

## The Role of Steroids in Endothelial Function in the Aging Male

Antonio Aversa, MD, PhD,<sup>1</sup> Roberto Bruzziches, MD,<sup>2</sup> Davide Francomano, MD,<sup>2</sup> Emanuela A Greco, MD,<sup>2</sup>  
Silvia Migliaccio, MD, PhD<sup>3</sup> and Andrea Lenzi, MD<sup>4</sup>

1. Senior Lecturer, Department of Experimental Medicine, Medical Pathophysiology Section, 'La Sapienza' University of Rome; 2. Research Fellow, Department of Experimental Medicine, Medical Pathophysiology Section, 'La Sapienza' University of Rome; 3. Researcher, Department of Health Sciences, Endocrinology Unit, Foro Italico University of Rome; 4. Full Professor, Department of Experimental Medicine, Medical Pathophysiology Section, 'La Sapienza' University of Rome

### 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 aging 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, estradiol and adrenal androgens in the pathophysiology, prevention, and treatment of male aging-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 that their deficiency may promote endothelial dysfunction. Testosterone decrease and increased mineralocorticoid activity in the aging 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 dyslipidemia.

### Keywords

Endothelial function, cardiovascular disease, hormones, atherosclerosis, aging

**Disclosure:** Antonio Aversa, MD, PhD, has received grants from Bayer Schering Pharma and Eli Lilly. Silvia Migliaccio, MD, PhD, is a consultant for Eli Lilly. The remaining authors have no conflicts of interest to declare.

**Received:** July 8, 2011 **Accepted:** July 29, 2011 **Citation:** *US Endocrinology*, 2011;7(2):145–9 DOI: 10.17925/USE.2011.07.02.145

**Correspondence:** Antonio Aversa, MD, PhD, Department of Medical Pathophysiology, 'La Sapienza' University of Rome, Viale Policlinico 155, 00161 Rome, Italy.  
E: antonio.aversa@uniroma1.it

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 ischemia, 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.<sup>1</sup> 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.<sup>2</sup> 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, estrogens 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 that 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 aging male.

### Endothelial Function and Dysfunction

The endothelium is characterized 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.<sup>3</sup> Through these multiple functions, the endothelium is primarily responsible for enabling the arterial system to deliver sufficient tissue perfusion.<sup>4</sup> 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.<sup>5</sup>

Endothelial dysfunction is characterized 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 (EPCs),<sup>6</sup> which may facilitate atherosclerotic processes and lead to an increased risk of cardiovascular events and death.<sup>7</sup> Recent studies suggest augmentation of circulating EPCs results in improved coronary collateral development in CAD.<sup>8</sup> 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), a 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 tumor necrosis factor (TNF);
- increased expression of markers of cell adhesion such as E-selectin,<sup>9</sup> intercellular adhesion molecule (ICAM),<sup>10</sup> 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 EPCs;
- 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 characterized by flow-mediated dilation (FMD).<sup>11</sup> This clinical measurement of endothelial dysfunction is strongly linked to coronary endothelial dysfunction and predicts cardiovascular events.<sup>12–14</sup> 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.<sup>15</sup>

## Steroid Hormones Regulate Endothelial Function

A considerable body of evidence exists linking sex steroid hormone deficiency to endothelial dysfunction.<sup>16,17</sup> Testosterone has a central role in endothelial health; in fact, its decline during the aging process may affect

either arterial reactivity or sexual function.<sup>18</sup> Hypogonadism and erectile dysfunction are common disorders found in aging men presenting to andrology clinics.<sup>19,20</sup> Both disorders have important associations with metabolic syndrome, type 2 diabetes mellitus, and CVD, all conditions with an increased morbidity and mortality in the aging male.<sup>21</sup> 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.<sup>22</sup> Testosterone inversely correlates with the severity of atherosclerosis and has beneficial effects upon vascular reactivity, inflammatory cytokine, adhesion molecules, insulin resistance, serum lipids, and hemostatic factors.<sup>23</sup> Interestingly, men with established CAD often have reduced circulating testosterone levels<sup>24</sup> 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.<sup>25</sup>

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.<sup>26</sup> Recent animal and *in vitro* studies have further documented that testosterone upregulates the expression of arterial androgen receptor (AR) messenger RNA (mRNA) and is associated with an inhibitory effect on neointimal plaque formation.<sup>27</sup> 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).<sup>28,29</sup> 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.<sup>30,31</sup>

The role of androgens in determining vasodilatation has been investigated recently and eNOS seems to be regulated by sex steroid hormones.<sup>32</sup> 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 [Ca<sup>2+</sup>] 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.<sup>33</sup> 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.<sup>34</sup>

Marin et al.<sup>35</sup> demonstrated that castration in rats reduced both neuronal NO synthase (nNOS) and eNOS expression and activity, and that 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.<sup>36</sup> 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.<sup>37</sup> 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.<sup>38</sup>

Estrogens in the male can be synthesized locally from testosterone by the CYP3A aromatase enzyme in many tissues. Estrogens may act via their classic nuclear receptors or via rapid membrane actions.<sup>39</sup> The rate of whole-body aromatization is higher in older men, but the precise mechanisms are unclear.<sup>40</sup> The age trends of estrogen levels in men have been reported variously as declining<sup>41</sup> or remaining steady during the aging process.<sup>42</sup> Estrogens were invariant with age in the Massachusetts male aging study (MMAS).<sup>38</sup> The ability of estrogen to enhance endothelium-dependent vasodilator function has been well described in females.<sup>43</sup> However, endogenous estrogen also influences vascular function in males. For example, a young man with an estrogen receptor- $\alpha$  (OR $\alpha$ ) mutation showed both CAD and endothelial dysfunction.<sup>44</sup> In healthy young men, inhibition of aromatase by administration of anastrozole impaired FMD, suggesting that endogenous estrogen production directly affects endothelial function;<sup>45</sup> moreover, the suppression of endogenous estrogens determined an impairment of FMD without significant changes in lipoproteins, homocysteine or CRP,<sup>45</sup> suggesting that endogenous estrogens play a direct regulatory role in endothelial function.

17 $\beta$ -oestradiol (E<sub>2</sub>) regulates NO synthase in the hypothalamus,<sup>46</sup> and modulates vascular endothelial growth/permeability factor in normal and tumor tissue<sup>47</sup> and glucose transporter-1 expression in the blood-brain barrier.<sup>48,49</sup> E<sub>2</sub> has an immediate action on median eminence endothelial cells via non-genomic signaling pathways, leading to NO-stimulated gonadotropin-releasing hormone (GnRH) release.<sup>50</sup> 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 E<sub>2</sub> in the anterior pituitary, but remains unchanged in the posterior pituitary.<sup>51</sup> OR $\alpha$  activation in cerebrovascular tissue resulted in increased eNOS activity and protein levels.<sup>52</sup> Increased NO production by eNOS may contribute to the neuroprotective effects of estrogens. Galea et al. hypothesized that the protective effects of E<sub>2</sub> in cerebral ischemia may be attributed to the blockade of leucocyte adhesion in cerebral endothelial cells.<sup>53</sup> *In vivo* estrogen treatment leads to a 100 % increase in eNOS mRNA copy number and increases eNOS protein levels by 47 % in mouse cerebral blood vessels,<sup>54</sup> suggesting that estrogen modulates eNOS at the transcriptional level in blood vessels *in vivo*. Low E<sub>2</sub> results in reduced nNOS and eNOS expression in the hippocampus and E<sub>2</sub> substitution reverses these effects,<sup>55</sup> suggesting that E<sub>2</sub> increases nNOS and eNOS expression and activity in the hippocampus and improves hippocampal function. Tivesten et al.<sup>56</sup> showed that circulating E<sub>2</sub> is a predictor of progression of carotid artery IMT in middle-aged men.

Only a few studies have addressed the relation between endogenous E<sub>2</sub> levels and atherosclerosis in men.<sup>36</sup> In a recent four-year longitudinal

study on the relation between baseline E<sub>2</sub> levels and progression of carotid artery IMT in men, Muller et al.<sup>57</sup> found a tendency toward a positive association between serum E<sub>2</sub> and IMT, but the data did not reach statistical significance. Further studies are needed to investigate the role of endogenous E<sub>2</sub> 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 estrogens 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.<sup>58</sup> The MMAS data show DHEA, DHEA-S, and androstenedione declining by 2–3 % per year, both cross-sectionally and longitudinally.<sup>59</sup> DHEA-S is thought to be of potential importance as a biomarker and protective factor against aging.<sup>60</sup> Clinical and experimental studies have shown that DHEA-S in humans and other mammals is a multifunctional 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.<sup>61,62</sup> DHEA is thought to be associated with life expectancy and anti-aging. 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.<sup>63,64</sup> Liu and Dillon<sup>65</sup> 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.<sup>66</sup> 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.<sup>67,68</sup> 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.<sup>69</sup>

Aldosterone is a steroid hormone that controls blood pressure by binding to the mineralocorticoid receptor (MR), a ligand-activated transcription factor, and regulates 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.<sup>70</sup> Such a mechanism may play a role in the pathogenesis of erectile dysfunction, especially in the aging 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.<sup>71</sup> 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 I<sub>2</sub> (PGI<sub>2</sub>), and endothelium-derived hyperpolarizing factor, is integral to the maintenance of physiological function. Endothelial damage due to various insults determines the production of potent

vasoconstrictors—such as ET-1, thromboxane-A<sub>2</sub>, and angiotensin-II—that contribute to vascular disease and increase the risk of morbidity and mortality in aging males with CAD. Considerable evidence now suggests that steroid hormones modulate endothelial function in all vascular beds and that their deficiencies promote endothelial dysfunction. Androgens and estrogens 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 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 hormone formulations. Better understanding of the role of steroid 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. ■

- Kathir K, Adams MR, Endothelial dysfunction as a predictor of acute coronary syndromes, *Semin Vasc Med*, 2003;3:355–62.
- Rosano GM, Androgens and coronary artery disease. A sex-specific effect of sex hormones?, *Eur Heart J*, 2000;21:868–71.
- Herrmann J, Lerman A, The endothelium: dysfunction and beyond, *J Nucl Cardiol*, 2001;8:197–206.
- Bruzziches R, Aversa A, Phosphodiesterase type 5 inhibitors and endothelial function, *Curr Sex Health Rep*, 2007;4:157–62.
- Aversa A, Bruzziches R, Francomano D, et al., Testosterone and phosphodiesterase type-5 inhibitors: new strategy for preventing endothelial damage in internal and sexual medicine?, *Ther Adv Urol*, 2009;1:179–97.
- Carlo-Stella C, Di Nicola M, Milani R, et al., Age and irradiation associated loss of bone marrow hematopoietic function in mice is reversed by recombinant human growth hormone, *Exp Hematol*, 2004;32:171–8.
- Hill JM, Zalos G, Halcox JP, et al., Circulating endothelial progenitor cells, vascular function, and cardiovascular risk, *N Engl J Med*, 2003;348:593–600.
- Vasa M, Fichtlscherer S, Adler K, et al., Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease, *Circulation*, 2001;103:2885–90.
- Blankenberg S, Rupprecht HJ, Bickel C, et al., Circulating cell adhesion molecules and death in patients with coronary artery disease, *Circulation*, 2001;104:1336–42.
- Witte DR, Broekmans WM, Kardinaal AF, et al., Soluble intercellular adhesion molecule 1 and flow-mediated dilatation are related to the estimated risk of coronary heart disease independently from each other, *Atherosclerosis*, 2003;170:147–53.
- Corretti MC, Anderson TJ, Benjamin EJ, et al., Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force, *J Am Coll Cardiol*, 2002;39:257–65.
- Anderson TJ, Uehata A, Gerhard MD, et al., Close relation of endothelial function in the human coronary and peripheral circulations, *J Am Coll Cardiol*, 1995;26:1235–41.
- Wu WC, Sharma SC, Choudhary G, et al., Flow-mediated vasodilation predicts the presence and extent of coronary artery disease assessed by stress thallium imaging, *J Nucl Cardiol*, 2005;12:538–44.
- Yavuz BB, Yavuz B, Sener DD, et al., Advanced age is associated with endothelial dysfunction in healthy elderly subject, *Gerontology*, 2008;54:153–6.
- Vita JA, Keaney JF Jr, Endothelial function: a barometer for cardiovascular risk?, *Circulation*, 2002;106:640–2.
- Miller VM, Mulvagh SL, Sex steroids and endothelial function: translating basic science to clinical practice, *Trends Pharmacol Sci*, 2007;28:263–70.
- Sader MA, Griffiths KA, Skilton MR, et al., Physiological testosterone replacement and arterial endothelial function in men, *Clin Endocrinol (Oxf)*, 2003;59:62–7.
- Allan CA, McLachlan RI, Age-related changes in testosterone and the role of replacement therapy in older men, *Clin Endocrinol (Oxf)*, 2004;60:653–70.
- Francomano D, Bruzziches R, Natali M, et al., Cardiovascular effect of testosterone replacement therapy in aging male, *Acta Biomed*, 2010;81(Suppl. 1):101–6.
- Aversa A, Bruzziches R, Francomano D, et al., Endothelial dysfunction and erectile dysfunction in the aging man, *Int J Urol*, 2010;17:38–47.
- Jones TH, Testosterone associations with erectile dysfunction, diabetes, and the metabolic syndrome, *Eur Urol*, 2007;6:847–57.
- Houglak H, Fleg JL, Najjar SS, et al., Relationship between androgenic hormones and arterial stiffness, based on longitudinal hormone measurements, *Am J Physiol Endocrinol Metab*, 2006;290:E234–42.
- Fukui M, Kitagawa Y, Ose H, et al., Role of endogenous androgen against insulin resistance and atherosclerosis in men with type 2 diabetes, *Curr Diabetes Rev*, 2007;3:5–31.
- Rosano GM, Sheiban I, Massaro R, et al., Low testosterone levels are associated with coronary artery disease in male patients with angina, *Int J Impot Res*, 2007;19:176–82.
- Akishita M, Hashimoto M, Ohike Y, et al., Low testosterone level is an independent determinant of endothelial dysfunction in men, *Hypertens Res*, 2007;30:1029–34.
- Aversa A, Bruzziches R, Spera G, A rationale for the use of testosterone salvage in treatment of men with erectile dysfunction failing phosphodiesterase inhibitors, *Endocrinologist*, 2005;15:99–105.
- Hanke H, Lenz C, Hess B, et al., Effect of testosterone on plaque development and androgen receptor expression in the arterial vessel wall, *Circulation*, 2001;103:1382–5.
- Orshah JM, Khalil RA, Gender, sex hormones, and vascular tone, *Am J Physiol Regul Integr Comp Physiol*, 2004;286:R233–49.
- Wynne FL, Khalil RA, Testosterone and coronary vascular tone: implications in coronary artery disease, *J Endocrinol Invest*, 2003;26:181–6.
- Chou TM, Sudhir K, Hutchison SJ, et al., Testosterone induces dilatation in canine coronary conductance and resistance arteries in vivo, *Circulation*, 1996;94:2614–9.
- Littleton-Kearney M, Hurn PD, Testosterone as a modulator of vascular behavior, *Biol Res Nurs*, 2004;5:276–85.
- Rosenfeld CR, Chen C, Roy T, Liu X, Estrogen selectively up-regulates eNOS and nNOS in reproductive arteries by transcriptional mechanisms, *J Soc Gynecol Invest*, 2003;10:205–15.
- Crews JK, Khalil RA, Antagonistic effects of 17 beta-estradiol, progesterone and testosterone on Ca<sup>2+</sup> entry mechanism of coronary vasoconstriction, *Arterioscler Thromb Vasc Biol*, 1999;19:1034–40.
- Murphy JG, Khalil RA, Decreased [Ca<sup>2+</sup>]<sub>i</sub>] during inhibition of coronary smooth muscle contraction by 17beta-estradiol, progesterone, and testosterone, *J Pharmacol Exp Ther*, 1999;291:44–52.
- Marin R, Escrig A, Abreu P, Mas M, Androgen-dependent nitric oxide release in rat penis correlates with levels of constitutive nitric oxide synthase isoenzymes, *Biol Reprod*, 1999;61:1012–6.
- Mäkinen J, Järvisalo MJ, Pöllänen P, et al., Increased carotid atherosclerosis in andropausal middle-aged men, *J Am Coll Cardiol*, 2005;45:1603–8.
- Malkin CJ, Pugh PJ, Jones RD, et al., Testosterone as a protective factor against atherosclerosis—immunomodulation and influence upon plaque development and stability, *J Endocrinol*, 2003;178:373–80.
- Malkin CJ, Pugh PJ, Jones RD, et al., The effect of testosterone replacement on endogenous inflammatory cytokines and lipid profiles in hypogonadal men, *J Clin Endocrinol Metab*, 2004;89:3313–8.
- Sharpe RM, The roles of oestrogen in the male, *Trends Endocrinol Metab*, 1998;9:371–7.
- Lakshman KM, Kaplan B, Travison TG, et al., The effects of injected testosterone dose and age on the conversion of testosterone to estradiol and dihydrotestosterone in young and older men, *J Clin Endocrinol Metab*, 2010;95:3955–64.
- Orwoll E, Lambert LC, Marshall LM, et al., Testosterone and estradiol among older men, *J Clin Endocrinol Metab*, 2006;91:1336–44.
- Araujo AB, Travison TG, Leder BZ, et al., Correlations between serum testosterone, estradiol, and sex hormone-binding globulin and bone mineral density in a diverse sample of men, *J Clin Endocrinol Metab*, 2008;93:2135–41.
- Gonzales RJ, Walker BR, Kanagy NL, 17β-Estradiol increases nitric oxide-dependent dilation in rat pulmonary arteries and thoracic aorta, *Am J Physiol Lung Cell Mol Physiol*, 2001;280:L555–64.
- Sudhir K, Chou TM, Messina LM, et al., Endothelial dysfunction in a man with disruptive mutation in oestrogen-receptor gene, *Lancet*, 1997;349:1146–7.
- Lew R, Komesaroff P, Williams M, et al., Endogenous estrogens influence endothelial function in young men, *Circ Res*, 2003;93:1127–33.
- Ceccatelli S, Grandison L, Scott RE, et al., Estradiol regulation of nitric oxide synthase mRNAs in rat hypothalamus, *Neuroendocrinology*, 1996;64:357–63.
- Nakamura J, Savinov A, Lu Q, Brodie A, Estrogen regulates vascular endothelial growth/permeability factor expression in 7,12-dimethylbenz(a)anthracene-induced rat mammary tumors, *Endocrinology*, 1996;137:5589–96.
- Shi J, Simpkins JW, 17 beta-Estradiol modulation of glucose transporter 1 expression in blood-brain barrier, *Am J Physiol*, 1997;272:E1016–E1022.
- Shi J, Zhang YQ, Simpkins JW, Effects of 17beta-estradiol on glucose transporter 1 expression and endothelial cell survival following focal ischemia in the rats, *Exp Brain Res*, 1997;117:200–6.
- Prevot V, Croix D, Rialas CM, et al., Estradiol coupling to endothelial nitric oxide stimulates gonadotropin-releasing hormone release from rat median eminence via a membrane receptor, *Endocrinology*, 1999;140:652–9.
- Ochoa AL, Mitchner NA, Paynter CD, et al., Vascular endothelial growth factor in the rat pituitary: differential distribution and regulation by estrogen, *J Endocrinol*, 2000;165:483–92.
- McNeill AM, Zhang C, Stanczyk FZ, et al., Estrogen increases endothelial nitric oxide synthase via estrogen receptors in rat cerebral blood vessels: effect preserved after concurrent treatment with medroxyprogesterone acetate or progesterone, *Stroke*, 2002;33:1685–91.

53. Galea E, Santizo R, Feinstein DL, et al., Estrogen inhibits NF kappa B-dependent inflammation in brain endothelium without interfering with I kappa B degradation, *Neuroreport*, 2002;13:1469-72.
54. Stirone C, Chu Y, Sunday L, et al., 17 Beta-estradiol increases endothelial nitric oxide synthase mRNA copy number in cerebral blood vessels: quantification by real-time polymerase chain reaction, *Eur J Pharmacol*, 2003;478:35-8.
55. Grohé C, Kann S, Fink L, et al., 17 Beta-estradiol regulates nNOS and eNOS activity in the hippocampus, *Neuroreport*, 2004;15:89-93.
56. Tivesten A, Hulthe J, Wallenfeldt K, et al., Circulating estradiol is an independent predictor of progression of carotid artery intima-media thickness in middle-aged men, *J Clin Endocrinol Metab*, 2006;91:4433-7.
57. Muller M, van den Beld AW, Bots ML, et al., Endogenous sex hormones and progression of carotid atherosclerosis in elderly men, *Circulation*, 2004;109:2074-9.
58. Belanger A, Candas B, Dupont A, et al., Changes in serum concentrations of conjugated and unconjugated steroids in 40- to 80-year-old men, *J Clin Endocrinol Metab*, 1994;79:1086-90.
59. Gray A, Feldman HA, McKinlay JB, et al., Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study, *J Clin Endocrinol Metab*, 1991;73:1016-25.
60. Villareal DT, Holloszy JO, Effect of DHEA on abdominal fat and insulin action in elderly women and men: a randomized controlled trial, *JAMA*, 2004;292:2243-8.
61. Barrett-Connor E, Khaw KT, Yen SS, A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease, *N Engl J Med*, 1986;315:1519-24.
62. Mitchell LE, Sprecher DL, Borecki IB, et al., Evidence for an association between dehydroepiandrosterone sulfate and nonfatal, premature myocardial infarction in males, *Circulation*, 1994;89:89-93.
63. Simoncini T, Mannella P, Fornari L, et al., Dehydroepiandrosterone modulates endothelial nitric oxide synthesis via direct genomic and non-genomic mechanisms, *Endocrinology*, 2003;144:3449-55.
64. Simoncini T, Mannella P, Pluchino N, Genazzani AR, Comparative effects of dydrogesterone and medroxyprogesterone acetate in critical areas: the brain and the vessels, *Gynecol Endocrinol*, 2007;23(Suppl. 1):9-16.
65. Liu D, Dillon JS, Dehydroepiandrosterone stimulates nitric oxide release in vascular endothelial cells: evidence for a cell surface receptor, *Steroids*, 2004;69:279-89.
66. Liu D, Dillon JS, Dehydroepiandrosterone activates endothelial cell nitric-oxide synthase by a specific plasma membrane receptor coupled to Galpha(12,3), *J Biol Chem*, 2002;277:21379-88.
67. Newcomer LM, Manson JE, Barbieri RL, et al., Dehydroepiandrosterone sulfate and the risk of myocardial infarction in US male physicians: a prospective study, *Am J Epidemiol*, 1994;140:870-5.
68. LaCroix AZ, Yano K, Reed DM, Dehydroepiandrosterone sulfate, incidence of myocardial infarction, and extent of atherosclerosis in men, *Circulation*, 1992;86:1529-35.
69. Yoshida S, Alharaa K, Azumab H, et al., Dehydroepiandrosterone sulfate is inversely associated with sex-dependent diverse carotid atherosclerosis regardless of endothelial function, *Atherosclerosis*, 2010;212:310-5.
70. Caprio M, Mammì C, Jaffe IZ, et al., The mineralocorticoid receptor in endothelial physiology and disease: novel concepts in the understanding of erectile dysfunction, *Curr Pharm Des*, 2008;14:3749-57.
71. Carneiro FS, Nunes KP, Giachini FR, et al., Activation of the ET-1/ETA pathway contributes to erectile dysfunction associated with mineralocorticoid hypertension, *J Sex Med*, 2008;5:2793-807.