

# Role of androgens in cardiovascular pathology

Dimitry A Chistiakov<sup>1</sup>  
 Veronika A Myasoedova<sup>2</sup>  
 Alexandra A Melnichenko<sup>2</sup>  
 Andrey V Grechko<sup>3</sup>  
 Alexander N Orekhov<sup>2,4</sup>

<sup>1</sup>Department of Neurochemistry, Division of Basic and Applied Neurobiology, Serbsky Federal Medical Research Center of Psychiatry and Narcology, Moscow, Russia; <sup>2</sup>Laboratory of Angiopathology, Institute of General Pathology and Pathophysiology, Moscow, Russia; <sup>3</sup>Federal Scientific Clinical Center for Resuscitation and Rehabilitation, Moscow, Russia; <sup>4</sup>Institute for Atherosclerosis Research, Skolkovo Innovative Center, Moscow, Russia

**Abstract:** Cardiovascular effects of androgen hormones in normal and pathological conditions can lead to either positive or negative effects. The reason for this variation is unknown, but may be influenced by gender-specific effects of androgens, heterogeneity of the vascular endothelium, differential expression of the androgen receptor in endothelial cells (ECs) and route of androgen administration. Generally, androgenic hormones are beneficial for ECs because these hormones induce nitric oxide production, proliferation, motility, and growth of ECs and inhibit inflammatory activation and induction of procoagulant, and adhesive properties in ECs. This indeed prevents endothelial dysfunction, an essential initial step in the development of vascular pathologies, including atherosclerosis. However, androgens can also activate endothelial production of some vasoconstrictors, which can have detrimental effects on the vascular endothelium. Androgens also activate proliferation, migration, and recruitment of endothelial progenitor cells (EPCs), thereby contributing to vascular repair and restoration of the endothelial layer. In this paper, we consider effects of androgen hormones on EC and EPC function in physiological and pathological conditions.

**Keywords:** cardiovascular disorders, atherosclerosis, androgens, testosterone therapy, risk factors

## Introduction

Androgens are a group of steroid hormones mostly represented by steroids produced by the testes and adrenal cortex. The main examples of androgens include testosterone, androstenedione, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate. Androgens play a key role in the establishment and maintenance of male properties in vertebrates, including sex activity and formation of secondary sex features in males.

In cardiovascular pathology, males have a higher risk of developing the disease during the reproductive period in comparison with females of the same age.<sup>1</sup> The sex-related difference in resistance to cardiovascular disease was hypothesized to be related to the cardioprotective role of estrogen hormones in females and harmful effects of androgen steroids in males.<sup>2</sup> This hypothesis was not initially supported by large-scale follow-up studies.<sup>3</sup> In contrast, many studies indicated the positive role of androgens in cardiovascular protection.<sup>1</sup> Furthermore, follow-up clinical studies showed that plasma concentrations of androgens were associated with various independent cardiovascular risk factors and mortality from cardiovascular pathology.<sup>1,4-6</sup> For example, decrease in blood testosterone was found to show a positive correlation with the elevation of arterial stiffness (an independent marker of cardiovascular pathology).<sup>7</sup> In a recent

Correspondence: Alexander N Orekhov  
 Laboratory of Angiopathology,  
 Institute of General Pathology and  
 Pathophysiology, Baltiyskaya st 8,  
 Moscow 125315, Russia  
 Tel +7 903 169 0866  
 Email a.h.opexob@gmail.com

meta-analysis, cardiovascular risk, in men with coronary artery disease (CAD) and heart failure who took testosterone as a drug compared with the control (placebo) group, was decreased.<sup>8</sup> Androgen steroids were found to attenuate atherogenesis in males via several mechanisms, including modulatory effects on lesion progression and plaque vulnerability to thrombosis,<sup>9</sup> decreasing fat accumulation in the arterial intima media,<sup>10</sup> and reducing carotid intima media thickness.<sup>11</sup> Treatment with testosterone was shown to induce arterial vasorelaxation in males affected with CAD.<sup>12,13</sup> In a randomized clinical trial, administration of testosterone in low doses was found to significantly improve cardiac function in men with stable angina when compared with the placebo group.<sup>14</sup> Treatment of hypogonadal men with testosterone led to reduced levels of inflammatory cytokines, decreased concentrations of total cholesterol but elevated interleukin (IL)-10, an anti-inflammatory cytokine.<sup>9</sup> Compared with the placebo group, regular injections of testosterone had beneficial effects on hypogonadal men affected with ischemic heart disease by improving mood and decreasing levels of total cholesterol and tumor necrosis factor (TNF)- $\alpha$ , a pro-inflammatory cytokine.<sup>15</sup> In cardiometabolic pathology, androgens exhibit pleiotropic positive effects by improving glucose metabolism,<sup>16</sup> serum lipid profile,<sup>17</sup> and hemostasis,<sup>18</sup> and inducing vasodilation.<sup>13</sup> Androgens are known to target muscle cells by promoting their growth and cardiomyocytes can respond to testosterone by increasing the cell size leading to cardiac hypertrophy.<sup>19</sup> In the vascular system, endothelial cells (ECs) and endothelial progenitor cells (EPCs) are primary targets of favorable actions of androgen hormones through the vascular lumen. We will discuss below the action of androgens on the cardiovascular system after reviewing the general role of androgens in physiological conditions.

## Biogenesis and biological actions of androgenic hormones

In mammals, two androgen hormones, testosterone and dihydrotestosterone (DHT) play a major role.<sup>20</sup> Both hormones interact with the single androgen receptor (AR). It has been found that testosterone binds to the AR with a stronger affinity than DHT.<sup>21</sup> Testosterone is the major sex hormone in males and is mainly produced by testes. Its synthesis is quite complicated and is converted from cholesterol by the action of five enzymes. Testosterone can then be transformed to DHT by 5 $\alpha$ -reductase (5 $\alpha$ RD) isozymes and the aromatase cytochrome P-450.<sup>22</sup> Testosterone is crucial in the control of catabolic reactions and general androgenic actions in males.<sup>23</sup> DHT is hardly involved in penis and prostate formation.<sup>24</sup>

In the cerebral arteries, ECs produce receptors that bind steroid hormones and steroid-metabolizing enzymes. For example, estrogen receptor (ER) $\alpha$ , 5 $\alpha$ RD isoform 2, and aromatase were found to be expressed by cerebral endothelium. Aromatase transforms testosterone to 17 $\beta$ -estradiol, a female sex hormone, thereby affecting the local equilibrium between androgens and estrogens in the brain.<sup>25</sup> It has been shown that the function of brain vessels can be influenced by local steroid hormones as well as by circulating steroids.<sup>26</sup>

AR mainly mediates the physiological effects of androgenic steroids. The binding of an androgen to AR leads to structural changes in the receptor molecule and causes the dissociation of chaperons that are bound to the inactive receptor. This induces AR dimerization, nuclear trafficking, and receptor activation through phosphorylation and promotion of transcriptional activity.<sup>27</sup> Described above was the DNA-dependent pathway of the androgen-mediated control of gene expression. The non-genomic mechanism of the action of androgens involves the modulation of numerous signaling pathways. One of them is the stimulation of cyclic adenosine monophosphate and protein kinase-A (PKA) via the sex hormone binding globulin (SHBG)/SHBG receptor complex.<sup>28</sup> This way leads to the elevation of cytoplasmic Ca<sup>2+</sup> concentration. Indeed, increase in intracellular Ca<sup>2+</sup> results in the stimulation of several signaling pathways, such as the PKA-dependent pathway,<sup>29</sup> the protein kinase-C-mediated mechanism,<sup>30</sup> and mitogen-activated protein kinases (MAPKs),<sup>31</sup> that finally induce the activation of AR and other transcription factors. The DNA-dependent pathway is slow to take effect, requiring several hours, while the non-DNA-dependent mechanism is much faster (taking only seconds or minutes) and can lead to posttranslational modulation of AR and other transcriptional factors. Additionally, AR can be stimulated by several growth factors including insulin-like growth factor-1 and epithelial growth factor. Because androgen-dependent non-genomic modifications are not involved in gene expression, these modifications can be manifested quickly. Cell type and AR ligands modulate the physiological action of androgen hormones to select genomic or non-genomic mechanisms of action.<sup>32</sup>

## Role of androgen hormones in ECs

Endothelial dysfunction is the initial stage in multiple cardiovascular disorders such as atherosclerosis and atherothrombosis. The prevalence of vasoconstrictors or vasodilators results in the promotion of the arterial stiffness.<sup>33</sup> In this context, the presence and viability of EPCs was considered an essential factor for productive vascular repair and recovery

of the endothelial layer after vessel denudation.<sup>34</sup> Deficiency and loss of function of EPCs were observed to be inversely associated with various cardiovascular risk factors, while higher levels of EPCs correlate with decreased mortality from cardiovascular events.<sup>35</sup>

The deficiency of EPCs reduces the regenerative potential of vascular endothelium and can lead to the progression of cardiovascular pathology. Since androgens generally have a protective role on ECs, androgen administration may prove beneficial for the improvement of endothelial function and prevention of atherosclerosis.

## Role of androgens and estrogens on proliferation of ECs

Androgens, such as testosterone and DHT, are involved in controlling EC proliferation and function.<sup>36</sup> In cultured human ECs, administration of DHT was shown to increase cell growth, stimulate MAPKs and creatine kinase.<sup>37,38</sup> Treatment with flutamide, an antagonist of AR, inhibited DHT actions, suggesting the involvement of AR-associated pathway. In a primary culture of aortic ECs, androgens were shown to induce EC proliferation and viability through AR/vascular endothelial growth factor (VEGF)-dependent and cyclin-dependent mechanisms.<sup>39</sup> In cultured ECs, the activation of VEGF synthesis was followed by the stimulation of cyclin expression and finally resulted in cell proliferation.<sup>39</sup> In ECs of female rats, testosterone can bind to AR and stimulate nitric oxide (NO) production that in turn potentiates EC growth.<sup>40</sup> Therefore, androgens support EC growth and proliferation that are important for endothelial repair in vascular damage and/or endothelial dysfunction, ie, are the important factors of cardiovascular disease.

In vascular beds, the population of ECs is rather heterogeneous and depends on the location.<sup>41</sup> For example, AR is not expressed in microvessels of certain tissues, such as skin or prostate, thereby preventing androgen-dependent EC proliferation through primary binding to AR.<sup>42</sup> However, androgens are able to influence the function of those cells in a paracrine manner through the stimulation of liberation of VEGF. A recent study showed that DHT could induce VEGF production by prostate cancer cells that in turn activated proliferation of ECs.<sup>43</sup> This effect could be defeated by estrogens.<sup>44</sup> The mechanism of VEGF induction in cancer cells may be important for the formation of tumor neovessels and further tumor propagation. However, vascular endothelium expresses both AR and ER. Indeed, the function of ECs can be modulated by both androgens and estrogens. Estrogens exhibit the opposite effects on EC proliferation, supporting

the quiescence of endothelium through the inhibition of cyclin A production and inducing the transformation of testosterone to 17 $\alpha$ -estradiol, a female hormone.<sup>44,45</sup>

According to recent findings, VEGF can act on ECs in both autocrine and paracrine fashions. For instance, estrogens are able to influence the effects of androgens on cell growth in ECs that do not express AR in a paracrine manner.<sup>43</sup> However, other androgen/estrogen-dependent vasoactive agents that explain the complexity of vasodilatory/vasoconstrictor effects of sex hormones on vascular endothelium, may exist.<sup>39</sup>

## Effects of androgens on proliferation of EPCs

Androgen hormones also stimulate the function of EPCs. Bone marrow-derived EPCs were shown to be involved in vessel remodeling, vasculogenesis, and reparation of endothelial denudation in injured vascular regions.<sup>33,46</sup> Decreased EPC numbers in the cardiovascular system correlate with elevated cardiovascular risk, while increased EPC counts correlate with decreased risk of cardiovascular mortality.<sup>35,47</sup> Furthermore, cell therapy with EPCs was shown to have beneficial effects on people with cardiovascular disease.<sup>48,49</sup> Increased circulating EPC numbers were found to correlate with higher blood levels of androgens.<sup>49,50</sup>

Some studies also showed that patients with central hypogonadism, a syndrome characterized by low production of sex hormones, had decreased levels of EPCs.<sup>51,52</sup> Treatment with testosterone can improve the state of men with central hypogonadism.<sup>53</sup>

Expression of AR was found in human EPCs. The binding of androgen hormones to the AR activates EPC proliferation and migration.<sup>51</sup> Androgen steroids control the proliferation of EPCs via the PI3K/Akt signaling.<sup>53</sup> Androgen-dependent stimulation of EC and EPC proliferation may, therefore, be suggestive of the cardioprotective effects of these hormones through the stimulation of vascular repair.<sup>53</sup>

## Effects of androgens on the vascular tone

The role of ECs in the regulation of vascular tone is crucial. Androgens cause arterial vasodilation by inducing relaxation of vascular smooth muscle cells (VSMCs) and also contribute to vasorelaxation through the induction of NO formation by ECs.<sup>54</sup> It was shown that in cultured ECs, DHEA stimulated endothelial NO production by up-regulating expression of endothelial NO synthase (eNOS) and ERK1/2 signaling.<sup>55</sup> In the ERK1/2-dependent pathway, DHEA regulates G protein-coupled receptors-ERK1/2-MAPK signaling to activate

eNOS protein production. DHEA also increases the duration of eNOS expression.<sup>56</sup>

In human umbilical vein endothelial cells (HUVECs), testosterone and DHT were observed to rapidly stimulate NO release in a dose-dependent manner through the activation of PI3K/Akt and ERK1/2 mechanisms. Androgen-dependent NO production was inhibited by flutamide, an AR-antagonist, indicating that stimulatory effects of androgens on ECs are mediated by the AR.<sup>57</sup> Identical effects of androgen hormones were also observed in cultured human arterial ECs.<sup>58</sup>

To stimulate endothelial NO production, testosterone activates release of Ca<sup>2+</sup> from the endoplasmic reticulum that then increases eNOS activity and NO generation. NO, in turn, causes elevated cyclic guanosine monophosphate (cGMP) production by up-regulation of guanylate cyclase. In turn, cGMP decreases Ca<sup>2+</sup> influx to VSMCs leading to vasodilation. In addition, testosterone up-regulates the liberation of endothelium-derived hyperpolarizing factor, a vasodilator, which blocks the activity of Ca<sup>2+</sup> channels and activates large-conductance K<sup>+</sup> channels. Finally, this results in VSMC hyperpolarization and relaxation.<sup>59</sup>

Data about the involvement of androgens in vasorelaxation through mediating the activity of cyclooxygenases are still contradictory.<sup>60-62</sup> It was demonstrated that testosterone-dependent vasodilation involves the activation of eNOS, inducible NO synthase, and increased production of prostacyclin.<sup>61</sup> Testosterone up-regulates cyclooxygenase-2, which in turn leads to increased prostacyclin formation. However, thromboxane A2 does not contribute to androgen-dependent vasorelaxation.<sup>63</sup> Androgens can also stimulate relaxation of vessels through induction of hydrogen sulphide (H<sub>2</sub>S) via an AR-HSP90-cystathionine- $\gamma$  lyase pathway.<sup>64</sup> Cystathionine- $\gamma$  lyase can produce H<sub>2</sub>S from amino acid L-cysteine. H<sub>2</sub>S is a physiologically active gas that is involved in cell signaling causing various biological effects including vasorelaxation.<sup>65</sup> Decrease in cystathionine- $\gamma$  lyase activity and insufficient production of H<sub>2</sub>S by HUVECs was shown to be associated with preeclampsia.<sup>66</sup>

Androgens can also induce vasoconstriction by up-regulation of the synthesis of arachidonic acid intermediates, including thromboxane A2 and 20-hydroxyeicosatetraenoic acid.<sup>60,62</sup> Androgenic steroids can also modulate production of endothelin-1 (ET-1), an endothelium-derived vasoactive molecule that increases blood pressure. In postmenopausal women, a direct correlation was shown between blood levels of testosterone, ET-1, and vascular tone.<sup>67</sup> Female-to-male transsexuals who are treated with high doses of testosterone

have increased ET levels.<sup>68</sup> However, in hypogonadal men, plasma ET is increased and tends to decrease after treatment with testosterone, suggesting that androgen hormones can reduce ET-1 formation.<sup>69,70</sup>

## Effects of androgens on the myocardium

The growth-promoting effect of androgens on muscle cells is currently well known.<sup>71</sup> Anabolic androgens are used in clinical practice to accelerate healing of wounds, promote skeletal muscle growth, and treat cachexia. Due to their effects on skeletal muscles, these hormones are often misused, for instance, by bodybuilders. High doses of anabolic androgens can induce pathological processes, most importantly, cardiac hypertrophy.<sup>72</sup> Moreover, animal studies have provided evidence that supra-physiological doses of testosterone induce cardiac fibrosis and apoptosis.<sup>73</sup> Antiandrogenic therapy was shown to be beneficial for the treatment of induced cardiac hypertrophy in model rodents.<sup>74</sup> However, at physiological levels, androgens are more likely to have a protective effect on the myocardium: testosterone deficiency was shown to be associated with cardiovascular risk in humans, while treatment with testosterone had beneficial effects.<sup>75</sup>

Treatment of aging males with physiological testosterone doses is known to have positive effects on the lipid profile, thus protecting them from atherosclerosis-related cardiovascular disease.<sup>76</sup> Indeed, androgen dysregulation associated with aging generally results in altered lipid metabolism, increased body fat and obesity that represent well-known risk factors of cardiovascular disease, and in many instances, can be attenuated by testosterone replacement therapy.<sup>77</sup> Direct protective effects of testosterone on cardiomyocytes were studied in animal models. For instance, testosterone could protect rat myocardium from ischemia/reperfusion injury through stimulation of  $\alpha(1)$ -AR.<sup>78</sup> The protective effects of androgens can also be conveyed in an AR-independent manner, as was shown in a mouse model, where physiological doses of testosterone attenuated aging of cardiomyocytes.<sup>79</sup>

In summary, it can be concluded that androgens at physiological levels appear to have a protective effect on the myocardium and cardiovascular system in general. The precise mechanisms of these effects remain to be studied in detail, but it is likely that it is pleiotropic and includes both direct pathways mediated by AR and indirect actions via improving lipid profile, reducing hypertension, fat deposition, chronic inflammation and other cardiovascular risk factors.

## The role of androgenic hormones in endothelial inflammatory responses

Endothelial inflammatory activation and dysfunction are involved in the initial steps of vascular impairment, including further atherogenesis. Androgenic hormones were shown to act as pro-inflammatory and anti-inflammatory messengers in circulation.<sup>54</sup> Many studies show that increase of plasma levels of androgen hormones is associated with elevated levels of inflammatory signaling molecules in humans.<sup>80-82</sup> Correlation between endothelial expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) and endothelial selectin, and testosterone levels was shown by several studies.<sup>83-86</sup> Therefore, these findings suggest that increased concentrations of androgenic steroids can enhance the adhesion of monocytes to ECs, an initial stage of vascular inflammation that can lead to the atherogenic changes in arterial vessels.

On the other hand, androgens exhibit anti-inflammatory properties through induction of endothelial vasodilation and production of anti-inflammatory cytokines, such as IL-10. Moreover, exposure of cultured human ECs to DHT or testosterone resulted in attenuated TNF- $\alpha$ /lipopolysaccharide-dependent inflammation and caused down-regulation of inflammatory molecules such as IL-6, VCAM-1, chemokine ligand 2, and caspases.<sup>87</sup> In cultured HUVECs, androgens were shown to suppress expression of caspase-3 and -9, and activity of p38/MAPK, and indeed decrease apoptosis of ECs induced by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).<sup>83,84,88</sup> It is difficult to explain these conflicting results, but the discrepancy can be influenced by the origin of ECs, donor's sex, and a variety of signaling pathways influenced by androgens. In a recent review attempting to summarize available knowledge, it was suggested that androgen dysregulation is likely to convey its deleterious effects on the cardiovascular system via a chronic inflammatory process, which is dependent on different signaling pathways, including nuclear factor- $\kappa$ B, and tightly linked to changes of lipid metabolism and increased blood pressure.<sup>89</sup>

## Anti-thrombotic actions of androgens

Quiescent ECs secrete a number of molecules involved in hemostasis and thrombosis such as plasminogen activator inhibitor type 1 (PAI-I), tissue plasminogen activator (t-PA), and tissue factor pathway inhibitor (TFPI). All these proteins are involved in the breakdown of blood clots, thereby indicating the anti-coagulant state of ECs in normal conditions. In elderly people, decreased plasma testosterone was shown

to be associated with reduced TFPI levels and accelerated hemostatic response.<sup>90</sup> Furthermore, serum testosterone was shown to directly correlate with t-PA but inversely correlate with PAI-I and factor VII.<sup>91,92</sup> In cultured HUVECs, stimulatory effects of testosterone on TFPI and t-PA expression levels were observed.<sup>93</sup> In summary, these results indicate that androgens show anti-thrombotic properties, thereby possessing the cardioprotective function.

## Effects of testosterone replacement therapy

Testosterone replacement therapy (TRT) of hypogonadal and aging men was introduced into clinical practice a long time ago. In normal physiological concentrations, testosterone as a therapeutic agent shows favorable vasculoprotective effects,<sup>94</sup> which have also been discussed above. Nevertheless, some clinical studies have reported adverse cardiovascular side effects of TRT in elderly men with low testosterone levels and various comorbidities.<sup>95,96</sup> For instance, treatment of old men fragility with testosterone was found to increase the frequency of cardiovascular events and overall cardiovascular mortality in the elderly. These results, however, were questioned by the scientific community.<sup>97</sup> Indeed, the retrospective design of the studies did not allow for proper comparison between groups, and the studied populations did not allow for generalization of the safety results. More studies are needed to properly evaluate the safety of TRT and risk/benefit balance of this therapeutic approach. Despite the fact that some clinical trials showed unfavorable effects of testosterone treatment, the majority of studies demonstrated beneficial role of therapy with androgen hormones on the cardiovascular health that is broadly appreciated.

## Conclusion

Androgenic steroid hormones act through genomic and non-genomic mechanisms and significantly influence the function of ECs and their progenitors. These hormones are involved in the regulation of the vascular tone, proliferation, mobility, adhesion, and anti-thrombotic properties of vascular endothelium. Androgens also participate in important pathogenic mechanisms such as atherogenesis and vascular inflammation. Many studies indicate that androgens play a vasculoprotective role through the anti-inflammatory, anti-apoptotic, and vasodilatory actions on endothelium and VSMCs and recruitment of EPCs essential for vascular repair. However, there are studies that report the adverse vascular effects of androgens, for example, via induction of several vasoconstrictors. The causes of these disparities are



obscure, but can involve the heterogeneity of the vascular endothelium and gender-specific effects of androgens. In addition, significant spatial and temporal changes in AR expression observed in ECs and their progenitors can also contribute to the opposite effects of androgens on vascular endothelium.

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## Author contributions

All authors contributed toward drafting and critically revising the paper and agree to be accountable for all aspects of the work.

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The authors report no conflicts of interest in this work.

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