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Androgen actions on endothelium functions and cardiovascular diseases

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Abstract

The roles of androgens on cardiovascular physiology and pathophysiology are controversial as both beneficial and detrimental effects have been reported. Although the reasons for this discrepancy are unclear, multiple factors such as genetic and epigenetic variation, sex-specificity, hormone interactions, drug preparation and route of administration may contribute. Recently, growing evidence suggests that androgens exhibit beneficial effects on cardiovascular function though the mechanism remains to be elucidated. Endothelial cells (ECs) which line the interior surface of blood vessels are distributed throughout the circulatory system, and play a crucial role in cardiovascular function. Endothelial progenitor cells (EPCs) are considered an indispensable element for the reconstitution and maintenance of an intact endothelial layer. Endothelial dysfunction is regarded as an initiating step in development of atherosclerosis and cardiovascular diseases. The modulation of endothelial functions by androgens through either genomic or nongenomic signal pathways is one possible mechanism by which androgens act on the cardiovascular system. Obtaining insight into the mechanisms by which androgens affect EC and EPC functions will allow us to determine whether androgens possess beneficial effects on the cardiovascular system. This in turn may be critical in the prevention and therapy of cardiovascular diseases. This article seeks to review recent progress in androgen regulation of endothelial function, the sex-specificity of androgen actions, and its clinical applications in the cardiovascular system.

1   Introduction

Epidemiological studies suggest that males are at greater risk for cardiovascular disease when compared to age-matched females during their reproductive years.[1–3] In the Framingham cohort, men developed cardiovascular diseases (CVD) at more than twice the rate of women in ages younger than 60-years old.[4] This gender difference has been attributed to an estrogen protective effect in women, and/or an androgen detrimental effect in men. However, this hypothesis has not been confirmed in large prospective studies.[5–7] More significantly, a growing body of evidence is suggesting the opposite, that androgens exhibit a protective action on the cardiovascular system.[3] Multiple prospective clinical trials and a recent meta-analysis have shown that endogenous androgen level is correlated with mortality and risk factors of CVD.[3,8–10] A reduction in plasma testosterone may contribute to increased arterial stiffness, a risk factor for CVD.[11,12] Two recent retrospective studies revealed that testosterone therapy is associated with reduced obesity, fat mass, and waist circumference, while improving glycemic control, and reducing mortality. Several retrospective clinical trials in men with coronary artery disease or heart failure have reported improved cardiovascular function in men who received testosterone compared to those treated with placebo.[13]

Androgen withdrawal in men has been associated with decreased central arterial compliance,[14,15] and increased risk for CVD and mortality.[3,16] Androgens have been shown to inhibit atherosclerosis through inhibiting carotid intima-media thickness,[17] atheroma formation,[17,18] and immunomodulation of plaque development and stability.[19] Administration of testosterone has been demonstrated to produce coronary vasodilation, increase the angina threshold in men with coronary artery disease,[20] and induce both endothelium-dependent and independent vasorelaxation.[21] English, *et al.*[22] discovered that, in men with stable angina, treatment with a testosterone patch increased the time to

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ST-segment depression on exercise stress testing when compared to placebo. In studying symptomatic hypogonadal men, Malkin, *et al*.[23] found that, compared with placebo, intramuscular injection of testosterone decreased proinflammatory cytokines (TNFα, IL-1β) and total cholesterol, while it increased anti-inflammatory cytokine IL-10, indicating a less atherogenic state. Moreover, a subjective and objective improvement in hypogonadal men with ischemic heart disease was observed when they were treated with testosterone, as compared to placebo treatment.[24] Taken together, this data suggests that the beneficial effects of androgens are mediated through favorable actions on lipid profile,[25,26] glycometabolism,[27] inflammation,[28] and haemostatic parameters.[14,26] This in turn translates into beneficial effects, including anti-angina,[22–24] anti-atherosclerosis, vasorelaxation,[21,29] and increased coronary blood flow.[30]

The understanding of cellular and molecular mechanisms of these beneficial androgen actions on the cardiovascular system will be critical for translation of androgen beneficial effects to the bedside. Since endothelial cells (ECs) and endothelial progenitor cells (EPCs) play a vital role in cardiovascular functions, this review is therefore focused on the current knowledge of androgen effects on ECs and EPCs along with the relevant intracellular mechanisms.

2 Biosynthesis and biological actions of androgens

In the mammalian system, there are two natural potent androgens: testosterone and dihydrotestosterone (DHT).[31] Both testosterone and DHT bind to the same androgen receptor (AR) in the cytoplasm of target cells, and DHT exerts higher affinity to the AR and stronger androgenic activity than testosterone.[32,33] Testosterone is the primary androgen and is mainly synthesized and secreted by the testes (> 95%) in males. The biosynthesis of testosterone initiates from cholesterol, involving five enzymatic reactions in the process: cholesterol 20,22-desmolase, 3β-hydroxysteroid dehydrogenases, 17α-hydroxylase, 17,20-desmolase, and 17β-hydroxysteroid dehydrogenase.[31] Testosterone can be converted to DHT under the actions of 5alpha-reductase (5αRD) isozymes, mainly in peripheral tissues. Testosterone can also be converted to estrogen by aromatase in the cytoplasm.[34] Testosterone plays an essential role in the anabolic process and androgenic effects in the male,[35,36] while DHT is critical in the formation of male external genitalia, prostate development and growth, and hair growth.[37] Interestingly, endothelium of the cerebral blood vessels also expresses sex steroid receptors and enzymes that metabolize sex steroids. Gonzales, *et al*.[38] found that estrogen receptor

(ER) alpha, AR, 5αRD-2 and aromatase were expressed in the endothelium of cerebral arteries, suggesting that local production of 17β-estradiol and DHT can occur within the cerebral arteries. The expression of aromatase that converts testosterone to 17β-estradiol alters the local balance of androgenic and estrogenic influences. Thus, the activity of cerebral vessels is affected by circulating sex hormones as well as locally produced sex steroids. The differential expression of hormone effectors in the vascular system may have important implications in the gender differences of cerebrovascular physiology and pathophysiology.[39]

The biological actions of androgens are mainly mediated through binding to the AR, the classical genomic pathway (see Figure 1). Androgen binding to the AR results in an AR conformational change that promotes the dissociation of chaperone proteins and facilitates receptor dimerization, nuclear transportation, phosphorylation, and DNA binding.[40] Upon the recruitment of co-regulators and general transcription factors, the transcription of target-genes is either induced or inhibited, leading ultimately to changes in androgen-target gene expression, and cellular or biological structures and functions.[32,41] This process usually takes hours before resulting in biological changes in target cells.

In addition to this classical genomic pathway, a rapid nongenomic pathway that is independent of DNA transcription has been demonstrated for androgen actions.[42–44] This nongenomic pathway of androgen actions may occur through multiple receptors. Androgens may activate cyclic adenosine monophosphate (cAMP) and protein kinase-A (PKA) through the sex hormone binding globulin (SHBG)/SHBG receptor complex,[45] or increase intracellular $Ca2^+$ levels through a G-protein coupled receptor non-voltage-gated $Ca2^+$ channel couple mechanism.[46–48] The elevation of intracellular calcium activates multiple signal transduction cascades, including PKA,[46,49,50] Protein kinase C,[51–53] and Mitogen-Activated Protein Kinase (MAPKs),[53–55] leading to a modification of AR activity and other transcription factors. AR may also interact with the intracellular tyrosine kinase C (Src), triggering cSrc activation and subsequent biological actions. The rapid stimulation of second messenger cascades by androgens via the nongenomic pathway may ultimately result in biological functions through posttranslational modulation of AR or other transcription factors. Since these posttranslational modifications do not involve gene transcription and new protein biosynthesis, it may take only seconds or minutes to occur. AR can also be activated by various growth factors, such as epithelial growth factor (EGF) and insulin-like growth factor-1(IGF-1), in the absence of its cognate ligands, which may also alter AR activity via posttransla-
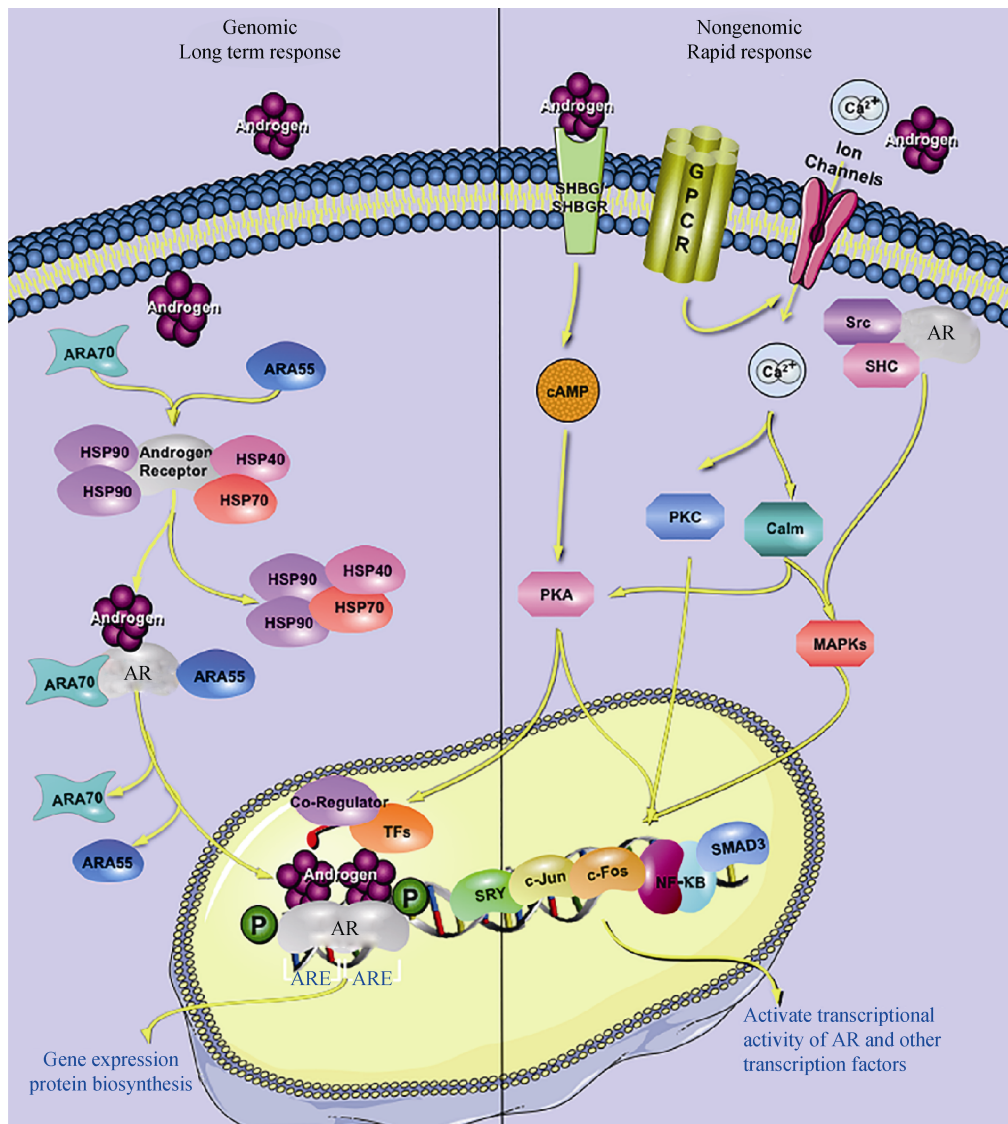
Figure 1.   The androgen signal pathways. The steroidal androgens, testosterone and DHT, mediate their biological effects predominantly through genomic pathway by binding to the AR and translocation into the nucleus, thereby facilitating the ability of AR to bind to its cognate response element, and recruiting coregulators to regulate the expression of target genes (left side). The nongenomic stimulation of second messenger cascades by androgens exerts biological effects through modulation of the transcriptional activity of AR or other transcription factors (right side). AR: androgen receptor; ARA: androgen receptor activator; ARE: androgen response element; cAMP: cyclic adenosine monophosphate; DHT: dihydrotestosterone; GPCR: G-protein coupled receptor; HSP: heat shock protein; MAPKs: mitogen-activated protein kinases; PKA: protein kinase A; PKC: protein kinase C; SHBG: steroid hormone binding globulin; SHBGR: SHBG receptor; Src: tyrosine kinase C; SHC: Src homology 2 domain-containing.

tional modification.[32] Whether the biological activity of androgens is mediated through the genomic or nongenomic pathway depends on cell type and AR-ligand.[56] The identification of molecules related to androgen-AR functions may facilitate the understanding of the cellular and molecular mechanisms of androgen actions.

3   Effects and mechanisms of androgens in the endothelium

Endothelium damage is thought to be an initiating step in formation of blood clotting, plaques, and atherosclerosis. It causes an imbalance between vasodilating and vasoconstricting substances produced by or acting on the vascular wall, resulting in increased arterial stiffness.[57–59] With mounting knowledge in vascular homoeostasis, EPCs, especially circulating EPCs, are considered an indispensable element for the reconstitution and maintenance of an intact endothelial layer.[60] The number and function of EPCs are correlated inversely with cardiovascular risk factors, and an increased level of EPCs is associated with a reduced risk of death from cardiovascular causes.[61] The deficiency of EC

and EPCs indicates an inadequate restoration of endothelium in pathological conditions that are underlying the development and progression of CVD. Since the endothelium plays a critical role in the cardiovascular system, the modulation of endothelial functions by androgens may be a promising mechanism for mediating androgen actions on cardiovascular physiology and pathophysiology.

3.1 Androgen regulation of EC proliferation and its modification by estrogens

Both DHT and testosterone have been shown to regulate cell proliferation and function via either a classical genomic pathway or nongenomic pathway in endothelial cells from a variety of origins.[62] Somjen, *et al*.[63,64] demonstrated that DHT at doses ranging from 3 to 3000 nmol/L produced a dose-dependent stimulation of [³H] thymidine incorporation and an activation of creatine kinase and MAPKs in a human endothelial cell line, ECV304. These androgen effects were blocked by the addition of flutamide, an AR-antagonist, indicating an AR-related mechanism. We have recently shown that both DHT and testosterone at doses within physiological range stimulated cell growth via an AR-VEGF (vascular endothelial growth factor)/cyclins mediated pathway in human primary aortic endothelial cells (HAECs).[65] Both DHT and testosterone produced a time- and dose-dependent increase in viable cell number and DNA biosynthesis in HAECs. This effect was blocked by the concomitant administration of casodex, a specific AR-antagonist, and by knockdown of AR using specific small interfering RNAs. This androgen action is mediated through upregulation of VEGF expression (Figure 2), which then upregulates cyclin expression in an autocrine fashion, resulting in the stimulation of cell proliferation.[65] Although 5α-reductase isozymes are expressed in HAECs, the effect of testosterone on HAEC proliferation is independent of DHT and estradiol since the testosterone effect was not blocked by dutarsteride, a specific 5α-reductase inhibitor that blocks the conversion of testosterone to DHT (Figure 3). In addition, 17β-estradiol does not stimulate cell proliferation in HAECs originated from males (Figure 4).[65] More recently, Campelo, *et al*.[66] reported that testosterone via a membrane-AR dependent mechanism enhanced cell growth through direct action on endothelial nitric oxide (NO) production in female rat ECs. Regardless of the exact mechanism by which androgen enhances EC growth, the stimulation of EC proliferation by direct androgen actions in the vascular system should assist in repair of endothelial injury and prevent endothelium impairments and dysfunction – a primary risk factor of vascular stiffness, hypertension, and atherosclerosis.

It is well known that ECs possess significant heterogeneity in the vascular system. Furthermore, the endothelium represents a group of small enterprises of cells located
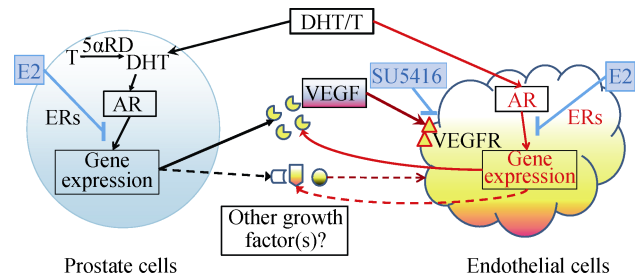

Figure 2. Illustration of androgen regulation of ECs via autocrine and paracrine mechanisms and its modification by estrogens. Androgens, T and DHT, regulate EC growth and functions through the regulation of gene expression of VEGF and other growth factors either directly in ECs (autocrine) or indirectly in other androgen-target cells such as prostate epithelial cells (paracrine). AR: androgen receptor; DHT: dihydrotestosterone; ECs: endothelial cells; ERs: estrogen receptors; E2: estrogens; SU5416: a specific VEGFR antagonist; T: testosterone; VEGF: vascular endothelial growth factor; VEGFR: VEGF receptor; 5αRD: 5alpha-reductases.
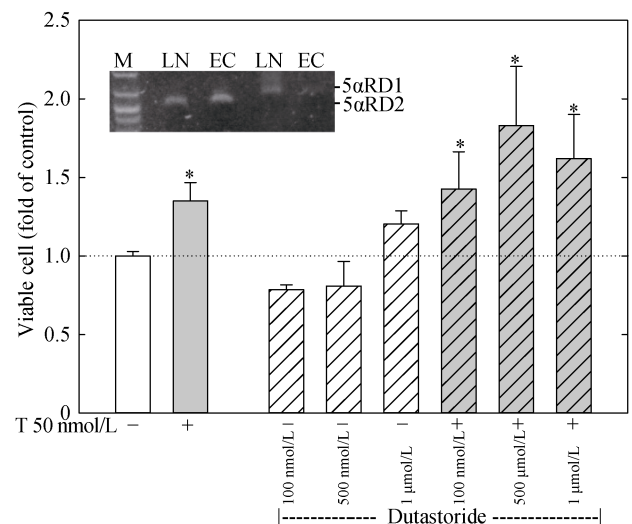
Figure 3. Dutarsteride fails to affect T stimulation of ECs growth. HAECs were plated in 96-wells and treated with or without T (50 nmol/L) and various doses of dutarsteride, a specific 5alpha-reductase inhibitor, for 48 h. The number of viable cells was determined. The insert shows a RT-PCR analysis of 5αRD1 and 5αRD2 expression in ECs and LN. *$P < 0.05$ compared to untreated control. ECs: human aortic endothelial cells; LN: LNCaP prostate cancer cells; M: makers; T: testosterone; 5αRD1/2: 5alpha-reductase type 1 or type 2.

within blood vessels of different tissues where each enterprise is uniquely adapted to meet the demands of the underlying tissue.[67] It has been shown that AR is not expressed in the ECs from microvessels of some organs such as the prostate gland and skin, and androgen is therefore unable to stimulate EC growth directly via AR.[68–70] However, androgen is able to affect EC proliferation indirectly through a paracrine fashion (Figure 2). By using prostate cancer cells
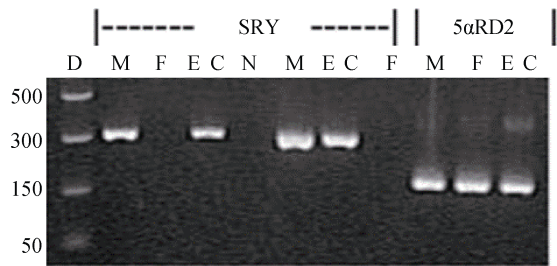
Figure 4. The determination of male originality of ECs by PCR analysis. Genomic DNA was isolated from M, F, or ECs and subjected to PCR amplification of the SRY gene (two specific primer pairs), a specific Y chromosome gene, and the 5alpha-reductase-2 gene (one specific primer pair). PCR products were analyzed by agarose gel electrophoresis. ECs: human aortic endothelial cells; F: females; Lane D: DNA size markers, M: males; N: negative control; 5αRD2: 5alpha-reductase type 2.

and ECs from mouse skin, we have demonstrated that androgen was able to upregulate VEGF expression in prostate cancer cells, which was secreted from these cancer cells and stimulated EC proliferation.[70,71] This paracrine action may be an important mechanism in synchronizing angiogenesis and tumor growth in tumor progression.

It is worthwhile to note that both direct and indirect androgen actions on EC proliferation (see Figure 2) may be modulated by other hormones such as estrogens.[70,71] We have shown that estrogens via ERs produce a ligand, receptor-isoform and cell specific modulation of androgen actions in multiple systems.[72–75] In ECs that express both AR and ERs, estrogens produce an ER-dependent modification of androgen actions on gene expression and cell proliferation in an ER-ligand specific manner.[71] On the other hand, in ECs that do not express AR, estrogens were able to modulate androgen-induced paracrine stimulation of cell growth via modification of androgen actions in paracrine cells.[70] Although VEGF as either an autocrine or paracrine hormone is the major factor in stimulating EC proliferation, other factors that remain to be elucidated may be involved in the androgen-estrogen regulation of gene expression and cell functions in ECs (see Figure 2).[70,76] The concept of ER-ligand, ER-isoform, and cell specific modulation of androgen actions by estrogens may have significant application for anti-androgen therapy of prostate cancer by estrogen analogs to maximize anti-tumor activity and to minimize cardiovascular side effects,[72,77,78] as well as for understanding the biology of sex steroid interaction in cardiovascular physiology and pathophysiology.[79,80]

3.2 Androgen regulation of EPC proliferation

The role of androgens in the cardiovascular system may also be displayed through regulation of EPC growth and functions (Figure 5). EPCs derived from bone marrow play an important role in vascular repair, angiogenesis, and replacement of damaged endothelial cells of blood vessels.[57–59] The circulating levels of EPCs correlate inversely with cardiovascular risk factors, and an increased level of EPCs is associated with a reduced risk of death from cardiovascular causes.[61,81,82] Moreover, EPC infusions may improve the outcome of the patients with cardiovascular diseases.[61,83,84] It is interesting to note that circulating EPC levels are correlated with plasma androgen concentrations.
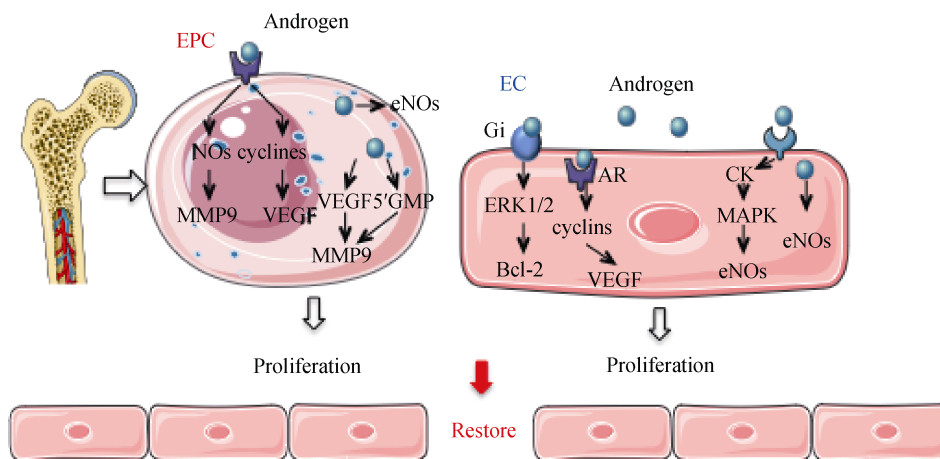
Figure 5. Potential mechanisms of androgens in regulating EC and EPC proliferation. In ECs, androgen diffuses into the cell directly activating a cascade of signaling creatine kinase and MAPK. The AR ligand may upregulate VEGF and cyclins through the genomic pathway. Androgen may also induce eNOS synthesis. In EPC, androgens may either upregulate VEGF and mitotic cyclins or increase the expression of MMP-9 and NO in the cytoplasm. Androgen activates a cascade of signaling resulting in increased NO and VEGF. AR: androgen receptor; CK: creatine kinase; EC: endothelial cells; EPC: endothelial projenitor cells; ERK1/2: extracellular signal-regulated kinase 1/2; eNOs: endothelial nitric oxide synthase; Gi: inhibitory regulative G-protein; MAPK: mitogen-activated protein kinases; MMP9: matrix metallopeptidase 9; NOs: nitric oxide synthase; VEGF: vascular endothelial growth factor.

A few of studies have demonstrated that, compared to normal controls, the circulating levels of EPCs were lower in patients with hypogonadotrophic hypogonadism, a condition with low plasma androgen levels, which was restored by testosterone replacement therapy (TRT).[85,86] Furthermore, AR is detected in human EPCs, and androgens via AR are able to stimulate EPC proliferation, migration, and colony formation.[85,87] Recently, it has been suggested that androgens regulate EPC proliferation and adhesion through the phosphatidylinositol 3-OH kinase (PI3K)/Akt pathway.[88] The effects of androgens on EC and EPC proliferation and function may be a significant factor mediating the beneficial actions of androgens in the cardiovascular system in males, and may explain findings that low levels of circulating androgens are associated with increased CVD morbidity and mortality in males.[88]

3.3 Androgens on endothelium-related vasodilatation and vasoconstriction

Endothelial tissue also plays a role in the regulation of vasoconstriction and vasodilatation, and hence the control of blood pressure. Although many studies have reported that androgens attenuate resistance of arteries predominantly by activation of K^+ channels and/or blockade of Ca^{2+} channels in vascular muscle cells, a growing number of studies have revealed that androgens induce vasodilation in resistance vessels mediated through endothelium-derived NO (see Figure 6).[89,90] Studies on *in vitro* ECs have found that dehydroepiandrosterone (DHEA) rapidly increased the expression of endothelial nitric oxide synthase (eNOS) and the activity of extracellular-signal-regulated kinases 1/2 (ERK1/2) via nongenomic pathway, which may increase NO secretion, leading to an increased flow-mediated dilation *in vivo*.[91] Moreover, Simoncini, *et al*.[92] revealed that DHEA triggered a G protein coupled receptors-ERK1/2-MAPK cascade to regulate eNOS protein synthesis in human umbilical venous endothelial cells (HUVECs); DHEA also initiated a prolonged genomic action to increase eNOS protein and NO synthesis. Goglia, *et al*.[93] revealed that both testosterone and DHT when administered at physiological concentrations,
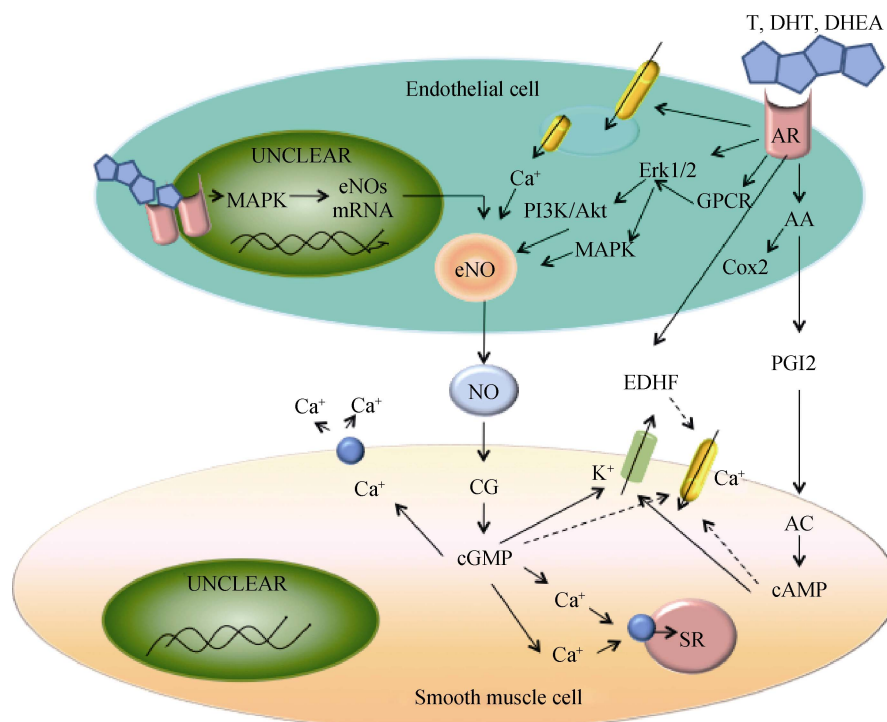
Figure 6. The mechanisms of androgen-induced endothelium-dependent VSM relaxation. T binds to endothelial cytosolic AR, leading to an activation of MAPK, gene transcription, endothelial cell proliferation, and eNOS production. Androgen also binds to endothelial surface membrane ARs, which is coupled to an increased Ca^{2+} release from the endoplasmic reticulum and a stimulation of the MAPK/Akt pathway, leading to an activation of eNOS and NO production. NO diffuses into VSM cells, binds to CG, and increases cGMP production. cGMP causes VSM relaxation by decreasing $[Ca^{2+}]i$ and myofilament sensitivity to Ca^{2+}. Endothelial AR activation may also activate COX2 and increase PGI2 production. PGI2 activates prostacyclin receptors in VSM, resulting in AC activation and cAMP production. cAMP causes VSM relaxation by mechanisms similar to cGMP. AR activation may also increase the production of EDHF, which activates K^+ channels and causes hyperpolarization and inhibition of Ca^{2+} influx via Ca^{2+} channels, leading to a VSM relaxation. Solid lines depict stimulatory effects and dashed lines indicate inhibitory effects. AA: arachidonic acid; AC: adenylate cyclase; Akt: protein kinase B; APK: mitogen-activated protein kinases; AR: androgen receptor; CG: guanylate cyclase; cGMP: cyclic guanosine monophosphate; COX2: cyclooxygenases; DHEA: dehydroepiandrosterone; DHT: dihydrotestosterone; EDHF: endothelium-derived hyperpolarizing factor; eNO: endothelial nitric oxide; ERK1/2: extracellular signal-regulated kinase 1/2; GPCR: G-protein coupled receptor; NO: nitric oxide; PGI2: prostacyclin; PI3K: phosphatidylinositol 3-OH kinase; SR: sarcoplasmic reticulum; T: testosterone; VSM: vascular smooth muscle.

produced dose-dependent genomic and nongenomic actions in HUVECs. Androgens rapidly increased endothelial synthesis of NO in HUVECs, which is mediated through a rapid recruitment of ERK1/2 and PI3K/Akt cascades.[93] It is worthy to note that both the genomic and nongenomic actions of testosterone and DHT were mediated via AR as evidenced by the fact that they were blocked by flutamide, a specific AR-antagonist, and the fact that testosterone effects were also mediated through conversion to estradiol.[93] Similar androgen effects have been reported by Yu, *et al*.[94] in HAECs.

Intracellular free calcium concentration is considered an important physiological regulator of NO production and vasodilatation. Testosterone binds to specific membrane receptor (s) in endothelial cells and stimulates Ca^{2+} release from the endoplasmic reticulum, eNOS activity, and NO production. NO activates guanylate cyclase and increases cyclic guanosine monophosphate (cGMP) production, causing vascular smooth muscle (VSM) relaxation by inhibiting Ca^{2+} influx and stimulating Ca^{2+} extrusion. Testosterone may also increase the release of endothelium-derived hyperpolarizing factor (EDHF), open large-conductance K^+ channels, cause VSM hyperpolarization and inhibit Ca^{2+} influx via Ca^{2+} channels.[95] This testosterone effect may be mediated, at least in part, via its aromatization to 17β-estradiol.[96,97]

It has been reported that the androgen effect on vasodilation may be mediated through modulation of arachidonic acid metabolism although the contribution of arachidonic acid metabolites to cardiovascular function is still controversial.[98–100] Marrachelli, *et al*.[98] found that testosterone induced an acute vasorelaxation that is dependent on endothelium-derived eNOS, iNOS, and prostacyclin. It has been shown that androgen induction of cyclooxygenase-2 was accompanied by a preferential increase in prostacyclin synthesis in regulation of vascular homeostasis without an impact on thromboxane A2 (TXA2) production,[101,102] which would favor vasodilation. Most recently, androgens have been demonstrated to induce vasodilation via induction of hydrogen sulphide, a gaseous mediator involved in cardiovascular homeostasis, mediated through an AR-hsp90-cystathionine-γ lyase pathway.[103] Moreover, androgens have also been shown to increase arachidonic acid metabolites such as 20-hydroxyeicosatetraenoic acid and TXA2, leading to an endothelium-dependent vasoconstriction,[99,100,104] which results in a detrimental effect on the cardiovascular system (Figure 6).

Androgens may also modulate the production and secretion of endothelium-derived contracting factors such as endothelin-1 (ET-1) and TXA2 to regulate vascular functions.

A cross-sectional study revealed a positive association between plasma ET-1, T, and blood pressure in postmenopausal women.[105] Meanwhile, studies in female-to-male transsexual subjects who received large doses of testosterone have shown high plasma levels of ET-1 in these subjects.[106] However, the association between androgens and ET-1 production is still obscure. It has been reported that plasma endothelin level was increased in males with hypogonadism,[107] and seemed to decrease after testosterone administration,[108] suggesting that androgens may decrease ET-1 production.

3.4 Androgens on endothelium-related inflammation

It has been documented that inflammation is involved in the process of endothelial dysfunction and impairment, and that androgens play a role in both pro-inflammation and anti-inflammation in the cardiovascular system.[90,109] Clinical studies have suggested that the elevation of inflammatory biomarkers is associated with endothelial cell dysfunction and CVD, and that there is a positive association between circulating androgens and inflammatory biomarker levels in humans.[1,2,105] Androgens have been shown to produce a dose-dependent induction of vascular cell adhesion molecule-1 (VCAM-1) expression and monocyte cell adhesion and to potentiate tumor necrosis factors (TNF)-induced VCAM-1 and cell adhesion molecule E-selectin expression and monocyte cell adhesion in ECs, which are presumably mediated through the activation of the transcriptional nuclear factor-kappa B (NF-κB).[110–114] In contrast, androgens have also been reported to produce anti-inflammatory effects by promoting endothelial cell survival, reducing endothelial expression of pro-inflammatory markers, and inhibiting proliferation and intimal migration of vascular smooth muscle cells. Norata, *et al*.[115] reported that incubation of human ECs with DHT or testosterone reduced the inflammatory response induced by TNF-α and lipopolysaccharide, which was mediated by AR and resulted in a decreased expression of VCAM-1, intercellular adhesion molecule-1 (ICAM-1), IL-6, monocyte chemoattractant protein-1 and proteases. These androgen effects were probably mediated through inhibition of the NF-κB signaling pathway. Furthermore, androgens have been reported to inhibit the expression of caspase-3, caspase-9, and phosphor-p38 MAPK induced by oxidative stress and attenuate cell apoptosis in HUVECs.[111,112,116] Although the reasons for these apparent discrepant findings are unclear, differences in the origins of ECs, the gender of donors, and the relevant variations of intrinsic molecules such as hormone receptors and hormone metabolic enzymes may account, at least in part, for the underlying mechanisms.

3.5   Androgens on endothelium-related anticoagulant

Tissue plasminogen activator (tPA), plasminogen activator inhibitor type 1 (PAI-1), and tissue factor pathway inhibitor (TFPI) are critical molecules released from ECs in the regulation of anticoagulant activity. Studies in humans have revealed that total and free testosterone levels are independent predictors of plasma TFPI concentrations and a low testosterone level in elderly men is associated with a low free TFPI antigen and a shortened initiation phase of TF-induced coagulation.[117] Moreover, human serum testosterone levels are positively correlated with tPA levels and negatively correlated with PAI-1 and clotting factor VII concentrations.[118–120] These findings are further supported by *in vitro* cell culture studies in which testosterone has been shown to increase TFPI and tPA expression while inhibiting PAI-1 secretion in the endothelium.[121] This data supports the concept that androgens possess anticoagulant activity, resulting in a protective action on the cardiovascular system.

4   Gender specificity of androgen actions in endothelium

Gender disparity in the incidence and progression of CVD indicates an intrinsic sexual dimorphism in the cardiovascular system, which is determined not only by gender-related differences in sex steroid levels, but also by gender-specific tissue, cellular, and molecular differences that mediate gender-specific responses. The effect of sex steroids on the cardiovascular system are complex via either direct or indirect influences on a multitude of cardiovascular biological processes, often in a gender-specific manner.[79] Accumulating evidence has demonstrated that both androgens and estrogens regulate various endothelium biological processes in a sex-dependent fashion.[79,113,122,123] Regarding the actions of androgens, both DHT and testosterone have been shown to induce gene expression and cell proliferation via multiple molecular mechanisms in the ECs of males but not females.[65,123,124] Maddox, *et al*.[125] found that the vascular rings from female rat aorta were much less sensitive and less contractile to prostaglandin F2 alpha (a vasoconstrictor) stimulation than those from male rats, which was endothelium-dependent and affected by pretreatment with testosterone and estradiol. McCrohon, *et al*.[113] reported that DHT increased human monocyte adhesion to vascular endothelium, mediated at least in part through an AR-dependent upregulation of VCAM-1 expression in ECs originating from males but not females. This sex-specific androgen effect may be related to an approximately two to five fold higher AR expression in ECs from males compared to those

from females.[112,126] However, a recent study by Annibalini, *et al*.[114] failed to demonstrate any gender-specific androgen action on TNF-α stimulated inflammatory responses. They showed that the inflammatory effect of TNF-α on VCAM-1, ICAM-1, and E-selectin gene expression and on cell adhesion was amplified by co-administration of testosterone or DHT while 17β-estradiol had no effect in HUVECs from both males and females under identical culture conditions. In sum, accumulating evidence supports the concept that androgens produce direct biological functions in ECs in a gender-specific manner though there is some inconsistent data. Further investigations are warranted to delineate the underlying reasons for these conflicting reports and to elucidate the clinical application of sex-specific endothelial actions of androgens.

5   Summary and prospective

The recent flourish of research into androgens in the cardiovascular system has made significant progress in revealing androgen effects and mechanisms. Findings suggest that androgens via genomic and/or nongenomic pathways possess significant effects on cell proliferation and migration, vessel contractility, and pathogenic processes such as inflammation, atherosclerosis, and clotting in the endothelium. Although a number of studies indicate that androgens drive beneficial effects in the endothelium, reports espousing the opposed conclusion are equally presented. The reasons for these apparent discrepancies are unclear although sex specificity of androgen actions and heterogeneity of ECs or endothelium are major factors to be considered. To further cloud the issue, the mechanisms of direct androgen actions on ECs or endothelium are poorly understood. Although it is clearly documented that androgens are able to interact with AR to modulate EC function, proliferation, and gene expression via the classical genomic pathway, there is also evidence for nongenomic androgen actions via various second messenger pathways or cascades without involvement of AR. Furthermore, significant species and sex differences, in addition to temporal and spatial variations of AR expression in ECs, EPCs, or endothelium have been documented. These may account, at least in part, for the conflicting androgen actions observed in the endothelial system.[62,70] Further studies with particular attention to experimental conditions that consider intrinsic EC differences such as the temporal and spatial origin of endothelium, sex and species specificity, and genetic variation in molecules along androgen signal pathways are necessary to clarify these discrepancies.

The role of androgens in cardiovascular system in humans is equally controversial. Although TRT of male hy-

pogonadism has been available since 1939, the benefits and safety of such therapy have become a major debate recently. Numerous meta-analyses suggest that most data supports the concept that endogenous normal testosterone and TRT, when used in physiological concentrations, displays favorable cardiovascular impact without an increased risk of adverse cardiovascular events or mortality, in both men and women.[13,127–130] However, two recent studies have raised new concerns about cardiovascular risk with TRT. The TOM trial, designed to investigate the TRT effect on frailty in elderly men, was prematurely terminated due to an increased incidence of cardiovascular events in the treatment arm.[131] A retrospective study, which was designed to assess the association between TRT and all-cause mortality, myocardial infarction, or stroke among male veterans, and to determine whether this association is modified by underlying coronary artery disease, has shown an increased risk of adverse outcomes in men treated with testosterone.[132] A most recent meta-analysis including data from 1940 to 2014 have found only four articles, including the two mentioned above, that indicated increased cardiovascular risks with TRT. On the other hand, dozens of reports, including randomized clinical trials and meta-analyses, suggest beneficial effects of endogenous normal androgens and TRT. While the reasons for this discrepancy are not clear, multiple factors should be considered such as sex difference, hormone interactions, routes of drug administration, androgen preparation, and individual genetic variations related to androgen pharmacokinetics and pharmacodynamics. The influence of sex and hormone milieu on androgen actions has been well documented as discussed above. The preparation and route of testosterone administration have also shown significant impact on androgen actions. Oral testosterone administration results in increased cardiovascular risks when compared to intramuscular and transdermal administration.[130] One explanation for discrepancies in these data is the individual genetic polymorphisms of participants in these trials, which may affect the disposition and efficacy of androgens. Individuals treated with the same regimen of TRT have shown large intra- and inter-individual variations in serum total testosterone and free testosterone levels, partially due to polymorphisms of genes related to testosterone disposition such as SHBG, phosphodiesterase (PDE7B) and uridine 5'-diphospho-glucuronosyltransferase.[133–135] Moreover, genetic polymorphisms along androgen signal pathways have been found to have a significant impact on androgen efficacy.[136] Genetic polymorphisms of AR gene have been demonstrated to alter the efficacy of androgens,[137,138] and may serve as a useful genetic marker(s) for the assessment of cardiovascular risk.[139] With advances in molecular ge-

netic technology and the emphasis of personalized/precision medicine, more genetic polymorphisms related to androgen disposition and efficacy will be revealed. Long-term, well-designed prospective trials with large cohorts and careful consideration of individual genetic polymorphisms are required to verify the cardiovascular efficacy/safety of androgens in both men and women. Until then, current data strongly suggests a beneficial relationship between normal androgens and cardiovascular health, a finding that remains to be widely appreciated.

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