

The Role of Steroids in Endothelial Function in the Ageing Male

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Abstract

Normal vascular endothelium is essential for the synthesis and release of substances affecting vascular tone, cell adhesion and the homeostasis of clotting and fibrinolysis. The degeneration of endothelial integrity promotes adverse events leading to atherogenesis. Circulating levels of endogenous hormones decline during ageing and this may contribute to the occurrence of major adverse cardiovascular events, independently of gender differences. During the last decade more attention has been drawn to the importance of testosterone, oestradiol and adrenal androgens in the pathophysiology, prevention and treatment of male ageing-associated diseases. A considerable body of literature is available indicating that steroid hormones, particularly the sex steroids, are known to modulate endothelial function in all vascular beds and their deficiency may promote endothelial dysfunction. Testosterone decrease and increased mineralocorticoid activity in the ageing male are frequent and may yield endothelial dysfunction and increased cardiovascular burden; we recommend careful hormonal investigations in men who present comorbidities such as diabetes, hypertension and dyslipidaemia.

Keywords

Endothelial function, cardiovascular disease, hormones, atherosclerosis, ageing

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The impairment of vascular endothelium is an early event in the development of diseases that may later become clinically overt, such as local and systemic atherosclerosis, myocardial infarction, cerebral ischaemia and erectile dysfunction. Normal vascular endothelium is essential for the synthesis and release of substances affecting vascular tone, cell adhesion and the homeostasis of clotting and fibrinolysis. The degeneration of endothelial integrity promotes adverse events leading to atherogenesis, such as infiltration of the vessel wall by macrophages loaded with oxidised lipoproteins.¹ The pathogenesis of endothelial dysfunction is intimately linked through increased expression and activation of endothelial nitric oxide (NO) synthase and the subsequent physiological actions of NO. Reduced biological activity of endothelium-derived NO links human atherosclerosis to cardiovascular disease (CVD) and underscores the role of altered endothelium in the pathogenesis of both conditions. Coronary artery disease (CAD) represents one of the most common and costly atherogenic diseases in the Western world. It is more common in men aged 30–50 years, compared with women of similar age, an observation that has often suggested harmful effects of steroids on the coronary circulation.² Although endogenous hormones have an impact on CVD, their role has remained largely unknown for many years. During the last decade more attention has been drawn to the importance of testosterone, oestrogens and adrenal androgens in the pathophysiology, prevention and treatment of male CVD. A considerable body of literature is available indicating that steroid

hormones, particularly the sex steroids, are able to modulate endothelial function in all vascular beds and their deficiency promotes endothelial dysfunction. These observations point to the clinical relevance of the role of steroids in vascular health, and to the treatment of patients with hormonal deficiencies and increased cardiovascular risk factors with appropriate hormone formulations with the aim of attaining physiological circulating levels. The aim of this article is to give an overview of the role of sex steroid hormones in the control of vascular tone and provide evidence that these mechanisms are involved in the atherogenic process in the ageing male.

Endothelial Function and Dysfunction

The endothelium is characterised by a dynamic single cell layer, which regulates vascular homeostasis, acts as a semipermeable layer and functions as a physical barrier. The endothelium possesses autocrine, paracrine and endocrine functions, which play a critical role in regulating vascular tone. Moreover, the endothelium regulates homeostatic processes including platelet activation, aggregation, inflammation, immune function, vascular permeability, vascular smooth muscle cell proliferation and angiogenesis.³ Through these multiple functions, the endothelium is primarily responsible for enabling the arterial system to deliver sufficient tissue perfusion.⁴ As the exact roles of each of these agents in health and disease are delineated, it is clear that the endothelium has a diverse role in maintaining vascular tone and arterial flow through a variety of

mechanisms. Furthermore, a disruption in these regulatory functions at any level could lead to impaired and insufficient tissue perfusion.⁵

Endothelial dysfunction is characterised by significant modifications in physiological and biochemical parameters. These include: vascular stiffness, increased vascular tone, production of inflammatory cytokines, increased permeability, susceptibility to invasion of immunocytes, a decrease in endothelial cell growth, and dysregulation of thrombotic factors. Endothelial injury and dysfunction are critical events in the pathogenesis of atherosclerosis. Advancing age is associated with a decreased number and impaired function of endothelial progenitor cells (EPC),⁶ which may facilitate atherosclerotic processes and lead to an increased risk of cardiovascular events and death.⁷ Recent studies suggest augmentation of circulating EPC results in improved coronary collateral development in CAD.⁸ Clinical and biochemical markers of endothelial dysfunction include:

- reduced expression and activity of endothelial NO synthase (eNOS), reduced synthesis of NO and increased production of asymmetric dimethylarginine (ADMA), competitive, endogenous inhibitor of eNOS;
- increased production of reactive oxygen species (ROS);
- increased synthesis and release of the vasoconstrictor peptide endothelin-1 (ET-1);
- increased production of inflammatory cytokines such as interleukin-6 (IL-6), C-reactive protein (CRP) and tumour necrosis factor (TNF);
- increased expression of markers of cell adhesion such as E-selectin,⁹ intercellular adhesion molecule (ICAM)¹⁰ and vascular cell adhesion molecule (VCAM);
- dysregulation of fibrinolytic factors such as von Willebrand factor (vWF), tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1);
- inability to regenerate from EPC;
- increased endothelial apoptosis;
- increased cellular permeability; and
- increased vascular tone.

In addition to the biochemical markers of endothelial dysfunction, diagnostic tools of endothelial dysfunction are characterised by flow mediated dilation (FMD).¹¹ This clinical measurement of endothelial dysfunction is strongly linked to coronary endothelial dysfunction and predicts cardiovascular events.^{12–14} Longitudinal observations confirmed that endothelial dysfunction in coronary and peripheral circulation is predictive of CVD, as the sensitivity and specificity are greater for coronary endothelial than for peripheral dysfunction.¹⁵

Steroid Hormones Regulate Endothelial Function

A considerable body of evidence exists linking sex steroid hormone deficiency to endothelial dysfunction.^{16,17} Testosterone has a central role in endothelial health; in fact, its decline during the ageing process may affect either arterial reactivity or sexual function.¹⁸ Hypogonadism and erectile dysfunction are common disorders found in ageing men presenting to andrology clinics.^{19,20} Both disorders have important associations with metabolic syndrome, type 2 diabetes mellitus and CVD, all conditions with an increased morbidity and mortality in the ageing male.²¹ A low testosterone level is positively associated with the presence and severity of atherosclerosis and a reduction in plasma testosterone might contribute to increased arterial stiffness, which in turn has been associated with increased cardiovascular risk.²² Testosterone inversely correlates with the severity of atherosclerosis and has beneficial effects upon vascular reactivity, inflammatory

cytokine, adhesion molecules, insulin resistance, serum lipids and haemostatic factors.²³ Interestingly, men with established CAD often have reduced circulating testosterone levels²⁴ that are often associated with a certain degree of endothelial dysfunction, independently of other vascular risk factors, suggesting a protective role of endogenous testosterone on the endothelium.²⁵

There are contradictory effects of androgens on the cardiovascular system and a large body of evidence suggests that several other physiological and pathophysiological processes in the arterial vessel wall are influenced by androgens.²⁶ Recent animal and *in vitro* studies have further documented that testosterone upregulates the expression of arterial androgen receptor (AR) mRNA and is associated with an inhibitory effect on neointimal plaque formation.²⁷ Arterial functions may be directly subject to testosterone influence and, most probably, two independent pathways of testosterone-induced effects within the vessel wall can be assumed (i.e. genomic and non-genomic).^{28,29} Vascular ARs may mediate these effects of testosterone on the arterial wall and testosterone has been shown to produce coronary, aortic and brachial vasculature dilatation by activation of both endothelium-dependent and -independent mechanisms.^{30,31}

The role of androgens in determining vasodilatation has been investigated recently and eNOS seems to be regulated by sex steroid hormones.³² Testosterone may activate the endothelium and stimulates the NO/cyclic guanosine monophosphate and/or the hyperpolarisation-mediated vascular relaxation pathway and may thus have potential beneficial effects against coronary artery atherosclerosis. Additional endothelium-independent effects of testosterone may involve inhibition of the signalling mechanism of vascular smooth muscle contraction, such as intracellular concentration [Ca²⁺] and protein kinase-C, whereas a significant portion of the vasorelaxant effect of testosterone appears to be endothelium-independent, because no significant difference is observed between the relaxant effects of the hormone in isolated vessels with and without endothelium.³³ Also, inhibition of NO synthase, prostaglandin synthase and guanylate cyclase does not appear to affect the vasorelaxant effect of testosterone, suggesting that the testosterone-induced vascular relaxation may involve inhibition of the mechanism of vascular smooth muscle contraction.³⁴

Marin et al.³⁵ demonstrated that castration in rats reduced both neuronal NO synthase (nNOS) and eNOS expression and activity and testosterone treatment restored eNOS in corpora cavernosa. These observations strongly suggested that low concentrations of testosterone or dihydrotestosterone (DHT) are associated with ultrastructural damage of the aortic endothelium. Mäkinen et al.³⁶ have shown that middle-aged men with symptoms of androgen deficiency are at risk of increased carotid intima media thickness (IMT) and suggested that normal testosterone levels may offer protection against the development of atherosclerosis in middle-aged men. Malkin et al.³⁷ hypothesised that the immune-modulating properties of testosterone are important in inhibiting atheroma formation and progression to acute coronary syndrome. The authors demonstrated that significant reduction in total cholesterol was recorded with testosterone therapy and demonstrated a shift in the cytokine balance to a state of reduced inflammation.³⁸

Oestrogens in the male can be synthesised locally from testosterone by the CYP3A aromatase enzyme in many tissues. Oestrogens may act via their classic nuclear receptors or via rapid membrane actions.³⁹ The rate of whole-body aromatisation is higher in older men, but the precise

mechanisms are unclear.⁴⁰ The age trends of oestrogen levels in men have been reported variously as declining⁴¹ or remaining steady during the ageing process.⁴² Oestrogens were invariant with age in the Massachusetts male aging study (MMAS).³⁸ The ability of oestrogen to enhance endothelium-dependent vasodilator function has been well described in females.⁴³ However, endogenous oestrogen also influences vascular function in males. For example, a young man with an oestrogen receptor- α (OR α) mutation showed both CAD and endothelial dysfunction.⁴⁴ In healthy young men, inhibition of aromatase by administration of anastrozole impaired flow-mediated dilation, suggesting that endogenous oestrogen production directly affects endothelial function;⁴⁵ moreover, the suppression of endogenous oestrogens determined an impairment of flow-mediated dilation without significant changes in lipoproteins, homocysteine or CRP,⁴⁵ suggesting that endogenous oestrogens play a direct regulatory role in endothelial function.

17 β -oestradiol (E₂) regulates NO synthase in the hypothalamus,⁴⁶ modulates vascular endothelial growth/permeability factor in normal and tumour tissue⁴⁷ and glucose transporter-1 expression in the blood-brain barrier.^{48,49} E₂ has an immediate action on median eminence endothelial cells via non-genomic signalling pathways, leading to NO-stimulated gonadotropin-releasing hormone (GnRH) release.⁵⁰ Vascular endothelial growth factor (VEGF) expression is higher in the neural lobe than in the anterior lobe and is undetectable in the intermediate lobe; it is rapidly upregulated by E₂ in the anterior pituitary but remains unchanged in the posterior pituitary.⁵¹ OR α activation in cerebrovascular tissue resulted in increased eNOS activity and protein levels.⁵² Increased NO production by eNOS may contribute to the neuroprotective effects of oestrogens. Galea et al. hypothesised that the protective effects of E₂ in cerebral ischaemia may be attributed to the blockade of leucocyte adhesion in cerebral endothelial cells.⁵³ *In vivo* oestrogen treatment leads to a 100 % increase in eNOS mRNA copy number and increases eNOS protein levels by 47 % in mouse cerebral blood vessels,⁵⁴ suggesting that oestrogen modulates eNOS at the transcriptional level in blood vessels *in vivo*. Low E₂ results in reduced nNOS and eNOS expression in the hippocampus and E₂ substitution reverses these effects,⁵⁵ suggesting that E₂ increases nNOS and eNOS expression and activity in the hippocampus and improves hippocampal function. Tivesten et al.⁵⁶ showed that circulating E₂ is a predictor of progression of carotid artery IMT in middle-aged men. Only a few studies have addressed the relation between endogenous E₂ levels and atherosclerosis in men.³⁶ In a recent four-year longitudinal study on the relation between baseline E₂ levels and progression of carotid artery IMT in men, Muller et al.⁵⁷ found a tendency towards a positive association between serum E₂ and IMT, but the data did not reach statistical significance. Further studies are needed to investigate the role of endogenous E₂ for incident CVD events.

The circulating adrenal androgen dehydroepiandrosterone (DHEA) represents a pathway that may be of increasing importance with age. In fact, it is a precursor for intracellular production of androgens and oestrogens in non-reproductive tissues. Studies agree that levels of DHEA and its sulphate (DHEA-S), the most plentiful steroid in serum, decline with age more markedly than other hormones. The adrenal steroid androstenedione follows a similarly sharp decline.⁵⁸ The MMAS data show DHEA, DHEA-S and androstenedione declining at 2–3 % per year, both cross-sectionally and longitudinally.⁵⁹ DHEA-S is thought to be of potential importance as a biomarker and protective factor against ageing.⁶⁰ Clinical and experimental studies have shown that DHEA-S in humans and other mammals is a multi-functional steroid implicated in a

broad range of biological effects, including obesity, diabetes, bone metabolism, neuroprotection and antitumorigenesis, and that plasma or serum DHEA-S levels are independently and inversely associated with the incidence of coronary heart disease and mortality in males.^{61,62} DHEA is thought to be associated with life expectancy and anti-ageing. However, its biological significance in atherosclerosis remains controversial. DHEA supplementation restored aortic eNOS levels and activity, suggesting that DHEA has direct genomic and non-genomic effects on the vascular wall.^{63,64} Liu and Dillon⁶⁵ demonstrated that physiological concentrations of DHEA acutely increase NO release from intact vascular endothelial cells, by a plasma membrane-dependent mechanism. This action of DHEA is mediated by a steroid-specific, G-protein-coupled receptor mechanism, which activates eNOS in both bovine and human endothelial cells.⁶⁶ This cellular mechanism may underlie some of the cardiovascular protective effects proposed for DHEA. According to other studies, there was no significant relationship between plasma or serum DHEA-S levels and atherosclerotic diseases, including coronary heart disease and carotid atherosclerosis, in males.^{67,68} Thus the clinical significance of DHEA-S in the prevention of atherosclerosis and cardiovascular diseases has not been fully elucidated in either males or females. However, in a recent study Yoshida et al. showed that although DHEA-S is not involved in endothelial function, it is inversely associated with sex-dependent signs of carotid atherosclerosis: increased maximum and mean IMT in males and decreased common carotid artery blood flow volume in females.⁶⁹

Aldosterone is a steroid hormone that controls blood pressure by binding to the mineralocorticoid receptor (MR), a ligand-activated transcription factor, and regulating genes that play a role in salt and water homeostasis in the kidney. The dysregulation of the mineralocorticoid system reveals its crucial role in various human diseases, including hypertension, atherosclerosis, cardiac failure, mineralocorticoid resistance and disorders of the nervous system. Recently, experimental animal models of mineralocorticoid/salt-induced hypertension and atherosclerosis have revealed an epithelial, pro-inflammatory role for MR activation. Extensive investigation has begun to elucidate the mechanisms underlying the vascular effects of MR activation, which involve its direct role in cardiomyocytes, vascular smooth muscle cells and endothelial cells. More specifically, in patients with cardiovascular risk factors and disease, including diabetes, hypertension and/or congestive heart failure, an excess of MR activation has been shown to have a negative impact on endothelial function, hence disrupting the physiological balance between vasoconstriction and vasodilatation.⁷⁰ Such a mechanism may play a role in the pathogenesis of erectile dysfunction, especially in the ageing male, whereas increased cardiovascular risk, hypertension and diabetes represent precipitating factors contributing to impaired vascular relaxation due to overactivation of the ET-1/endothelin receptor-A (ETA) pathway.⁷¹ ETA receptor blockade as well as mineralocorticoid receptor blockade may represent alternative therapeutic approaches for erectile dysfunction associated with salt-sensitive hypertension and in pathological conditions where increased levels of ET-1 are present.

Conclusions

The endothelium plays a critical role in the physiological function of all vascular beds, maintaining vascular homeostasis and thus preventing initiation or progression of vascular disease. Any insult or injury to the endothelium may produce pathological states and dysfunction. Synthesis and release of vasodilators from the endothelium, such as NO, prostaglandin I₂ (PGI₂) and endothelium-derived hyperpolarising factor,

is integral to maintenance of physiological function. Endothelial damage due to various insults determines the production of potent vasoconstrictors such as ET-1, thromboxane-A2 and angiotensin-II that contribute to vascular disease and increase the risk of morbidity and mortality in CAD in aging males. Considerable evidence now suggests that steroid hormones modulate endothelial function in all vascular beds and that their deficiencies promote endothelial dysfunction. Androgens and oestrogens produce specific and marked biological effects on endothelial function as demonstrated by the changes in the endothelial markers of function and dysfunction. Low testosterone and DHT are associated with ultrastructural damage of the aortic endothelium. Also endothelial dysfunction in men is associated with low

plasma testosterone levels, independent of other risk factors, suggesting a protective effect of testosterone on the endothelium. Several studies have also corroborated that DHEA and the mineralocorticoid system play a crucial role in CVD, but extensive investigation is necessary to elucidate the mechanisms underlying the vascular effects of MR activation. These observations point to the clinical relevance of steroid hormones in vascular health and to treating patients with hormonal deficiencies with appropriate levels of physiological hormones in regulating endothelial function is critical for translation of the basic research into treatment of patients with metabolic syndrome, vascular disease and erectile dysfunction. ■

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