Androgen therapy and atherosclerotic cardiovascular disease

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Abstract: Cardiovascular disease (CVD) remains the leading cause of death in Western society today. There is a striking gender difference in CVD with men predisposed to earlier onset and more severe disease. Following the recent reevaluation and ongoing debate regarding the estrogen protection hypothesis, and given that androgen use and abuse is increasing in our society, the alternate view that androgens may promote CVD in men is assuming increasing importance. Whether androgens adversely affect CVD in either men or women remains a contentious issue within both the cardiovascular and endocrinological fraternities. This review draws from basic science, animal and clinical studies to outline our current understanding regarding androgen effects on atherosclerosis, the major CVD, and asks where future directions of atherosclerosis-related androgen research may lie.

Introduction

Epidemiological studies have shown there is a striking gender difference in cardiovascular disease (CVD) with men having higher rates of clinical events than women (Kalin and Zumoff 1999). These findings, together with the increased incidence of CAD in women after menopause (Tracy 1966), have led to the dogma that female hormones protect against the development of CVD (Jeanes et al 2007). The opposite hypothesis, that male hormones may promote CVD in men, has been little investigated. With prospects of androgens being introduced widely for non-classical therapeutic applications, an important clinical question is: do androgens increase the risk or severity of CVD? Such androgen therapy is being targeted towards our aging population, a population that would have pre-existing CVD. The recent proposal that stems from the female hormone replacement therapy (HRT) studies is that HRT appears beneficial in females only when it is initiated before the development of significant atherosclerosis (Rossouw et al 2007). Whether this is also true for androgen-based therapies is unknown. This review explores what is known about androgens and their gender-specific effects on the pathogenesis of atherosclerosis to try and highlight those questions that need to be answered for the safe use of androgen-based HRT in both men and women.

Risk factors of atherosclerosis

Primary risk factors for atherosclerosis include elevated levels of low-density lipoprotein (LDL), increased levels of homocysteine, hypertension, diabetes mellitus, obesity, smoking, increasing age and also male gender (Ross 1999). In all developed countries, men have an earlier and greater incidence of heart disease than women (Liu et al 2003; Wu and von Eckardstein 2003; Isidori et al 2005). This may be one of the oldest clues to the underlying pathogenic mechanisms of atherosclerosis and suggests that gender-related differences between men and women drives, at least in part, the disparate atherosclerotic plaque formation (Liu et al 2003). There remains no clear

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evidence, to date, that there is a genetic contribution to the male predisposition to atherosclerosis, however the androgenic milieu may underlie plaque formation in men.

Molecular mechanisms of androgen action

The molecular machinery mediating cellular responses to androgens is complex and involves both genomic and nongenomic effects that are still far from being clearly understood (Figure 1). Genomic effects of androgens are mediated by a specific receptor, the androgen receptor (AR). In response to the binding of androgens to AR, it switches to a transcription factor that regulates target gene expression (Davison and Bell 2006). Non-genomic effects of androgens occur independently of AR. Instead, membrane-bound receptors have been proposed to trigger rapid effects of androgens that lead to 2nd messenger signaling (Benton et al 2004). This, in turn, triggers a variety of cell responses (Wierman 2007). These nongenomic pathways underlie the rapid vasodilation of coronary arteries by testosterone (Malkin et al 2006; Cooper et al 2007; Seyrek et al 2007).

Regulation and tissue expression of AR

AR function and transactivation ability is regulated by post-translational modifications such as phosphorylation (Zhou et al 1995; Gioeli et al 2002), acetylation and sumoylation (Thomas et al 2004). AR expression itself is regulated at both the mRNA and protein levels by androgens (Lee and Chang 2002). Androgens predominantly decrease AR mRNA at

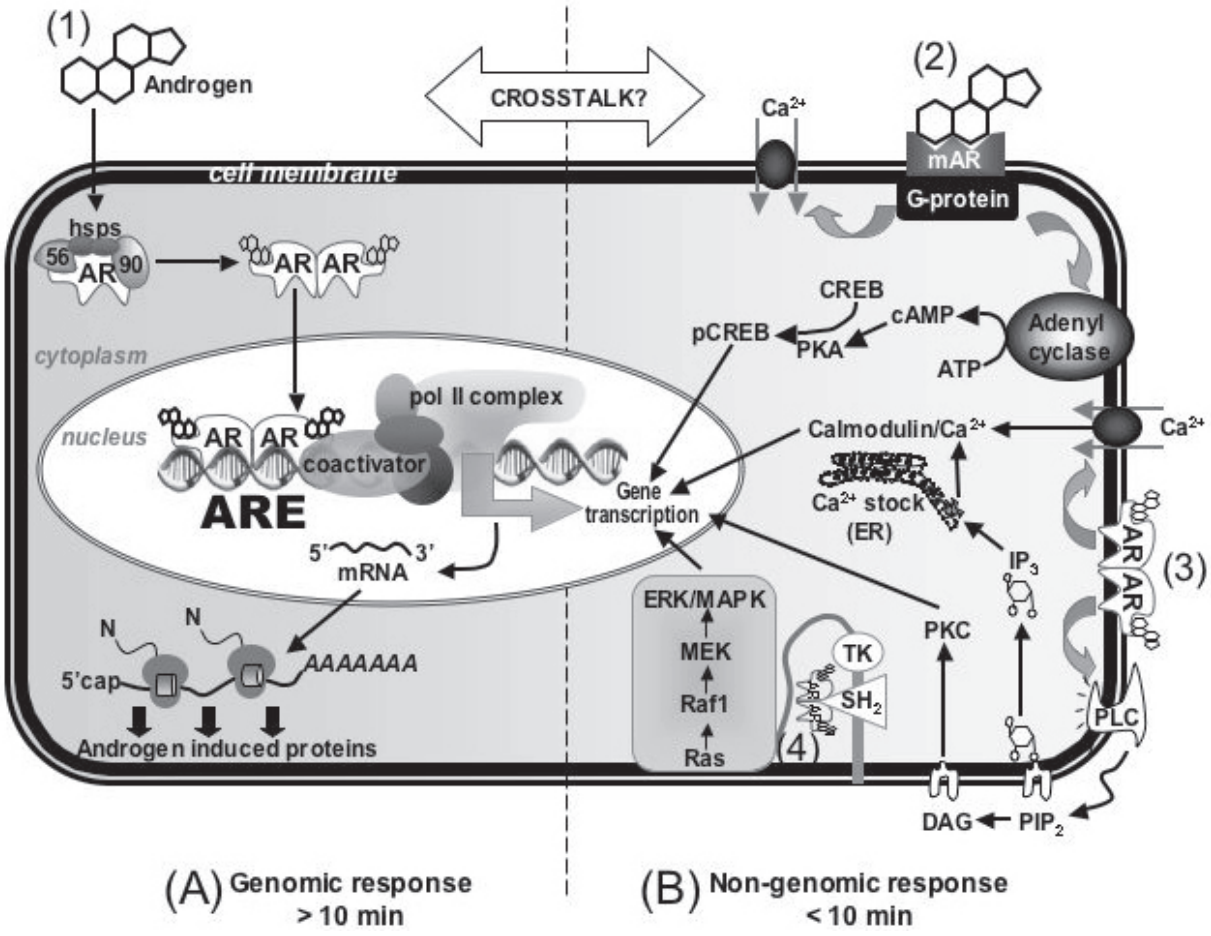

Figure 1 Molecular mechanisms of androgen action. (1) Androgens mediate gene transcription via binding to the classical cytosolic AR in the genomic pathway; (2) Androgens mediate rapid effects through a novel membrane receptor; (3) Androgens interact with the classical cytosolic AR associated with the plasma membrane; (4) Androgens act through a multi-protein complex associated with the plasma membrane.

Abbreviations: AR: androgen receptor; hsps: heat shock proteins; ARE: androgen response element; mRNA: messenger RNA; mAR:putative membrane AR; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; ATP: adenosine triphosphate; CREB:cAMP response element binding protein; pCREB: phosphorylated CREB; PLC:phospholipase C; PIP2: phosphatidyl inositol bisphosphate; DAG: diacylglycerol; IP3: inositol 3-phosphate; PKC: protein kinase C; ER: endoplasmic reticulum; SH2: Src homology domain; TK: tyrosine kinases; MEK: mitogen activated protein kinase-extracellular signal regulated kinase; ERK: extracellular signal regulated kinase; MAPK: mitogen-activated protein kinase.

the transcriptional level (Trapman et al 1990; Krongrad et al 1991) however, they simultaneously increase AR stability and translational efficiency thereby even in the presence of decreased AR mRNA levels, androgens increase AR protein levels in most cell types (Yeap et al 1999).

AR has been detected in the majority of tissues throughout the body (Quigley et al 1995). AR is evident in vascular cells and gender-specific expression of AR has been shown in monocyte-derived macrophages (Ng et al 2003), endothelial cells (Death et al 2004) and vascular tissue (Death et al 2004), where cells or tissue from male donors had significantly higher AR protein levels. The gender dichotomy in AR expression may underlie gender-specific effects of androgens on atherosclerosis.

Metabolic activation of testosterone

Testosterone and other androgens can mediate effects via metabolic activation (Figure 2). This involves the conversion of testosterone at peripheral nongonadal tissues to active metabolites, estradiol or DHT. Conversion of testosterone to estradiol involves a P450-dependent aromatase enzyme (CYP19) and acts to diversify androgen action, since estradiol binds to the estrogen receptor (ER), and not AR, thereby regulating the expression of a completely different set of genes. The conversion of testosterone to DHT is catalyzed by 5α-reductases. DHT has greater binding affinity for AR than testosterone and a slower dissociation rate, therefore has a higher molar potency (Grino et al 1990). Hence, the conversion of testosterone to DHT effectively amplifies AR action.

Androgens and vascular cell effects

Androgens have been shown to promote-, and suppress-, pro-atherogenic, pro-inflammatory effects on all cell types involved in atherogenesis. Given current evidence it would appear that androgen effects are dependent on cell type, dose, type of androgen, and time of exposure. For example, T suppresses vascular cell adhesion molecule-1 (VCAM-1) expression in human endothelial cells, via an aromatase/estrogen receptor-dependent mechanism (Hatakeyama et al 2002; Mukherjee et al 2002); however, DHT, a non-aromatisable androgen, induces VCAM-1 expression in human endothelial cells (Death et al 2004). Similarly, T has been shown to enhance reverse cholesterol transport (Langer et al 2002) whilst DHT promotes cholesteryl ester accumulation in monocyte derived macrophages (Ng et al 2003). In addition, T has been shown to inhibit nitric oxide release from monocytes via inhibition of inducible nitric oxide synthase (Friedl et al 2000). This decrease in NO potentially increases thrombosis risk via increased platelet aggregation. Additionally, T has adverse effects stimulating the proliferation of rat vascular smooth muscle cells (Fujimoto et al 1994), inducing proteoglycan synthesis and the elongation of glycosaminoglycans (GAG) chains on these proteoglycans (Hashimura et al 2005) and T increases apoptotic damage of vascular smooth muscle cells (Ling et al 2004). Importantly, some of the effects on both endothelial cells and MDMs were gender-specific, occurring in cells derived from males but not females, and associated with increased AR expression in male-derived cells (Ng et al 2003; Death et al 2004). This suggests that steps in atherogenesis could be markedly different between genders, mediated by androgen exposure and AR expression levels.

Therefore, both T and DHT can have effects that could lead to the development of atherosclerosis, associated with male-dependent AR expression. However, T can have equally anti-atherogenic effects, associated with aromatisation. Obviously, more work is required for us to understand how androgens act at the cellular level. One of the major questions that has recently emerged is whether aromatisation is an important protective mechanism? There is now a real need to study and understand the metabolic activation pathways of T, in those cell types associated with atherosclerosis, and to determine if manipulating those pathways can switch between the atheroprotective versus atherogenic effects of T?

Androgens and atherosclerosis: evidence from animal model studies

As with the cellular studies, androgen treatment has been shown to both promote and retard lesion formation in animal studies of atherosclerosis (Table 1). The effects of T appear to be gender-, steroid/dose/administration-, and/or species- specific. For the most part, T treatment of male animals has led to a decrease in atherosclerotic lesion size or the atherosclerosis-related end point studied (e.g. aortic cholesterol content) (Bruck et al 1997; Elhage et al 1997; Alexandersen et al 1999; Nathan et al 2001). Similarly, DHEA treatment of male animals has led to a decrease in atherosclerosis (Gordon et al 1988; Arad et al 1989; Eich et al 1993). Both T and DHEA are readily aromatisable to estradiol, and aromatase inhibition has been shown to block the atheroprotective effects of T (Nathan et al 2001). In keeping with the importance of aromatase to mediate the atheroprotective effects of T, a study showed that a 3-month treatment with an anabolic androgenic steroid, stanozolol, had no effect on atherosclerosis or blood lipids in cholesterol-fed rabbits (Fogelberg et al 1990). Stanozolol is a 5α-reduced substrate so it cannot be converted to estrogen

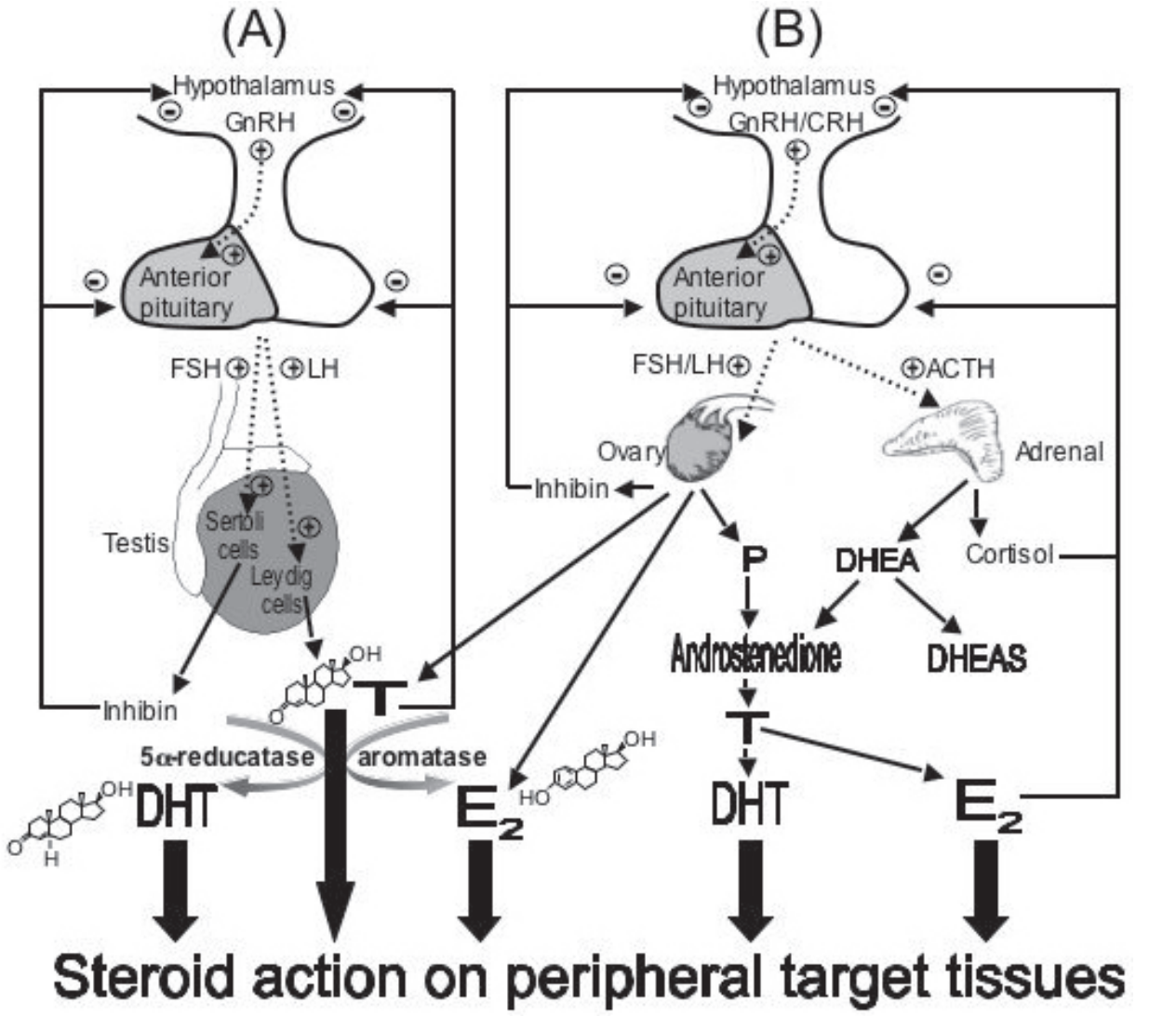

Figure 2 Schematic representation of the major sources of androgens in men and women. (MEN) The hypothalamic-pituitary-gonadal axis in men. The dotted lines represent the pulsatile release of gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH) and luteinising hormone (LH). LH serves to stimulate the testis to produce testosterone (T) or FSH stimulates production of inhibin. In turn, these exert a negative feedback on both the pituitary and hypothalamus regulating LH and FSH secretion. T can be reduced to the more active dihydrotestosterone (DHT) or aromatised to estradiol (E2) in target tissues. (WOMEN) The hypothalamic-pituitary-ovarian and hypothalamic-pituitary-adrenal axis represents the major sources for androgen synthesis in women. In addition to inhibin and cortisol, high levels of E2, progesterone (P) and adrenal androgens exert a negative feedback to regulate the secretions of GnRH, corticotropin-releasing hormone (CRH), FSH, LH and adrenocorticotropic hormone (ACTH).

by aromatase and therefore, only has androgenic effects. Interestingly, 2/10 stanozolol-treated normal diet fed rabbits developed atherosclerosis versus 0/72 control rabbits, thereby suggesting that stanozolol may increase the propensity for atherosclerotic lesion development. This was not followed up by these investigators.

In contrast to the studies that showed T was atheroprotective, two studies (out of 12) demonstrated increased atherosclerotic plaque formation after exogenous T treatment (Toda et al 1984; von Dehn et al 2001). However, both of these studies used an experimental approach that differed from usual practice. The first studied chicks, rather than rodents, and they only observed increased atherosclerotic lesion when T was administered at 150 mg for 7 weeks (compared to 50 mg/day/rabbit for 3 months, Bruck et al 1997). No studies have subsequently been performed in chicks to confirm the original findings. The second study demonstrating adverse effects of T used chemical, rather than surgical, castration of apoE-deficient mice. In this model, T treatment (35 mg dosage) was observed to increase atherosclerotic lesion area by a significant, but small, extent. This study did not examine the aromatase pathway so in this animal model it is not clear

Table 1 Effects of androgens on atherosclerosis in animal models

Animal model	Treatment duration	Hormone	Endpoints	Effect on atherosclerosis
Male odx rabbits Larsen 1993	17 wk	T[i]	Abdominal aorta cholesterol	T null
Male apoE$^{-/-}$ odx mice Female apoE$^{-/-}$ ovx mice Elhage 1997	8 wk	T[iii], E_2[1]	Aortic fatty streak lesions	T and E_2 decreased in both sexes
Male odx rats Female ovx rats Chen 1996	2 wk	T[iv], E_2[1]	Myointimal proliferation after balloon injury of carotids	T null, E_2 decreased in both sexes
Male odx rabbits Female ovx rabbits Bruck 1997	12 wk	T[i], E_2[2], T[i]+ E_2[2]	Aortic plaque size	T decreased in male, E_2 decreased in female, T+ E_2 decreased in both sexes
Male apoE$^{-/-}$ mice Female apoE$^{-/-}$ mice von Dehn 2001	8 wk	Cetrorelix*. T[ii]	Aortic fatty streak lesions	Cetrorelix decreased in both sexes, T[iii] decreased in female
Male LDLR-/- mice Nathan 2001	8 wk	Odx, T[iii], E_2[1], anastrazole[###]	Aortic fatty streak lesions	E_2 decreased, T decreased but reversed by anastrazole
Male rabbits Fogelberg 1990	12 wk	Stanozolol[Δ]	Aortic atherosclerosis	Null
Male odx rabbits Alexanderson 1999	30 wk	T[i,ii], DHEA[δ]	Aortic atherosclerosis	T and DHEA decreased
Male rabbits Gordon 1988	12 wk	DHEA[δ]	Aortic atherosclerosis following balloon-induced intimal injury	Decreased
Male rabbits Arad 1989	8 wk	DHEA[δ]	Aortic fatty streak	DHEA decreased
Male rabbits heterotopic cardiac transplants Eich 1993	5 wk	DHEA[δ]	Graft atherosclerosis	DHEA decreased
Female ovx monkeys Adams 1995	2 yrs	T[iii]	Coronary artery plaque size	T increased
Male chicks Toda 1984	7 wk	T[iii]	Aortic atherosclerosis	T increased
Male apoE$^{-/-}$ mice Female apoE$^{-/-}$ mice von Dehn 2001	8 wk	T[iii] + Cetrorelix*	Aortic fatty streak lesions	T increased in male
Female ovx monkeys Obasanjo 1996	1 – 2 yrs	Nandrolone[Δ]	Coronary plaque and lumen size	Nandrolone increased

[1]7β-estradiol, [2]estradiol valerate, [i]testosterone enanthate, [ii]testosterone undecanoate, [iii]testosterone not specified, [iv]testosterone proprionate, [*]cetrorelix - gonadotropin-releasing hormone (GnRH) antagonist, [###]anastrazole - aromatase inhibitor, [δ]DHEA - dehydroepiandrosterone, [Δ]stanozolol and nandrolone are anabolic steroids.

if aromatase would be expressed, which could help explain the disparate result.

As with the male animal model data, testosterone effects on atherosclerotic plaque formation in female animal models is also contentious. The data that exists is very limited with only 5 studies in total. Three of these studies were performed in rodents and testosterone was found to decrease lesion size in 2/3 of them (Elhage et al 1997; von Dehn et al 2001). The third rodent study showed no effect of testosterone treatment on atherosclerotic plaque development (Chen et al 1996). The other two female animal studies were performed with primates and showed that testosterone or the anabolic androgen, nandrolone, induced atherosclerosis over a 2-year treatment period (Adams et al 1995; Obasanjo et al 1996). These primate studies, whilst only have a small number of animals in the experimental groups, remains the strongest evidence that exogenous androgen treatment may be atherogenic in females.

Importantly, all of the animal studies have targeted the effects of androgens on atherosclerotic plaque development without examining an effect of T on existing plaque. As has now been highlighted by the recent estrogen therapy trials, the timing of hormone therapies can have different outcomes on CVD. Although contentious, and remains to be proven, estrogen-based therapies given after plaque has developed leads to adverse effects (increased myocardial infarction, stroke) in the short-term. Whether androgen-based therapies have similar outcomes dependent on timing and age of patient has not been investigated.

Figure 3 summarizes the primary effect of androgen and estrogen treatment on atherosclerotic lesion development, as measured in animal studies. Note that many more studies have targeted estradiol effects and the number of androgen studies are relatively small in comparison, especially those that focus on T effects in females. Therefore, it remains unclear whether T is anti- or pro-atherogenic in female animal models, whilst in males T is atheroprotective, most probably via aromatisation to estradiol. Direct T or stanozolol effects appear to be atherogenic, although the limited number of studies do not allow for any firm conclusions to be made. Therefore, androgens, atherosclerosis, and gender-specific effects remains an important area for future research development.

Androgens and atherosclerosis: evidence from clinical studies

To date, the major clue that androgens may drive CAD in men remains the gender dichotomy in the earlier incidence of atherosclerosis. However, epidemiologic studies report no association between high physiologic androgen levels and atherosclerosis (English et al 2000; Hak et al 2002; Muller et al 2004). Instead, the inverse has been reported namely that hypoandrogenemia associates with CAD (Malkin et al 2003), or an atherogenic lipid profile (Tchernof et al 1997; Zmuda et al 1997), metabolic syndrome (Kupelian et al 2006), type 2 diabetes (Haffner et al 1996; Stellato et al 2000), systolic and diastolic hypertension (Svartberg et al 2004), visceral obesity (Khaw and Barrett-Connor 1992), increased fibrinogen (Bonithon-Kopp et al 1988), arterial stiffness (Hougaku et al 2006) and all-cause or cardiovascular deaths (Barrett-Connor and Khaw 1988). Hypoandrogenemia is common and it has been reported that 10% of men between 40 and 60 years of age and 25% between 60 and 80 years of age have low levels of free T (Vermeulen and Kaufman 2002) therefore hypo- rather than hyper- androgenemia may be the gender-specific factor driving atherosclerosis. However, not all studies have found an association between hypoandrogenemia and increased CVD (Contoreggi et al 1990) therefore it is difficult to draw any firm conclusions. It is of interest that castration of male rodents has been shown to increase atherosclerosis in animal models of atherosclerosis (Nathan et al 2001).

Given that low T levels appear harmful for CVD and its important risk factors, T supplementation would be expected to be beneficial. Meta-analysis review of cardiovascular safety of T replacement therapy has reported that T supplementation was relatively safe in terms of cardiovascular health (Haddad et al 2007). However, this meta-analysis needs to be interpreted with caution as none of the randomized controlled trials that were included in the analysis were designed to assess cardiovascular safety and therefore adverse outcomes may have been censored and/or not reported, therefore, weakening the meta-analysis conclusions. However, other studies have shown that T replacement therapy has demonstrable beneficial effects on CVD risk factors, including waist measurements (Marin et al 1992), visceral abdominal fat mass (Marin et al 1992), as well as positive effects on numerous metabolic parameters including insulin sensitivity, glucose control, and hyperlipidemia (English et al 2000; Malkin et al 2006). Direct effects on the arterial tree have also been described with consistent improvement in both anginal symptoms and ischemia on electrocardiograms in men treated with injectable T preparations (Rosano et al 1990; Webb et al 1999; English et al 2000; Pugh et al 2003).

However, while the observations above would suggest T supplementation improves CVD risk factors, meta-analysis

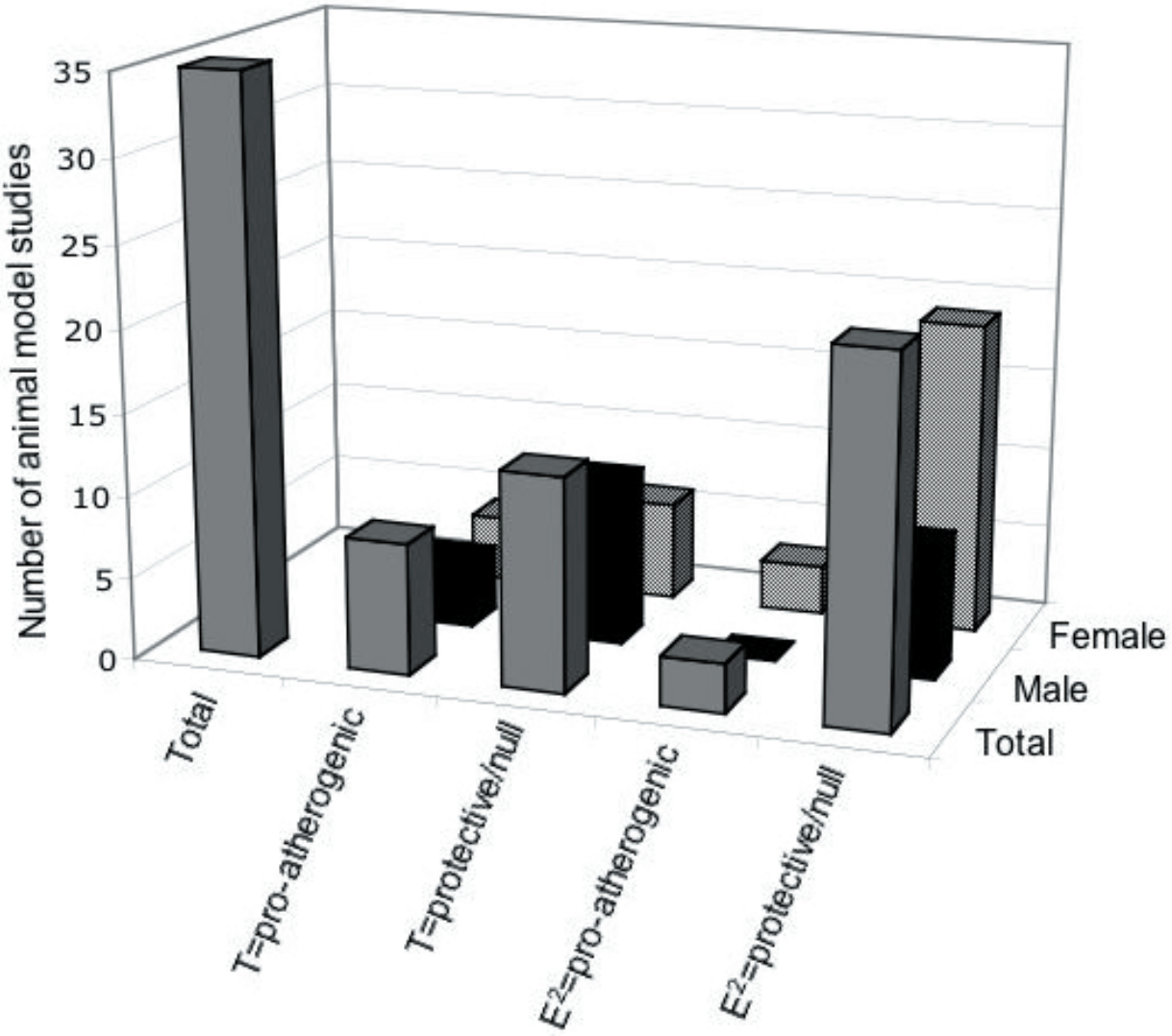

Figure 3 Animal model studies examining the effects of androgens on atherosclerosis. Total number of animal model studies are represented by the solid grey bar. Of the total number of studies, male studies are represented by the black solid bar. Of the total number of studies, female studies are represented by the hatched bar. T=pro-atherogenic – represents the number of animal model studies showing pro-atherogenic effects of T treatment or castration; T=protective/null – represents the number of animal model studies showing protective or null effects of T treatment or castration; E2=pro-atherogenic – represents the number of animal model studies showing pro-atherogenic effects of T treatment or castration; E2=protective/null – represents the number of animal studies showing protective or neutral effects of T treatment or castration.

by Whitsel et al (2001) found a dose-dependent decrease in HDL-C and total cholesterol levels with T use in hypogondal men. Similarly, small intervention trials have demonstrated that exogenous T supplementation in young men lowers HDL (Meriggiola et al 1995; Wu et al 1996) but in older men T did not affect HDL (Snyder et al 2001; Page et al 2005). The disparite findings may indicate that the effect of T replacement on HDL may be age-dependent. Any effect of T on lowering HDL-C needs to be considered as low HDL levels are a strong risk factor for CVD (Gordon et al 1997).

Other cardiovascular diseases that often coexist with CVD, including hypertension and ischemic stroke, also show a similar gender bias, with males at higher risk. Androgens have been reported to adversely affect both conditions, with reports of prohypertensive effects (Jenkins et al 1994; Reckelhoff 2005) and to worsen the acute phase of stroke (Hawk et al 1998). Therefore, when considering T replacement therapy in aging men, the effect on atherosclerosis cannot be considered without simultaneously investigating hypertension and stroke (Figure 4).

Anabolic androgenic steroid (AAS) use has been anecdotally associated with various forms of cardiovascular disease. Self-administration of AAS has been linked with sudden cardiac death, androgen-induced vasospasm, platelet

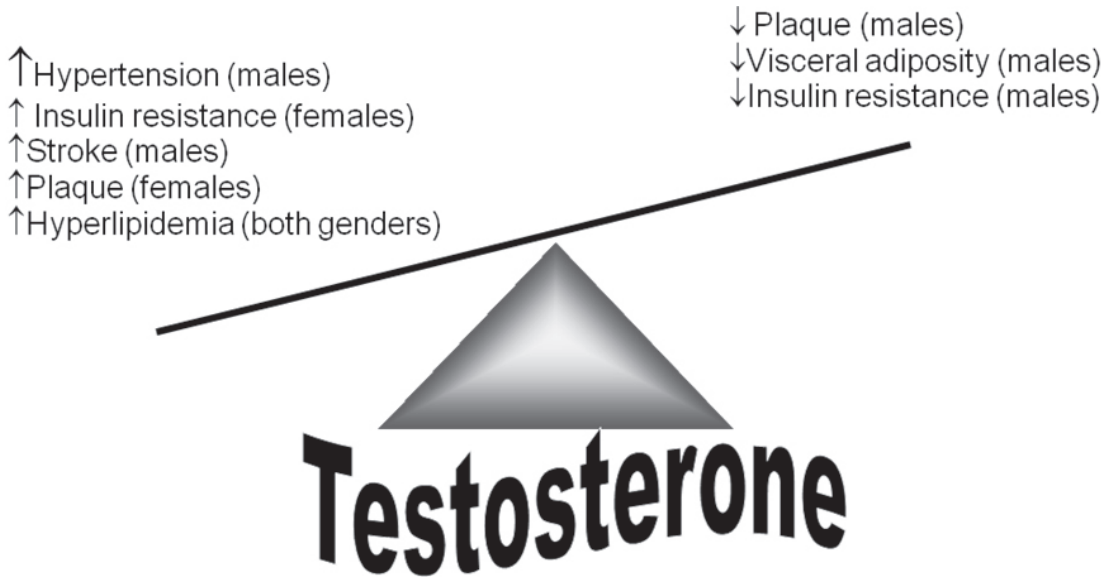

Figure 4 Summary of the effects of exogenous T treatment on cardiovascular endpoints.

aggregation, activation of the coagulation cascade, and abnormal left ventricular function and hypertrophy (Maron et al 1996). It has also been reported that self-administration of several AAS simultaneously for 8 or 14 weeks produces profound unfavorable effects on lipoproteins and lipids, leading to an increased atherogenic profile (Hartgens et al 2004). However, any link between the adverse lipid profile induced by AAS use and increased atherosclerosis remains to be established.

In women, it is much clearer that androgen excess is linked to the burden of CVD risk factors. The most well studied of such risk factors is insulin resistance. It has been hypothesized that insulin resistance is a consequence of androgen effects. Excessive androgenic steroid exposure of female rats (Holmang et al 1990), normal females (Polderman et al 1994), transsexual females (Bjorntorp 1993), and patients with aplastic anemia (Woodard et al 1981) can lead to insulin resistance and may at least be partly reversed by estrogen administration (Andersson et al 1997). In keeping with this hypothesis, it was recently demonstrated that postmenopausal women with well-controlled type 2 diabetes that are insulin resistant show evidence of biochemical and clinical androgen excess, compared to non-diabetic, postmenopausal women with no known risk factors for diabetes other than obesity (Korytkowski et al 2005). Further evidence of a link between high androgen levels and CVD or CVD risk factors is observed in women with polycystic ovary syndrome (PCOS). Women with PCOS have a sustained exposure to high physiologic androgen levels. This condition is associated with endothelial dysfunction, obesity and metabolic abnormalities such as insulin resistance and dyslipidaemia, all of which may predispose PCOS women to premature atherosclerosis (Paradisi et al 2001; Krentz et al 2007).

However, despite the association between excess androgen in women and insulin resistance, CVD risk factors and angiographical evidence of atherosclerosis, there remains no evidence of increase cardiovascular mortality in these women. Additionally, in female-to-male transsexuals, testosterone therapy has not been linked to excess cardiovascular mortality or morbidity (van Kesteren et al 1997). Therefore, the question of the cardiovascular safety of androgen therapy in women remains unanswered. Based on existing observations, androgen use may increase insulin resistance in women with a consequent sequalae of cardiovascular effects however apart from the observations in women with PCOS and type 2 diabetes, and animal data suggesting androgens promote atherosclerosis in females, there is no solid data to support the claim (Figure 4). More work is necessary to establish a real link between androgens, insulin resistance and atherosclerosis in women.

Summary

From our current understanding of the effects of androgens on atherosclerosis, it has become apparent that the view androgens are harmful is too simplistic. This is made most evident by the erratic nature of the findings reported in cellular, animal and clinical studies. Clearly, much more work is needed in both the basic science and clinical arenas to

fully elucidate the effects of androgens on the development of atherosclerosis. For men, exogenous T treatment appears largely beneficial, at least in part via aromatization of T to estradiol, especially if physiological T levels are deficient. However, self-administered AAS usage remains a major CVD safety concern, especially given reported adverse lipid profile effects. As clinicians consider the use of T in management of symptoms associated with the aging male, there remains inconsistent and poorly reported data on cardiovascular risk of long-term T use. For T treatment in aging women, the current data would suggest androgen excess has adverse effects on CVD risk factors, especially in women with diabetes. In summary, there remains limited knowledge about exogenous androgen treatments in both men and women. Despite this, androgen use and abuse is increasing in our society, either for therapeutic or recreational reasons. Whether androgens adversely affect CVD in either men or women remains a contentious issue that is in desperate need of more research.

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