaemia [21] and hypertension [22]. The prevalence of functional hypogonadism in the general population of men over 40 years of age ranges from 2.1-12.3% [23], while it reaches approximately 50% in males with type 2 diabetes mellitus and/or obesity [24]. Low testosterone levels are associated with increased mortality and shortened lifespan [25], and CV disease (CVD) accounts for the largest proportion of deaths [26]. Population-based studies have found an inverse association between endogenous testosterone levels and all-cause and CV mortality, particularly in older men [27]. However, observational studies looking at low total or low free testosterone and PAD have yielded mixed results (Table **1**).

The Edinburgh artery study (40 men and 43 women with PAD were compared with 88 sex-matched subjects without PAD) did not show differences in total testosterone, free testosterone, oestradiol or sex-hormone binding globulin (SHBG) between cases and controls of either sex [28]. (In males with PAD compared with male control subjects, the serum total testosterone was 13.6 (95% confidence interval (CI) 12.0-15.3) *vs.* 14.9 (95% CI 13.3-16.5) nmol/l, p=0.28, and the serum free testosterone 0.40 (95% CI 0.35-0.45) *vs.* 0.38 (95% CI 0.34- 0.42) nmol/l, p=0.47 [28]. Among 1,422 male participants of the Framingham Heart Study Offspring

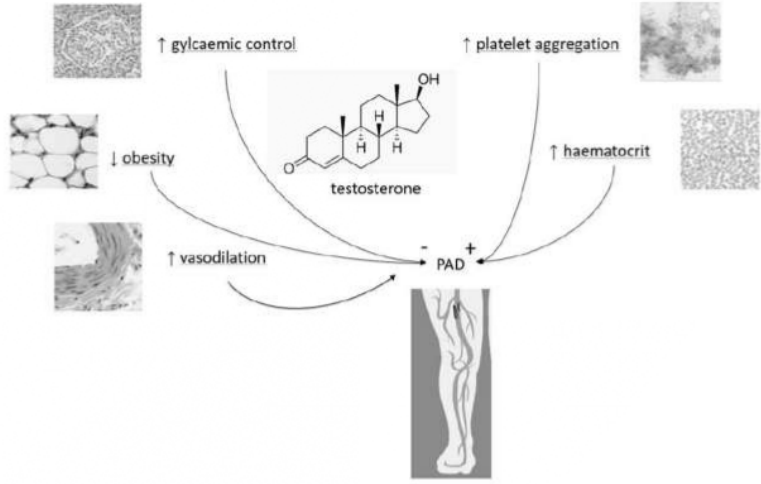

Fig. (1). Schematic representation of the effects of testosterone on metabolism and vascular function with relation to incidence of peripheral arterial disease (PAD). (↑ denotes an increase, ↓ decrease, - an inhibitory effect and + an enhancing effect). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Table 1. Epidemiologic observational studies on the association of serum testosterone and peripheral arterial disease (PAD) in men.

First Author, Year, Country of Study, Reference	Type of Study, (Study Acronym)	Measured and Calculated Laboratory Parameters	Main Findings
Price JF *et al.* 1977, Scotland (UK), [28].	Case-control study (Edinburgh artery study)	TT SHBG calculated FT	No difference in TT, FT and SHBG between men with PAD and controls
Harring R *et al.*, 2011, USA, [29].	Cross-sectional and longitudinal cohort study (Framingham Heart Study Offspring Study)	TT SHBG calculated FT	Lower FT may be associated with PAD and the progression of PAD in men
Tivesten A *et al.*, 2007, Sweden, [30].	Cross-sectional cohort study (Swedish arm of the MrOS Study)	TT SHBG calculated FT	Low FT in older men associated with PAD
Collet TH *et al.*, 2020; USA, [31].	Cross sectional and longitudinal cohort study (American arm of the MrOS Study)	TT SHBG	TT and SHBG not associated with PAD in older men
Maggio M *et al.*, 2012, Italy, [32].	Cross sectional and longitudinal cohort study (inCHIANTI study)	TT SHBG	TT not associated with PAD in men, lower SHBG associated with PAD in older men
Hernandez-Mijares A *et al.*, 2010, Spain, [33,34].	Cross sectional and longitudinal cohort study	TT SHBG calculated FT	TT not associated with PAD in diabetic men
Yeap BB *et al.*, 2010, Australia, [35].	Cross-sectional cohort study (Health In Men Study)	TT SHBG calculated FT	Low TT and low FT associated with AAA in older men

TT – total testosterone, SHBG – sex-hormone binding globulin, FT – free testosterone, AAA – abdominal aortic aneurysm.

cohort with average total serum testosterone of 28.8 (standard deviation (SD) 4.4) nmol/l, serum free testosterone 0.30 (SD 0.12) nmol/l and serum estrone 189.7 (SD 67.4) pmol/l, low free testosterone and high estrone concentrations were associated with PAD - defined as an ankle-brachial index (ABI) <0.90, intermittent claudication or lower extremity revascularization [29]. Men with age-adjusted free testosterone levels in the lowest quartile, compared with men in the highest quartile, had an odds ratio of PAD 1.92 (95% CI 0.96-3.87), men in the second quartile had an odds ratio of PAD 1.57 (95% CI 0.76-3.23), while men in third and fourth quartile did not differ [29]. In contrast, men with age-adjusted estrone concentrations above the 75th percentile and those with estrone below the 25th percentile had an odds ratio of PAD 1.57 (95% CI 0.80-3.07) [29]. Low SHBG in men was also associated with PAD in the Framingham Heart Study Offspring cohort [29]. In women participants of the Framingham Heart Study Offspring cohort, no significant associations between sex hormone concentrations, including testosterone and PAD, were found [29].

The Swedish arm of the Osteoporotic Fractures in Men (MrOS) study with 3,014 participants (average age 75.4 (SD 3.2) years, serum total testosterone 16.9 (SD 7.0) nmol/l, serum free testosterone 0.31 (SD 0.14) nmol/l) reported that ABI was significantly and positively associated with free testosterone and negatively associated with free oestradiol in a linear regression model that included age, current and previous smoking, diabetes, hypertension and body mass index [30]. On the other hand, the American arm of the MrOS study, a prospective follow-up of 522 men with an average serum total testosterone of 14.4 (SD 5.4) nmol/l, failed to show an association between endogenous levels of testosterone, oestradiol, and SHBG with coronary heart disease, cerebrovascular disease or PAD [31]. In the Italian Aging in the CHIANTI Area study, low SHBG was associated with the presence of PAD in older men and women, while total testosterone levels were not associated with PAD in men (participants with PAD compared with participants without PAD 13.4 (SD 4.0) *vs.* 15.0 (SD 4.5) nmol/l, p=0.09), but were higher in older women with PAD compared with control women (3.3 (SD2.9) *vs.* 2.1 (SD 2.5) nmol/l, p=0.02) [32]. For elderly women, these results seem discordant with a sub-study of the Australian trial Aspirin in Reducing Events in the Elderly (ASPREE) which focused on the association between serum testosterone and major adverse CV events (MACE), not including major adverse limb events, in apparently healthy Australian women over 70 years of age [33]. Elderly women with total testosterone and dehydroepiandrosterone levels in the lowest quartile were at the highest risk for MACE, while no association was found between SHBG and MACE [33].

In a Spanish cohort of 192 diabetic men (average age 56 years on inclusion) without a history of previous vascular

disease, among which 44 men had low total testosterone (<12 nmol/l) or low free testosterone (<0.225 nmol/l), low testosterone was associated with body mass index, waist circumference, neuropathy, triglycerides, C-reactive protein, glucose, insulin levels, but not with an increase in silent myocardial ischaemia or PAD [34].

In a cross-sectional study of 3,620 community-dwelling Australian men aged 70-88 years, low free testosterone and high SHBG concentrations were independently associated with the prevalence of abdominal aortic aneurysm. This condition can be associated with PAD due to common risk factors [35].

Overall, the association between low free testosterone and PAD remains equivocal since two large observational studies found an association [29, 30], while three other observational studies failed to do so [28, 31, 32]. The reasons for the discrepancies may go beyond differences in baseline characteristics of the studied cohorts and may involve methodological issues [27].

4. METHODOLOGICAL ISSUES REGARDING DETERMINING FREE AND BOUND TESTOSTERONE IN THE CIRCULATION

Only 1-2% of total testosterone circulates in blood free, while the vast majority is bound to carrier proteins: approximately 60% to SHBG, approximately 40% to albumin, and a small portion to cortisol-binding globulin and orosomucoid [27, 36]. Free testosterone is directly bioavailable to exert its hormonal effects [27, 36]. Testosterone bound to albumin is also considered bioavailable because of its low binding affinity [27, 37]. The properties of testosterone binding to proteins are incompletely understood. Some widely used assumptions are oversimplified and contribute to inaccurate linear models for calculating free testosterone [27]. Methods for direct measurement of free testosterone are currently plagued by numerous problems; more reliable assays for measuring free testosterone are needed [38].

5. TESTOSTERONE TREATMENT OF MALE HYPOGONADISM

Testosterone treatment is most commonly offered to men aged 40-65 years with low total testosterone levels (<12 nmol/l) [39]. A systematic review and meta-analysis of >3000 patients with hypogonadism from randomised placebo-controlled trials concluded that testosterone treatment with any formulation, dose frequency and route of administration for a minimum duration of 3 months, had potentially favourable effects on CV risk, such as increased lean-to-fat body mass, as well as improved insulin sensitivity and glycaemia [39]. Testosterone replacement by injectable testosterone undecanoate (1000 mg at the first visit, after 6 weeks and thereafter every 10 weeks for 1 year) in obese hypogonadal men with diabetes mellitus improved insulin sensitivity with a reduction of glycated haemoglobin A1c by 0.94 (SD 0.88) % and improvement of flow-mediated dilation of the brachial artery by 2.40 (SD 4.16) %, without exerting ill effects on the study population [40]. A meta-analysis that included the aforementioned study and four other older studies failed to show a significant relation between flow-mediated dilation and testosterone replacement, although a trend toward improvement was found with acute administration and a trend towards worsening with chronic administration [13]. The Testosterone Trials (TTrials), a coordinated set of seven placebo-controlled, double-blind trials in 788 men with a mean age of 72 years evaluated the efficacy of 1-year testosterone replacement by transdermal gel (1% testosterone, 5 g/daily) in older men with low testosterone, and demonstrated that treatment improved all aspects of sexual function, slightly improved mood and depressive symptoms (assessed by a telephone call-administered questionnaire), corrected mild to moderate anaemia (assessed as an increase in haemoglobin by ≥10 g/l in 52% of testosterone-treated men compared with 19% of control men), markedly increased volumetric bone mineral density by 6.8%, increased the noncalcified coronary artery plaque volume by 41 (95% CI 14-67) mm^3, but was not associated with more CV or prostate adverse events; however, the number of participants and the duration of treatment were not sufficient to draw definitive conclusions about any possible risks associated with testosterone treatment [41].

There is some uncertainty about the safety of testosterone treatment since it increases haematocrit, and lowers high-density lipoprotein cholesterol (HDL-C), and some studies have reported an increased risk of CV events [39]. A recent systematic review and meta-analysis of 109 randomized controlled trials with 5,601 participants found no increase in CV risk with testosterone replacement during 3-9 months of follow-up. However, the studies were heterogeneous, long-term data were lacking, and no interventional randomised studies have been adequately powered to assess CV safety [39]. In addition, there is no evidence to date that short- or long-term testosterone replacement has beneficial effects on the course and complications of PAD. However, this may not be an actual effect but rather the result of limited data [42]. Therefore, sufficiently powered, well-designed, randomized trials are needed to clarify the effects of testosterone substitution on vascular function and CVD, including PAD.

Currently, the United States Food and Drug Administration (US FDA) recommends that men on testosterone treatment be advised of the potential CV risks [43]. Still, the European Medicines Agency has concluded that there is insufficient evidence to link testosterone treatment with increased CV risk [44]. Based on the available evidence, concerns about CV safety should not discourage the initiation of testosterone treatment in men with low CV risk, whereas men with high CV risk should be counseled that the CV safety of testosterone therapy is uncertain. The US FDA-mandated trial Testosterone Replacement therapy for Assessment of long-term Vascular Events and efficacy ResponSE in hypogonadal men (TRAVERSE) will provide robust evidence on this topic [45].

Novel and possibly safer ways of testosterone replacement for hypogonadal males are being developed, including nasal and oral medications, that - together with gels and patches - avoid long-term supraphysiological testosterone levels, characteristic of injectable preparations which strongly suppress the hypothalamic-pituitary-gonadal axis [46].

6. ANDROGEN DEPRIVATION THERAPY (ADT)

ADT is the standard of care for advanced prostate cancer. Artificial testosterone lowering can be achieved through

gonadotropin-releasing hormone (GnRH) agonism, GnRH antagonism, anti-androgens, and inhibitors of steroid synthesis [47]. The effects of artificially lowering testosterone levels on CV outcomes have been studied in observational studies and randomized control trials. In a cohort of 73,196 prostate cancer patients aged 66 years or older who were followed for a median of 4.55 years, patients receiving GnRH agonists were at increased risk for coronary artery disease (hazard ratio (HR) 1.16 (95% CI 1.10-1.21)), myocardial infarction (HR 1.11 (95% CI 1.01-1.21)), and sudden cardiac death (HR 1.16 [95% CI 1.05-1.27]) compared with men receiving no ADT [48]. Men treated with orchiectomy were more likely to develop diabetes, but not coronary heart disease, myocardial infarction, or sudden cardiac death [48]. A pooled analysis of six trials in which patients were randomized to GnRH agonists or GnRH antagonists showed that men with pre-existing coronary artery disease had a significantly lower risk of CV events on GnRH antagonists compared with GnRH agonists (6.5 *vs.* 14.7%, p=0.002) during 1 year of follow-up (p=0.002), while no difference in CV events was observed in men without a history of coronary artery disease [49]. Overall, most observational studies and randomized trials found an increased risk of CV events with ADT [47,50]. Some heterogeneity in the results might be due to different selections of patients in terms of age, pre-existing CVD, CV risk factors, and comorbidities, as well as the duration of observation [47, 50]. It should also be emphasized that adverse CV effects associated with ADT may vary depending on the type of therapy. As mentioned, GnHR antagonists seem to be associated with lower CV risk than GnHR agonists [49]. This important assumption was tested in the Trial Comparing the Cardiovascular Safety of Degarelix Versus Leuprolide in Patients With Advanced Prostate Cancer and Cardiovascular Disease (PRONOUNCE), which was unfortunately terminated prematurely due to the COVID-19 pandemic and therefore could not clarify the question under investigation [51].

7. ABUSE OF TESTOSTERONE AS A PERFORMANCE-ENHANCING DRUG

Abuse of androgenic anabolic steroids, among which testosterone is most widely available as a performance-enhancing drug, is associated with adverse CV effects [52]. Repetitive injection of supraphysiologic doses of androgenic anabolic steroids has been associated with increased low-density lipoprotein cholesterol (LDL-C), decreased HDL-C, hypercoagulability, leading to myocardial infarction and cerebrovascular disease, and myocardial hypertrophy with fibrosis, leading to cardiac arrhythmias and heart failure [52]. Long-term anabolic-androgen abuse in strength-trained men aged 29-37 years resulted in lower maximal exercise capacity than in control subjects, and 20% of abusers had signs of coronary artery disease on CT angiography [53].

8. TRANS-MEN

In older comparisons with cis-gender people, trans-people showed an increased risk for myocardial infarction and death due to CVD, but the risk was consistently higher in trans-women than in trans-men [54, 55]. Newer data, albeit limited by the relatively small sample sizes and imperfect control populations, do not show an increased risk of CVD in transgender males receiving testosterone, indicating that appropriate testosterone dosing in trans-men is probably safe regardless of consistently increasing systolic blood pressure and LDL-C and lowering HDL-C levels [56].

CONCLUSION

Testosterone improves glycaemic control, has anti-obesity effects and induces vasodilation, but it also stimulates platelet aggregation and increases haematocrit. The association of low testosterone levels in men with PAD is not unequivocally proven.

Androgen deprivation treatment for advanced prostate cancer may be associated with elevated CV risk, as is testosterone abuse for performance enhancement. On the other hand, judicious treatment of male hypogonadism or testosterone treatment of trans-men appears to be safe.

LIST OF ABBREVIATIONS

ADT	=	Androgen Deprivation Therapy
CV	=	Cardiovascular
GnRH	=	Gonadotropin-Releasing Hormone
LDL-C	=	Low-Density Lipoprotein Cholesterol
PAD	=	Peripheral Arterial Disease

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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