The Brain, the Penis and Steroid Hormones: Clinical Correlates with Endothelial Dysfunction

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Abstract: Erectile function is a complex neurovascular process that depends on the health of the central and peripheral nervous systems and the vasculature. Thus, signaling from the central nervous system (brain) to the peripheral nervous system (penis) is critical and is modulated by a set of complex interactions that depend on cerebral and vascular circulation. The cerebral and peripheral vasculatures are target tissues for sex steroid hormones. Gonadal, adrenal and neurosteroids regulate the function and physiology of the endothelium and modulate vascular and cerebral circulation by genomic and non-genomic dependent mechanisms. Recent advances in cell and molecular biology have defined a critical role of endothelium in vascular function. A host of biochemical and clinical markers of endothelium function and dysfunction have been identified to assess vascular pathology. Emerging evidence suggests that sex steroid hormones play an important role in maintaining endothelial health and sex steroid deficiency is associated with endothelial dysfunction, vascular disease and erectile dysfunction. Such information has important clinical implications in patient management with sex steroid hormone insufficiency, diabetes, metabolic syndrome, vascular disease and erectile dysfunction. In this review, we discuss the role of sex steroid hormones in modulation of the biochemical and clinical markers associated with endothelial dysfunction. Specifically the regulation of endothelial nitric oxide synthase, assymetric dimethylarginine, reactive oxygen species, endothelin-1, inflammatory cytokines, tumor necrosis factor-α, markers of cell adhesion, dysregulation of fibrinolytic factors and the inability to regenerate from endothelial progenitor cells concomitant with increased endothelial apoptosis, increased cellular permeability and increased vascular tone.

Key Words: Sex steroids, neurosteroids, endothelium dysfunction, erectile dysfunction, nitric oxide, nitric oxide synthase, vascular tone, vascular disease.

INTRODUCTION

I. Central Nervous System & Erectile Function

Considerable evidence exists on the potential role of neurosteroids and neuroactive steroids on sexual function and behavior [1]. In addition, gonadal and adrenal steroids, upon conversion into neuroactive steroids, in the brain, may modulate sexual function and behavior. Furthermore, *de novo* synthesis of neurosteroids in the central nervous system has been well documented and enzymatic machinery necessary for the biosynthetic pathway exists [1]. Sexual desire, arousal, and orgasm are modulated by a complex set of interactions between the somatic and autonomic nervous systems, operating at cerebral, spinal, and peripheral levels [2]. Neurosteroids elicit specific responses in select target neuronal pathways and modulate sexual function [1]. The exact details of such interactions, however, remain, at best, poorly understood. Neurosteroids and neuroactive steroids as well as peptide hormones modulate neural activities and modify the sexual responses. Further, dopaminergic and serotonergic systems play an important role in various components of the sexual response cycle at the central level. Other neurotransmitters including adrenergic, cholinergic, nitergic, gamma-aminobutyric acidergic, and neuropeptides also contribute to the sexual response. Temel *et al.* [3] reviewed data from animal and human studies and proposed that within the cortical areas, parts of the frontal lobe (medial and inferior) and cingulate gyrus (anterior) and within the subcortical areas, parts of the amygdala [corticomedial, medial and bed nucleus of the stria terminalis (BNST)], thalamus (medial dorsal, and Cm-parafascicular [Pf] complex), hypothalamus paraventricular nucleus (PVN), medial and lateral, preoptic areas (POAs) and mamillary bodies, nucleus accumbens, fornix and striatum are involved in erection. The authors suggested that brain centers are potent modulators of the spinal centers responsible for generation of penile erection. Salas *et al.* [4] suggested that the laterodorsal tegmental nucleus (LDT) and surrounding region appear to be involved in regulation of penile erection and different anatomical areas in the mesopontine tegmentum may have specific roles in this physiological process. Melis *et al.* [5] showed that oxytocin in the ventral tegmental area (VTA) activates mesolimbic dopaminergic neurons, which may be involved in the appetitive and rewarding effects of sexual activity. Suzuki *et al.* [6] investigated the effects of castration and testosterone (T) replacement on intracavernous pressure (ICP) elicited with electrical stimulation of the medial preoptic area (MPOA) and cavernous nerve (CN) in male rats. The authors suggested that T plays an important role not only in the central nervous system but also in the peripheral neural pathways for the maintenance and restoration of erectile capacity.

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The cerebral vasculature is a target tissue for sex steroid hormones. Estrogens, androgens, and progestins modulate the function and pathophysiology of the cerebral circulation [7]. Estrogens decrease cerebral vascular tone and increase cerebral blood flow by enhancing endothelial nitric oxide synthase (eNOS) expression and activity and facilitating the prostacyclin pathways. Estrogens have important protective effects on cerebral endothelial cells by increasing mitochondrial efficiency, decreasing free radical production, promoting cell survival, and stimulating angiogenesis. Although much has been learned regarding hormonal effects on brain blood vessels, most studies involve young, healthy animals. It is becoming apparent that hormonal effects may be modified by aging or disease states, such as diabetes and atherosclerosis. Furthermore, the effects of T are complicated because this hormone is also converted to estrogen and DHT, systemically and possibly within the vessels themselves.

Estradiol (E_2) regulates Nitric Oxide (NO) synthase (NOS) in the hypothalamus [8] and modulates vascular endothelial growth permeability factor in normal and tumor tissue [9] and glucose transporter-1 expression in blood-brain barrier [10, 11]. E_2 has an immediate action on median eminence endothelial cells *via* non-genomic signaling pathways leading to NO-stimulated GnRH release [12]. Vascular endothelial growth factor (VEGF) expression is higher in the neural lobe than in the anterior lobe undetectable in the intermediate lobe and is rapidly up-regulated by E_2 in the anterior pituitary but remains unchanged in the posterior pituitary [13]. Estrogen receptor alpha (ERα) activation in cerebrovascular tissue resulted in increased eNOS activity and protein levels [14]. Increased NO production by eNOS may contribute to the neuroprotective effects of estrogens. Galea *et al.* [15] hypothesized that the protective effects of E_2 in cerebral ischemia may be attributed to the blockade of leukocyte adhesion in cerebral endothelial cells. E_2 inhibited the basal and interleukin-1β (IL-1β)-mediated expression of the intercellular adhesion molecule type-1 (ICAM1) and NF-κβ activation, in cultured brain endothelial cells. *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 [16]. The authors suggested that estrogen modulates eNOS at the transcriptional level in blood vessels *in vivo*. Low E_2 results in reduced neuronal nitric oxide synthase (nNOS) and eNOS expression in hippocampus and E_2 substitution reversed these effects [17] suggesting that E_2 increases nNOS and eNOS expression and activity in hippocampus and improves hippocampal function.

II. A Vascular Bed with a Unique Physiological Function: The Penis

The penis is comprised of two cylindrical chambers, the corpora cavernosa, which comprises the erectile tissue. The tunica albuginea, a thick fibroelastic tissue surrounds the corpora cavernosa. The vascular bed of the erectile tissue encompasses several cellular and non-cellular elements, including interconnecting sinusoidal spaces, the endothelium lining the lacunar spaces, the trabecular smooth muscle and the fibroelastic connective tissue matrix. The cavernosal arteries provide arterial blood flow to the corpora through the resistance helicine arteries and arterioles. Venules located in the subtunical region permit venous blood outflow from the sinasoids. Corporal smooth muscle relaxation is considered essential for penile erection *via* increased arterial inflow and restriction of blood out flow. The endothelial cells of the cavernosal arteries, helicine arteries and arterioles as well as the endothelium lining the lacunar spaces play a critical role in regulating the physiological function of the penis. Thus, modulation of endothelium function in the penis by sex steroid hormones plays an important physiological role in erectile function and dysfunction.

III. Role of Endothelium in Vascular Function

The endothelium is characterized by a dynamic single cell layer, which regulates vascular homeostasis, acts as a semi-permeable layer, and functions as a physical barrier. The endothelium possesses autocrine, paracrine, and endocrine functions, which play a critical role in regulating vascular tone. The endothelium responds to various stimuli such as shear stress by releasing NO [18] and synthesizes and secretes vasoconstrictor molecules such as endothelin-1 (ET-1) and prostaglandin E_2 (PGE2). Moreover, the endothelium regulates homeostatic processes including platelet activation, aggregation, inflammation, immune function, vascular permeability, vascular smooth muscle cell proliferation, and angiogenesis [19]. Endothelial dysfunction is characterized by an imbalance in the expression and activity of the various signaling molecules producing alterations in the biochemical pathways regulating endothelial function therefore resulting in vascular disease such as atherosclerosis and hypertension [20].

IV. Biochemical and Clinical Markers of Endothelial Dysfunction

Endothelial dysfunction is characterized by significant modifications in the 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 fibrinolytic factors.

Clinical and biochemical markers of endothelial dysfunction include: a) reduced expression and activity of eNOS, reduced synthesis of NO, and increased production of asymmetric dimethylarginine (ADMA), a competitive, endogenous inhibitor of eNOS, b) increased production of reactive oxygen species (ROS) c) increased synthesis and release of the vasoconstrictor peptide ET-1, d) increased production of inflammatory cytokines such as interluekin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor alpha (TNF-α), e) increased expression of markers of cell adhesion such as E-selectin [21], intracellular adhesion molecule (ICAM) [22], and vascular cell adhesion molecule (VCAM), f) dysregulation of fibrinolytic factors such as Von Willebrand Factor (vWF), tissue plasminogen activator (tPA), and plasminogen activator inhibitor (PAI-1); g) inability to regenerate from endothelial progenitor cells (EPC); h) increased endothelial apoptosis; i) increased cellular permeability; j) increased vascular tone. In addition to the biochemical markers of endothelial dysfunction, diagnostic tools of endothelial dysfunction are characterized by flow mediated dilation (FMD) [23]. This clinical measurement of

endothelial function is strongly linked to coronary endothelial dysfunction and predicts cardiovascular events [24-26].

V. Role of Endothelial Dysfunction in Vascular Disease

The mechanisms by which vascular endothelium regulates vascular function involve multiple signaling pathways. Expression and activity of eNOS is critical for vascular function and decreased expression or reduced NO synthesis coupled with increased scavenging of NO by ROS or increased concentration of the competitive inhibitor, ADMA, contributes to vascular pathology [27]. Reduced NO synthesis and increased ADMA production has been linked to coronary artery disease and erectile dysfunction [28]. Similarly, increased production of ET-1 enhances vascular tone and promotes loss of vasodilatory properties [29, 30]. Endothelial dysfunction is also characterized by increased expression of markers of cell adhesion, E-selectin [21], soluble intercellular adhesion molecule (sICAM) [22], and VCAM [31]. Increased serum levels of these factors are indicative of endothelial dysfunction. Increased endothelial permeability has also been implicated as a risk factor for cardiovascular disease and this is attributed to invasion of the endothelium by lipoproteins, monocytes, and macrophages. This invasion promotes smooth muscle cell migration and proliferation [32] and facilitates the formation of lesions and atherosclerotic plaques [33]. With biochemical insult or injury, the endothelium becomes susceptible to apoptosis and loses its ability to regenerate. In addition, endothelial progenitor cells growth is regulated and may not compensate for the loss of endothelium *via* apoptosis, thus, exacerbating vascular permeability. Finally, dysregulation of fibrinolytic factors such as vWF, tPA, and PAI-1 also characterize endothelial dysfunction.

V1. Sex Steroid Hormones Regulate Endothelial Function

Considerable body of evidence exists linking sex steroid hormones deficiency to endothelial dysfunction [34, 35]. Low plasma T level was associated with endothelial dysfunction in men independent of other risk factors, suggesting a protective effect of endogenous T on the endothelium [36]. Gonadal hormones affect myogenic tone in male rat cerebral arteries through NOS- and/or endothelium-dependent mechanisms [37]. Low serum free T, estrone, and free Insulin-like Growth Factor (IGF) were inversely related to intima media thickness (IMT) [38]. Similarly, an inverse relationship exists between T level and thoracic IMT [39]. Examination of the endothelium from castrated rats by transmission electron microscopy demonstrated significant endothelium damage, in which the cell surface appeared crumpled, rough, adhesive and ruptured [40]. This pathology was partially restored by treatment of castrated rats with T or DHT. These observations strongly suggested that low concentrations of T or DHT are associated with ultrastructural damage of the aortic endothelium. Mäkinen *et al.* [41] have shown that middle-aged men with symptoms of androgen deficiency are at risk of increased carotid IMT and suggested that normal T levels may offer protection against the development of atherosclerosis in middle-aged men. Malkin *et al.* [42, 43] hypothesized that the immune-modulating properties of T are important in inhibiting atheroma formation and progression to acute coronary syndrome. The authors demonstrated that significant reduction in total cholesterol was recorded with T therapy and demonstrated a shift in the cytokine balance to a state of reduced inflammation. DHEA restored aortic eNOS levels and eNOS activity suggesting that DHEA may have direct genomic and non-genomic effects on the vascular wall [44, 45]. Liu & Dillon [46, 47] 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. This cellular mechanism may underlie some of the cardiovascular protective effects proposed for DHEA. Parenteral T therapy improves both endothelial-dependent (flow-mediated) and endothelium-independent brachial artery vasodilation in postmenopausal women using long-term estrogen therapy [48]. Suppression of endogenous estrogens with aromatase inhibitors resulted in impairment of FMD without significant changes in lipoproteins, homocysteine or CRP [49], suggesting that endogenous estrogens play a direct regulatory role in endothelial function in young healthy men.

VII. Biochemical and Clinical Markers of Endothelial Dysfunction

Fig. (**1**) provides a scheme of the potential mechanisms by which sex steroid hormone modulate endothelial and vascular function by multiple, and overlapping, signaling pathways. These reactions involve genomic and non-genomic mechanisms which stimulate endothelial function to produce endocrine and/or paracrine factors that affect the underlying vascular bed. Sex steroid deficiency contributes to endothelial and smooth muscle dysfunction and vascular disease. Fig. (**2**) illustrates the relationship between endothelial dysfunction, biochemical markers and the role of sex steroids on these parameters. In the proceeding sections, we discuss the effects of sex steroid hormones on expression and activities of endothelial biomarkers of function and dysfunction.

1. Endothelial Nitric Oxide Synthase (eNOS) Expression and Activity

Considerable evidence exists suggesting that eNOS is regulated by sex steroid hormones [44, 34, 50]. Marin *et al.* [51] demonstrated that castration reduced both nNOS and eNOS expression and activity and T treatment restored eNOS in corpora cavernosa. Treatment of bovine aortic endothelial cells (BAEC) with DHEA increased expression of eNOS [45, 47, 52] and stimulated an increase in NO secretion *via* PI3 kinase-dependent pathways [53] and in another study *via* non-genomic pathway with concomitant increase in cyclic guanine monophoshate (cGMP) release from endothelial cells [47]. Simoncini *et al.* [44] showed DHEA treatment in human umbilical vein endothelial cells (HUVEC) induced a concentration dependent increase in NO release in cultured medium *via* activation of eNOS *via* a non-genomic signaling pathway.

E_2 increases eNOS protein expression in rat cerebral microvessels *via* receptor mediated signal pathways [54] and E_2 treatment of BAEC caused eNOS translocation from the intracellular membrane to the nucleus *via* a Ca^{2+} dependent

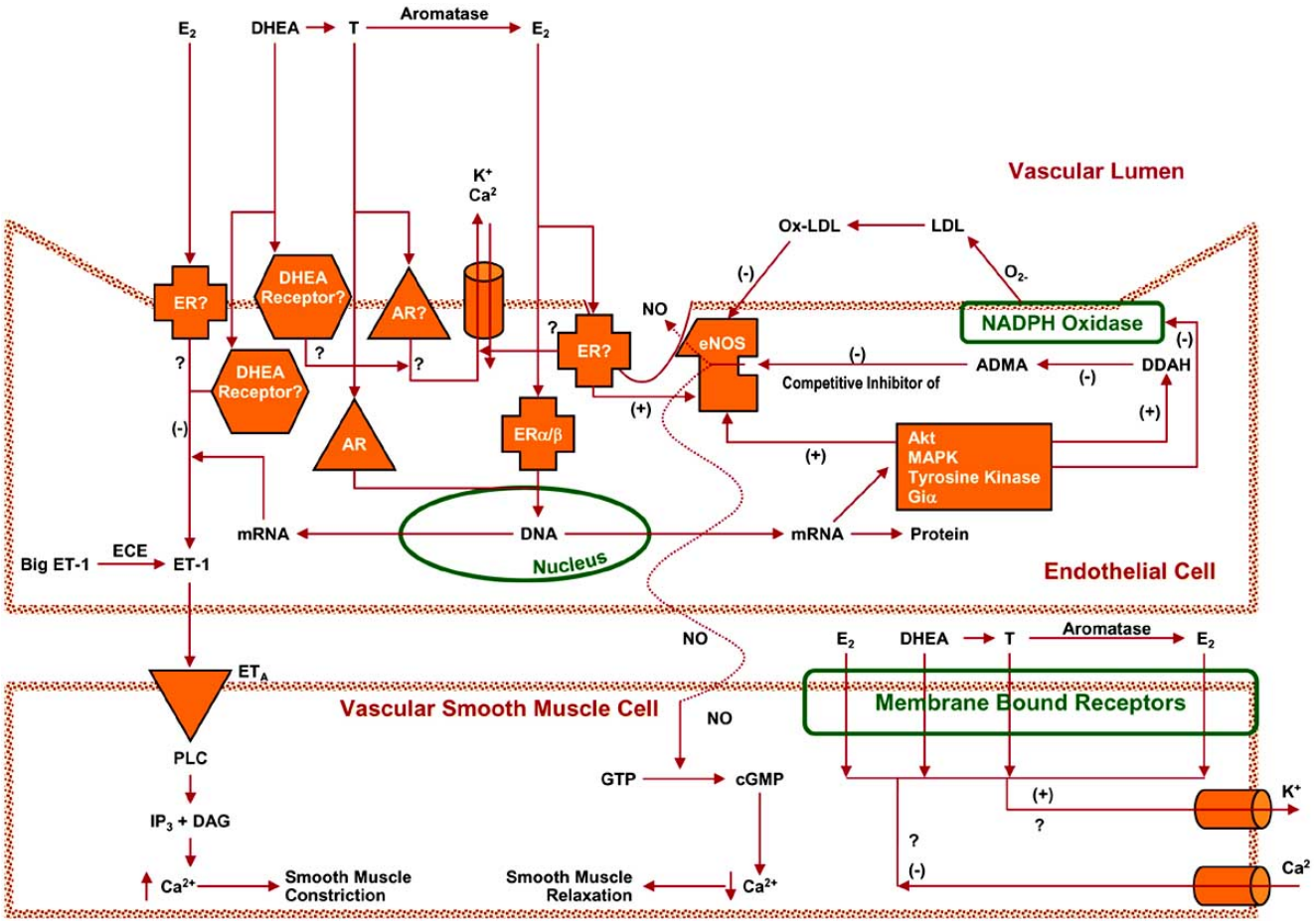

Fig. (1). Potential mechanisms of sex steroid hormones in the endothelium

This is a summary of the various mechanisms that could be involved in sex steroids and their influence on endothelial dysfunction. This depiction displays the vascular lumen, the endothelial cell, and the smooth vascular muscle cell and delineates the purported interplay between them. This figure points to the existence of non-genomic receptor elements of the sex steroids and theorized mechanisms. Abbreviations: Ox-LDL = Oxidized-LDL, eNOS = Endothelial Nitric Oxide Synthase, NO = Nitric Oxide, E_2 = Estradiol, DHEA = Dehydroepiandrosterone, T = Testosterone, P = Progesterone, AR = Androgen Receptor, ER = Estrogen Receptor, ECE = Endothelin Converting Enzyme, ET_A = Endothelin-1 Receptor, ET-1 = Endothelin Type-1, PLC = Phospholipase C, DAG = Diacylglycerol, IP3 = Inositol triphosphate , ADMA = Assymetrical Dimethylarginine, DDAH = Dimethlyarginine Dimethylaminohydrolase, GTP = Guanine Triphosphate, cGMP = Cyclic Guanine Monophosphate, ? = to show that the pathway is not fully elucidated.

mechanism [55]. Human endothelial cells treated with E_2 and progesterone for 48 hours showed increased eNOS activity and expression [56]. E_2 increased eNOS activity in aortic strips from Wistar rats [57] and HUVEC cells [58]. The mechanism purported here is activation of MAPK and PI3-Kinase which is coupled to eNOS.

2. Synthesis and Activity of ADMA

A study by Cakir *et al.* [59] involving 18 men with idiopathic hypogonadal hypogonadism showed higher serum levels of ADMA and L-arginine. Serum levels of ADMA decreased (5.72±2.64 to 4.36±1.12) and NO production increased 24 hours after treatment with T [59]. Leifke *et al.* [60] made similar observations in which a significant decrease in ADMA was noted in hypogonadal men treated with T gel. E_2 treatment decreased oxidized-LDL induced increase in ADMA and increased NO production in HUVEC cells [61]. E_2 treatment in HUVEC cells and in women with high plasma E_2 levels (> 2000 pg/ml) showed reduced (48%) ADMA and increased (56%) NO production [62]. Balloon injury to the common carotid of female rats that were bilaterally ovariectomized had an increase of ADMA and a decrease of NO production that was rescued with E_2 treatment (90 day sustained release pellet containing 1.5 mg) [63]. Dai *et al.* [64] treated male Sprague-Dawley rats with LDL and E_2 showed an attenuation of the subsequent ADMA rise and increased vasodilator response to acetylcholine compared to LDL pretreatment alone. Furthermore, it was shown that E_2 increased expression of Dimethylarginine Dimethylaminohydrolase (DDAH), an enzyme that catalyzes the metabolic breakdown of ADMA [65].

3. NADPH Oxidase and Reactive Oxygen Species

Increased production of superoxide is a marker of endothelial dysfunction due to the ability of superoxide to inhibit NO signaling and cause damage to endothelial cell organ-

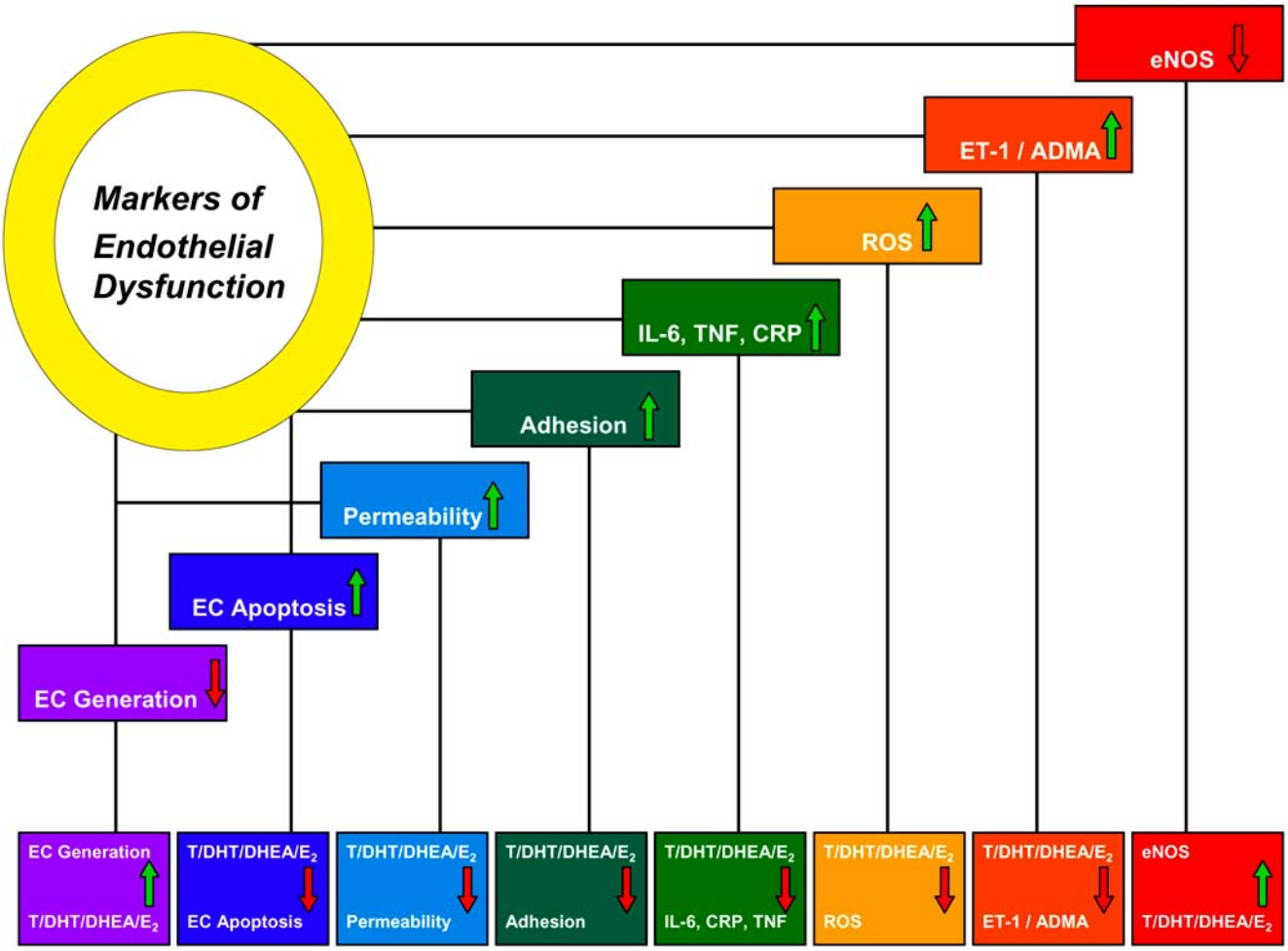

Fig. (2). Effects of sex steroid hormones on endothelial biomarkers

Endothelial dysfunction is characterized by decreased expression of eNOS, increased synthesis of ET-1, ADMA, ROS, IL-6, TNF-α, CRP, adhesion molecules, increased permeability, endothelial apoptosis, and reduced endothelial progenitor cell generation (depicted by arrows to denote increase or decrease). The effects of sex steroid hormones on the expression, synthesis and secretion of endothelial markers are denoted by arrows to indicate the changes (increase or decrease).

elles. Thus, reduction in superoxide levels represents a protective function. DHEA inhibited macrophage superoxide production as well as neutrophil and granulocyte proliferation [66] and inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulated superoxide anion (O_2-) formation by human neutrophils [67]. Brignardello *et al.* [68] showed that in ten patients with type-2 diabetes treatment with DHEA resulted in decreased ROS. DHEA also decreased TNF-α-induced ROS in human endothelial cells [69]. Male Wistar rats undergoing ischemic reperfusion of the kidneys show increased oxidative stress levels, which were reduced upon DHEA treatment [70].

E_2 inhibited NADPH oxidase in human monocytic cells and prevents accumulation of ROS [71] by inhibiting precursors of the NADPH oxidase reaction whereas the other sex steroids did not have an effect. Also, increased ROS were noted in ovariectomized mice and ROS levels were attenuated by E_2 treatment [72]. E_2 significantly reduced superoxide induced VSCM proliferation *via* E_2 dependent mechanisms in aortic smooth muscle cells of male rats [73]. E_2 and P treatment in female porcine coronary arteries decreased superoxide anion production by 67% while P treatment increased superoxide production by 59% respectively [74]. The purported theory for why P increased superoxide may be linked to regulation of NADPH oxidase.

4. ET-1 Expression and Activity

Takahashi *et al.* [75] demonstrated that endothelin converting enzyme and endothelin receptor subtypes A and B were up-regulated in castrated male Sprague-Dawley rats, suggesting an inhibitory effect of T. Kumanov *et al.* [76] reported that 33 patients with an average age of 21.58±0.69 years with various forms of hypogonadism had significantly higher ET-1 levels than 14 age-matched healthy controls. When these patients were treated with T depot intramuscularly over 6 months, ET-1 levels decreased, yet not significantly. DHEA treatment stimulated increased ET-1 protein expression *via* a non-genomic MAPK-dependent pathway in BAEC [53]. Peng *et al.* [77] showed that DHEA reversed ET-1 induced tension in human pulmonary artery ring smooth muscle cells *via* up-regulation of K_{ca} channel. DHEA also stimulated eNOS production and thus the balance of

DHEA between MAPK and PI 3-kinase signaling pathways may explain DHEA's conflicting reports discussing beneficial cardiovascular effects. Ba *et al.* [78] showed that E_2 attenuated ET-1 induced vasoconstriction in male rat aortic vessels following trauma-hemorrhage *via* an estrogen receptor type-beta (ERβ) mediated pathway that is independent of endothelium derived eNOS when compared to sham operated controls. E_2 administration in male Sprague-Dawley rats with aneurysmal subarachnoid hemorrhage (SAH) showed significantly reduced serum levels of ET-1 as compared to untreated SAH and control groups [79]. As can be seen, E_2 treatment consistently reduces ET-1 levels, *in vitro* and *in vivo* studies, *via* an estrogen receptor-dependent mechanism.

5. Vascular Tone

T treatment caused vasodilation in porcine coronary arteries [80] and T and DHT caused vasodilation in human umbilical arteries *via* a non-androgen receptor (AR) pathway [81]. Supraphysiological doses of T relaxed isolated radial arteries *via* activation of ATP/potassium channels [82] and caused vasodilation in internal mammary arteries [83]. T had a vasodilatory effect on rabbit tracheal smooth muscle mediated *via* eNOS [84]. T caused arterial relaxation *via* inhibition of Ca^{2+} entry into the smooth muscle cells [85, 86]. Furthermore, T has also been able to cause vasodilation in denuded vessels [83, 87, 88]. Sader *et al.* [89] has shown that 23 men with an average age of 32 years old were randomized into three groups receiving either 10 mg of T alone or in combination with 10 or 20 mg of E_2 and showed a dose-dependent increase in FMD. Webb *et al.* [90] showed that T treatment into the left coronary artery caused vasodilation and increased flow. The underlying molecular mechanism for the effect of T has yet to be completely elucidated. Yildiz & Seyrek [91] hypothesized that since denuded vasculature produced the same result the major effect of T is thought to be mediated directly by the vascular smooth muscle. The postulated mechanism suggests that T either activates K^+ channels to increase efflux and/or inhibits Ca^{2+} channels causing hyperpolarization and subsequent vasodilation. Because these changes occur in seconds to minutes, it is suggested that this action is likely to be mediated *via* the interactions with receptors on the membrane (non-genomic effect) rather than interaction with the nucleus (genomic effect). Furthermore, other studies have shown the presence of AR receptors on the membrane of vascular smooth muscle cells [92, 93] suggesting that the proposed mechanism is likely.

DHEA produced vasodilation in human umbilical arteries [81] and in porcine coronary arteries [80]. E_2 resulted in arterial relaxation *via* inhibition of Ca^{2+} entry into the smooth muscle cells [85] and inhibited intracellular Ca^{2+} increase *via* a non-genomic pathway [94] and E_2 produced vasorelaxation in human coronary arteries [95]. The purported mechanism for DHEA, E_2 and P are similar to those of T, non-genomic and with wide variation of potential mechanisms most likely due to variations in the vasculature. The major theme is that a Ca^{2+} transporter is inhibited, in the case of E_2, L-type Ca^{2+} channel is inhibited on the membrane of vascular smooth muscle cells [94]. Thus, sex steroid hormones have an important role in regulating vascular tone *via* endothelium dependent and independent pathways.

6. CRP, IL-6 and Inflammatory Markers

In men over the age of 70 years old, total T was inversely correlated with CRP [96]. No correlation was found between total or free T or DHEA and CRP in middle-aged and elderly men [97] and CRP did not change with T or DHT treatment. In 61 eugonadal men (ages 18-35) treated with T-enthanate for 20 weeks no changes in CRP levels were noted [98]. CRP levels also did not change with DHT treatment in androgen deficient men over the age of 60 [99]. DHT treatment did not affect IL-6 in human osteoblastic cells [100]. In eugonadal men, exogenous therapy with DHT and T did not increase inflammatory markers [99]. The overall data on CRP is inconclusive. T, DHT, and DHEA either elicits no effect or mitigates release of inflammatory cytokines and thus may have a protective role. Thus far, there is no consensus on this issue. In NC/Nga mice, a model for human atopic dermatitis, DHEA treatment prevented an age-induced increase in IL-6 production [101]. DHEA and DHEAS inhibited IL-6 production in healthy human derived monocytes [102]. Castrated mice receiving DHT treatment showed a decrease in release of pro-inflammatory cytokines interleukin IL-1β and IL-6 from splenic and peritoneal macrophages [103].

7. Expression and Function of VCAM, ICA and E-Selectin

T inhibited VCAM-1 mRNA and protein expression in HUVEC most likely *via* conversion to E_2 with endogenous aromatase [104]. Interestingly, T increased TNF-α-induced expression of E-selectin and VCAM-1 in HUVEC cells [105]. T treatment of castrated rabbits showed a decrease in sICAM, matrix metalloproteinase (MMP) and reduced plaque atherogenesis and aortic intimal thickness [106]. DHT treatments promoted vascular cell adhesion *via* up-regulation of VCAM-1, which is thought to occur mainly in the male endothelial cells but not in the female [107]. Incubation of HUVEC with DHT for 48 hours caused monocyte adhesion in a dose-dependent manner by increasing expression of VCAM-1. This reaction was blocked by addition of an anti-VCAM-1 antibody [108]. Thus it appears that T exhibited an endothelial protective effect whereas DHT had a deleterious effect. Aortic endothelial cells incubated with DHEAS for 48 hours and then treated with TNF-α caused up-regulation of ICAM and attenuation of VCAM *via* inhibition of NF-κβ [109]. DHEA inhibited oxidized LDL induced expression of VCAM/ICAM/PECAM-1 and U937 cells adhesion to HUVEC cells [110]. DHEA had no effect on VCAM/ICAM/E-Selectin in HUVEC cells [111]. DHEA inhibited adhesion of HUVEC cells with and without TNF-α induction and also inhibited ICAM-1 expression but not E-selectin expression [69]. E_2 showed no modulatory effect on ICAM expression in HUVEC [112] but increased TNF-α-induced expression of E-selectin and VCAM-1 [105]. E_2 reduced VCAM-1 expression *via* reduction of NF-κβ, activator protein-1 (AP-1), and GATA in human saphenous endothelial vein cells [113].

8. Expression and Function of TNF-α

T inhibited a myriad of leukocyte cytokine secretions including IL-2, IL-4, IL-10, IFN-δ, and TNF-α on peripheral leukocytes of healthy males [114]. Male rats with low serum T presented with higher incidences of cardiac failure and

also T lowered TNF-α mRNA expression [115]. Zhang attributed the mechanism to direct action of T on macrophages. However, a study in macrophages showed that T did not affect TNF-α release [116]. Furthermore, T treatment of castrated rabbits showed a decrease in TNFα and IL-6 [106]. DHEA and its analogs were shown to inhibit TNF-α production in J774A.1 cells, a murine macrophage cell line [117]. DHEA reduced TNF-α and TNF-α receptor system [68]. DHEA treatment of RAW 264.7 cells, a murine macrophage culture, significantly reduced TNF-α levels [118]. DHEA administration to NMR1 mice following induced sepsis is accompanied by a decrease in TNF-α release [119]. Male Wistar rats undergoing ischemic reperfusion of the kidneys showed increased TNF-α production that was reduced upon DHEA treatment *via* improvement of oxidative balance [70]. Lipopolysaccharide (LPS) induced TNF-α levels were significantly decreased with DHEA treatment in CD1 female mice [120]. This result was purported to be obtained by mitigation of endotoxic shock effects of the TNF-α pathway.

9. Endothelial Cell Apoptosis

Treatment of human umbilical vein cells (EA.H926) with T reduced Bcl-2 protein expression [121]. DHT had no effect in blocking fluvustatin induced apoptosis of endothelial cell line EA.H926 [122]. Supraphysiological doses of T given to HUVEC cells induced apoptosis [123]. DHEA treatment protects against endothelial cell apoptosis by up-regulating transcription and translation of the anti-apoptotic protein Bcl-2 [124]. HUVEC cells were treated with TNF-α and oxidized LDL to induce apoptosis. TNF-α–induced apoptosis, which was not altered by E_2 treatment after 6 hours, whereas oxidized LDL caused apoptosis at 24 hours and this was attenuated by E_2 *via* increases in the anti-apoptotic proteins, Bcl-2 and Bcl-xL [125]. E_2 treatment enhanced growth and reduced TNF-α induced apoptosis in EC's [126]. In human endometrial endothelial cells (HEEC), E_2 and P inhibited apoptosis [127]. Intracarotid artery injection of 0.01 mmol/L of hydrogen peroxide into eight-week female old rats caused endothelial cell apoptosis. Treatment with E_2 reduced the rate of apoptosis of EC's by 50%. However, treatment with P did not have an effect [128]. A study with excised resistance arteries from 66 post-menopausal women showed that E_2 treatment decreased signs of endothelial cell apoptosis [129]. The majority of findings point to a protective effect of sex steroids by inhibition of apoptotic genes and/or up-regulation of anti-apoptotic genes.

10. Endothelial Progenitor Cell Growth

Treatment of E304 endothelial cells with DHT increased endothelial cells and had a bimodal effect on vascular smooth muscle cells (VSMC's) in which high doses decreased proliferation and low doses increased proliferation [130]. T treatment increased endothelial cell proliferation *via* a MAPK kinase signaling pathway perhaps mediated by a putative G protein-coupled receptor on the plasma membrane [52]. Foresta *et al*. [131] showed that in 10 men with idiopathic hypogonadotropic hypogonadism had a lower serum EPC's compared to normal controls (37.3 and 98.1 cells/ml) and when T was administered the number of EPC's rose to 170.5 cells/ml in 6 months. The mechanism was attributed to an androgen receptor on CD34-positive cells [132]. DHEAS inhibited endothelial cell growth, whereas none of the other sex steroids had an effect [133]. DHEA also inhibited human umbilical vein cell proliferation in a dose-dependent manner *via* up-regulation of p53 and p21 and androgen/estrogen receptor independent mechanisms. E_2 increased cell proliferation, where as T inhibited cellular proliferation [134]. DHEA treatment increased EC proliferation independent of androgen receptor in BAEC [52]. It appears that DHEA or DHEAS effect on endothelial cell regeneration is pro-atherogenic and is mediated independent of androgen or estrogen pathways. E_2 enhances growth and reduces TNF-α induced apoptosis in EC's. The enhanced EC growth may be mediated *via* telomerase activity and attenuation of MAPK signaling [126]. Overall, it appears that sex steroids increase cellular regeneration through a host of mechanisms dependent or independent of genomic action of sex steroid receptors.

11. Vascular Permeability

Increased endothelial permeability is attributed to increased phosphorylation of occludin, the main component of gap junction content. When HUVEC cells were pretreated with E_2 and DHT for 24 hours occludin expression was increased which decreases permeability *via* increased MAPK signaling and perhaps *via* cytochrome C-oxidase modulation and this protects against endothelial dysfunction [135]. DHEAS substantially increased vascular permeability in male ddY mice [136]. This response was blocked by Diphenhydramine (DPH), a histamine receptor antagonist, implying that DHEAS induces histamine release and this affects permeability. Interestingly, DHEAS-induced increase in permeability was blocked by P. Topical application of Fluasterone (DHEA analog) increased vascular permeability in mouse skin [137]. The proposed mechanism is that DHEA inhibits glucose-6-phosphate dehydrogenase (G6PD), which reduces the supply of NADPH required species, which would subsequently lower permeability. E_2 treatment of HUVEC cells showed a decrease in endothelial cell permeability whereas P reversed the effect of E_2 [138]. V-Cadherin, which is known to be associated with vessel permeability [139], was up-regulated by E_2 and down-regulated by P. Sex steroids decrease permeability either with direct influence on occludins, cadherins, tight junctions, and other related compounds as well as indirectly through increased eNOS or reduction in ROS.

12. Expression and Function of PAI-1, vWF factor and tPA

HUVEC cells treated with physiological doses of T decreased PAI-1 levels [140] and increased the antigen levels of tPA. However, at a larger dose, antigen levels of tPA were decreased. BAEC treated with T showed biphasic modulation of PAI-1. At low concentrations PAI-1 was up-regulated and at high concentrations PAI-1 was down-regulated [141]. T treatment with two 2.5 mg patches daily for 12 weeks did not affect tPA and PAI-1 in 46 men (average age 62 years old) with chronic stable angina [142]. A study of 28 hemodialysis patients showed that there was no correlation between vWF and T [143]. T treatment in female to male transsexuals did not alter tPA or PAI-1 levels. During venous occlusion (VO) tPA increased whereas PAI-1 did not change at baseline and after 4 months of T treatment. Transdermal treatment was not effective compared to oral treatment [144]. In HEEC, E_2 and P showed no change in PAI-1 levels [127].

E_2 or P treatment of BAEC showed biphasic modulation of PAI-1. At low concentrations PAI-1 was up-regulated and at high concentrations PAI-1 was down-regulated [131]. In male to female transsexuals treated with oral ethinyl E_2 and cyproterone acetate (CA), an anti-androgen, reduced tPA and PAI-1 sharply. Serum levels of tPA changed during the (VO) before and 4 months into the ethinyl E_2 whereas PAI-1 did not change in either cases. Transdermal treatment was not effective compared to oral treatment [144]. DHEA treatment increased cGMP activity, a marker for NO production, which decreased PAI-1 in 24 healthy elderly men (65 year old) [52]. DHEA treatment (50 mg 3xper day for 12 days) for 18 men reduced PAI-1 (55.4±3.8 ng/ml to 38.6±3.3 ng/ml) and tPA (from 8.1±1.9 ng/mL to 5.4±1.3 ng/mL) [145]. Serum levels of DHEAS did not change as vWF, PAI-1 and tPA changed [146]. DHEA treatment (150 mg/daily 40 days duration) in men with DHEAS levels < 2000 mg/l and verified coronary heart disease (CHD) did not influence PAI-1 and tPA plasma concentrations [147].

VII. Implications of Sex Steroid Hormone Deficiency in Endothelial Dysfunction

With age, circulating levels of sex steroid hormones decrease in both men [148, 149] and women [150]. The Rotterdam study, a population based cohort study, showed low levels of endogenous androgens are associated with increased likelihood of atherosclerosis in elderly men [151]. Svartberg demonstrated an inverse relationship between total T levels and carotid IMT [152]. This finding was not independent of body mass index (BMI). Accumulating evidence has shown an association of low T with cardiovascular mortality, morbidity in men of varying age, and cardiovascular risk factors [36, 39, 41, 153, 154]. Men have a higher rate of cardiovascular diseases (CVD) than females. The likely culprits appear to be T, DHT, DHEA and their metabolites. Capaldo *et al.* [155] showed that men with sex steroid deficiency had a greater IMT thickness. According to Akishita *et al.* [36], low plasma T levels were associated with endothelial dysfunction independent of other factors. Low plasma T has also been associated with cardiovascular risk in healthy men [156]. Akishita *et al.* [157] also found that DHEAS levels correlate with FMD analysis and it was irrespective of other confounding factors in women.

A poignant view on the effects of androgen deficiency on vascular function can be seen with the adverse effects of androgen deprivation therapy (ADT) in prostate cancer patients. ADT, whether *via* orchiectomy or use of GnRH agonists or antagonists results in low circulating T/DHT with concomitant changes in body composition, insulin resistance and vascular disease [158]. There is a decrease in lean muscle mass and an increase in fat mass. A long-term study (1-8 months) comparing men undergoing ADT to eugonadal men found an increase in fat mass compared to controls of eugonadal men [159]. ADT has been implicated in inducing metabolic syndrome [160]. Overall, ADT in men with prostate cancer has been shown to increase risk of cardiovascular events [161]. Keating *et al.* [162] reported that men undergoing ADT had 25% increase in risk of coronary artery disease compared to non-ADT. In a large study consisting of 23,000 men undergoing ADT for at least 12 months showed an increase of cardiovascular morbidity by 20% compared to non-ADT men after controlling for confounding factors [163]. A recent report found that men receiving ADT were approximately 2.6 times at greater risk of cardiovascular mortality than non-ADT controls after adjusting for confounding factors [164]. Interestingly a new study by D'amico *et al.* [165] have suggested that elderly men with T-1 to T-2 localized prostate cancer should not be given primary ADT due to reduced overall survival in these patients. Montalcini *et al.* [166] showed that post-menopausal women in the lowest T tertile had the least FMD which implies that not only does estrogen deficiency play a role in cardiovascular disease, but T deficiency as well.

VIII. Endothelium Dysfunction Contributes to Erectile Dysfunction

Erectile dysfunction (ED) and atherosclerosis share similar risk factors [167]. It has been hypothesized that ED may be an early warning marker for cardiovascular disease [168, 169] Gazzaruso *et al.* [170] has shown a higher incidence of ED among men with diabetes and overt and silent cardiovascular artery disease (CAD). In patients with CAD, the prevalence of ED was 8 times more likely [171]. Montorsi *et al.* [172] showed that in men with CAD, the incidence of ED was approximately 49%. It was also shown that there was a correlation between ED and cardiovascular morbidity in 132 men [173]. Nurkalem *et al.* [174] showed reduced coronary blood flow in ED patients. In a study comprising 9,000 men, ED was found to independently predict cardiovascular disease at a rate similar to smokers, patients with familial cardiovascular disease or hypercholesteremia [169]. It is known that ED and CAD both arise from the underlying endothelial dysfunction [175, 176]. An important cause of both ED and endothelial dysfunction arises out of a decreased production of NO or down-regulation of eNOS, NO is vital in vasorelaxation as well as modulating smooth muscle cells and inhibiting cellular adhesion [177].

DISCUSSION AND CONCLUSIONS

Erectile dysfunction is a neurovascular process that requires healthy central and peripheral nervous systems and the peripheral vascular beds. Erectile dysfunction has received great attention over the past decades and this is attributed to the advances in research made in vascular biology of the erectile tissue. Further, new information is emerging suggesting that the central nervous system play a critical role in the regulation of the mechanism involved in sexual function and behavior *via* neurosteroids.

The endothelium plays a critical role in the physiological function of all vascular beds, maintaining vascular homeostasis 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, and EDHF are integral to maintenance of physiological function. Endothelial damage due to various insults contribute to vascular disease and erectile dysfunction.

Considerable body of literature is available indicating that steroid hormones modulate endothelial function in all vascular beds including the brain and the penis and their deficiency 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 T and DHT are associated with ultrastructural damage of the aortic endothelium. Also, endothelial dysfunction in men is associated with low plasma testosterone level independent of other risk factors, suggesting a protective effect of testosterone on the endothelium. Furthermore, free testosterone level is inversely correlated with VCAM-1 concentration and IMT, which are indicators of endothelial function. Several studies have also corroborated that DHEA also improved endothelial function in vascular beds. These observations point to the clinical relevance of sex steroid in vascular health and to treating patients with hormonal deficiencies with appropriate physiological hormone levels formulations. Better understanding of the role of sex steroid hormones in regulating endothelial function is critical to translation of the basic research into treatment of patients with metabolic syndrome, vascular disease and erectile dysfunction.

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ABBREVIATIONS

ADMA = Assymetric Dimethylarginine

ADT = Androgen Deprivation Therapy

AP-1 = Activator Protein-1

AR = Androgen Receptor

ATP = Adenosine Triphosphate

BAEC = Bovine Aortic Endothelial Cells

Bcl-2 = Anti-Apoptotic Protein

Bcl-xL = Anti-Apoptotic Protein

BMI = Body Mass Index

BNST = Bed Nucleus of the Stria Terminalis

CAD = Cardiovascular Artery Disease

cGMP = Cyclic Guanine Monophosphate

CHD = Coronary Heart Disease

CN = Cavernous Nerve

CRP = C-Reactive Protein

CVD = Cardiovascular Disease

DDAH = Dimethlyarginine Dimethylaminohydrolase

DHEA = Dihydroepiandrosterone

DHT = Dihydrotestosterone

DPH = Diphenhydramine

E_2 = Estradiol or 17β-estradiol

EA.H926 = Human Umbilical Vein Cells

EC = Endothelial Cells

ED = Erectile Dysfunction

eNOS = Endothelial Nitric Oxide Synthase

EPC = Endothelial Progenitor Cells

ERα = Estrogen Receptor Alpha

ERβ = Estrogen Receptor Beta

ET-1 = Endothelin-1

FMD = Flow Mediated Dilation

G6PD = Glucose-6-phosphate-dehydrogenase

GATA = GATA Transcription Factor

GnRH = Gonadotropin Releasing Hormone

HEEC = Human Endometrial Endothelial Cells

HUVEC = Human Umbilical Vascular Endothelial Cells

ICAM1 = Intercellular Adhesion Molecular Type 1

ICP = Intracavernous Pressure

INF-γ = Interferon-γ

IGF = Insulin-like Growth Factor

IL-1β = Interleukin-1 Beta

IL-2 = Interleukin-2

IL-4 = Interleukin-4

IL-6 = Interleukin-6

IL-10 = Interleukin-10

IMT = Intima Media Thickness

J774A.1 = Murine Macrophage cell line

LDL = Low Density Lipoprotein

LDT = Laterodorsal Tegmental Nucleus

LPS = Lipopolysaccharide

MAPK = Mitogen-Activated Protein (MAP) Kinases

MMP = Matrix Metalloproteinase

MPOA = Medial Preoptic Area

NADPH = Nicotinamide Adenine Dinucleotide Phosphate

NC/Nga = A model animal for human atopic dermatitis

nNOS = Neuronal Nitric Oxide Synthase

NO = Nitric Oxide

O_2- = Superoxide anion

P = Progesterone

P21 = Cyclin-Dependent Kinase Inhibitor 1A

P53 = Tumor Protein 53

PAI-1 = Plasminogen Activator Inhibitor type 1

PECAM-1 = Platelet Endothelial Cell Adhesion Molecule

PGE2 = Prostaglandin E2

PI-3 Kinase = Phosphate Inisitol-3-Kinase

POA = Preoptic Area

PVN = Paraventricular Nucleus

RAW 264.7 = A murine Macrophage Cell Line

ROS = Reactive Oxygen Species

SAH = Aneurysmal Subarachnoid Hemorrhage

T = Testosterone

TNF-α = Tumor Necrosis Factor-alpha

tPA = Tissue Plasminogen Activator

TPA = Tetradecanoylphorbol-13-acetate

VCAM = Vascular Cell Adhesion Molecule

VEGF = Vascular Endothelial growth factor

VO = Venous Occlusion

VSMC = Vascular Smooth Cell Muscle

vWF = Von Willebrand Factor

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