Pharmacological Reports
2008, 60, 49–60
ISSN 1734-1140

Review

Gender and the endothelium

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Abstract:
The understanding of the basis of gender differences in vascular function is of critical importance to establish gender targeted interventions in cardiovascular medicine. In this review we concentrate on the central role of the endothelium in respect to gender differences in cardiovascular physiology and pathophysiology. The role of estrogen and its receptors is introduced not only as key players in gender-related differences in incidence of cardiovascular abnormalities but also in endothelium-dependent maintenance of vascular tone through the release of endothelium-derived vasodilators and vasoconstrictors. An improved understanding of the distinct processes that confer vascular maintenance in women and men will help to develop new treatment alternatives and improve the use of existing drugs.

Key words:
gender, cardiovascular, endothelium, estrogen, NO, EDHF, HRT

Introduction

Gender is one of the factors linked to differences in cardiovascular morbidity and mortality. In the younger age groups (25 to 49 years) coronary heart disease (CHD) is two to five times more common in men than in women [51] suggesting that premenopausal women are protected from cardiovascular diseases (CVD), however this protection is lost after menopause. It is well accepted that the risk for CHD increases markedly with age in both men and women, yet this is more pronounced in women older than fifty [51, 52]. In Europe, about 55% of all female deaths are caused by CVD, particularly CHD and stroke, compared with 44% of all male deaths [39]. Age-adjusted mortality for CVD is continuously declining in the last four decades, but this occurs to a lesser extent in women [111]. Until the last decade, CVD in women have been underestimated due to a lower prevalence rates in younger age groups, and due to representation of CVD as a male disorder. It was probably simply assumed that the knowledge derived from studies on men is applicable to women [79]. Recent retrospective analyses, however, indicate that there are clinically relevant differences between women and men in terms of prevalence, presentation, management and outcomes of CVD [90] and enhanced understanding to why CVD affects women and men differently is highly warranted. In this review we concentrate on the central role of the endothelium in gender-related differences in cardiovascular physiology and pathophysiology.

The endothelium as a major contributor for gender differences in vasculature

The vascular endothelium is a highly active tissue regulating vascular tone, leucocytes and platelet acti-

vation, angiogenesis, inflammatory response, permeability and the metabolism of vascular mediators. Growing appreciation of its physiological activities is accompanied by recognition that endothelial dysfunction contributes to a wide range of pathophysiological processes including hypertension, CHD, stroke, diabetes and atherosclerosis in both genders. In this review we focus on an ability of endothelial cells (EC) to release vasoactive substances that confer the control of vascular tone.

The vasodilator capacity of the endothelium is accounted mainly by three powerful factors.

1) Prostacyclin (PGI_2) – a cyclooxygenase (COX)-dependent metabolite of arachidonic acid (AA) [25]. PGI_2 elicits relaxation through binding to specific cell-surface PGI_2 receptors resulting in G-protein mediated activation of adenylate cyclase and formation of cAMP. This phosphorylates protein kinase A and resuls in the reduction of calcium in vascular smooth muscle cells (SMC) and vasodilatation [78].

2) Nitric oxide (NO) is produced by NO synthase (NOS) from the semi-essential amino acid L-arginine and molecular oxygen [89]. There are three isoforms of NOS – two constitutive forms NOSI or neuronal (nNOS) and NOSIII or endothelial (eNOS) and one inducible form NOSII (iNOS). NOSI and NOSIII are expressed constantly and require calcium/calmodulin interaction for activation, whereas NOSII is inducible by cytokines and it is calcium-independent. Several co-factors are required for regulation of NOSIII activity, including tetrahydrobiopterin, NADPH, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN) [32], and heat-shock protein 90, which acts as a chaperone and facilitates NOSIII activity [33]. Once released by the endothelium NO activates soluble guanylyl cyclase of SMC, with following increase in intracellular cGMP and activation of protein kinase G leading to reduced intracellular calcium and relaxation [42].

3) Endothelium-derived hyperpolarizing factor (EDHF) has a variable nature and mechanisms of action that are related to the endothelium-dependent hyperpolarisation of SMC. Currently EDHF is appreciated as a substance and/or electrical signal that is generated or synthesized in and released from the hyperpolarized endothelium, which in turn hyperpolarizes SMC with consequent relaxation [30]. To date the cytochrome P450 (CYP450) products of AA [95], potassium ions [26], hydrogen peroxide [72] and C-type natriuretic peptide (CNP) [13] have been introduced as potential candidates for EDHF. EC hyperpolarization could be also transmitted to SMC through myoendothelial gap junctions (MEGJ) [14] that are clusters of intercellular channels formed by connexin (Cx) proteins.

It is important to mention also endothelium-derived vasoconstrictiors and a 21-amino-acid peptide endothelin (ET)-1 has been introduced as the most powerful vasoconstrictor described so far. Endothelium is the main place for its generation within the vascular wall and ET-1 acts as the natural counterpart to endothelium-derived vasodilators [105]. ET-1 interacts with two different G protein coupled receptors, ET-A and ET-B. ET-A receptors are expressed predominantly on the membranes of SMC and they mediate vasoconstriction [21]. ET-B receptors are expressed predominantly on EC [23] and they mediate vasodilatation through generation of NO [110] and PGI_2 [83]. ET-B receptors are also found on SMC, where they induce constriction [10].

Current evidence supports the role of endothelium in gender-related differences, and the cardiovascular protection in pre-menopausal women is mainly attributed to an enhanced vasodilative capacity of the endothelium. This is reflected by a number of studies, which demonstrate that basal and agonists-induced NO release from endothelium is elevated in vasculature from females compared to males [55, 85, 121]. The vascular bed specificity for gender differences may also exist, since recent study of Ahmed at al. [1] suggests that particularly renal vasculature of men becomes more dependent on NO with age compared with that of women, indicating that any renal disease that interferes with NO production may, over time, cause existent kidney damage to progress more quickly in men relative to women.

The pro-oxidant environment is also less pronounced in women, as reflected by different activity and expression of vascular NADPH oxidase [76] and plasma markers of oxidative stress [50, 93] further suggesting an enhanced *in vivo* inactivation of NO in males rather than females. An endogenous inhibition of NOS is also important, as concentrations of asymmetric dimethylarginine (ADMA) in female volunteers younger than 50 years is lower than that in males of comparable age, but increases significantly after menopause [100]. Gender related difference in vascular effects to ET-1 is also observed, and ET-1 concentrations are usually higher in men *vs.* women [91], and it is reduced in male to female transsexuals and increased in female to male transsexuals after hormonal replacement therapy (HRT) [77].

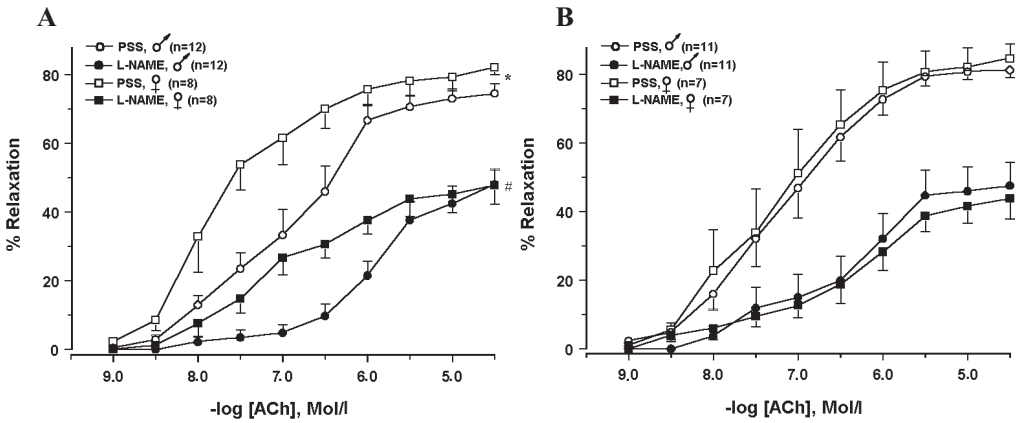

Fig. 1. Concentration-response curves to ACh in femoral arteries isolated from WT mice in physiological salt solution (PSS) and after incubation with combination of N^{W}-nitro-L-arginine methyl ester (100 µmol/l) plus N^{W}-nitro-L-arginine (300 µM) plus 10 µM of indomethacin (L-NAME). (**A**) Responses to ACh in distal femoral arteries (DFA, internal diameter < 200 µm) in female *vs.* male mice in PSS and after L-NAME. * $p < 0.05$ female *vs.* male in PSS; # $p < 0.05$ female *vs.* male after L-NAME (ANOVA). (**B**) Concentration-response curves to ACh in proximal femoral arteries (PFA, internal diameter > 200 µm) in female *vs.* male mice in PSS and after L-NAME. (Reprinted with permission from The Journal of Physiology [69])

An enhanced production of COX-dependent products [5] and increased contribution of EDHF-mediated responses are also pertinent to female vasculature. Experimental evidence has suggested that EDHF appears to be more important in female small arteries to confer endothelium-dependent dilatation, while NO plays a predominant role in arteries from males, as demonstrated in several vascular beds (mesenteric, tail) from rats [73, 88, 116]. If under normal physiological conditions the functional role of EDHF is more significant in females, it has been suggested that in pathological conditions EDHF may serve as a compensatory pathway against the loss of NO in female rather than in male arteries [73].

Our group has also reported an enhanced endothelium-dependent relaxation in female *vs.* male murine femoral arteries [69]. It is important to stress that this gender-related difference in endothelium-dependent relaxation was a distinguishing feature of arteries with internal diameter (ID) < 200 µm, as in larger arteries no difference was observed (Fig. 1) [69]. Since gender related differences in ACh-induced relaxation persisted after inhibition of NOS and COX products, we suggested that EDHF, rather than NO or PGI_2, is involved. However, we failed to obtain gender-related differences in ACh-induced relaxation in small (ID < 200 µm) arteries from the mesentery in the same animal model, further indicating the importance of vascular bed studied or experimental systems used (wire *vs.* pressure myograph) when determining gender related differences in endothelium-dependent dilatation [24].

Recently EDHF has been also implicated in gender-related differences in blood pressure control. The generation of animals, which lack both eNOS and COX-1, i.e., the "EDHF mouse", has allowed a direct assessment of the involvement of EDHF to endothelium-dependent relaxation in small arteries [101]. In eNOS/COX-1 double knockout mice, EDHF-mediated response appeared to compensate the absence of endothelial NO in females, but not in males. In female mice, the deletion of eNOS and COX-1 did not affect mean arterial blood pressure, while males become hypertensive [101]. The same group has also reported the presence of gender differences in the increased blood pressure in eNOS knockout mice [101], the observation that contrasts to previous reports about similar level of hypertension between females and males in eNOS knockout mice [48, 63, 104].

The role of estrogen

Estrogen has been introduced not only as a key player in gender-related differences in incidence of CVD but also in endothelium-dependent maintenance of vascular tone [75]. Number of reports have shown that this hormone may enhance NO-mediated relaxation [11, 64, 80, 96]. The estrogen's complex effect on NO/NOS

system is also corroborated by its direct anti-oxidant effect, thus increasing the lifespan of NO. The aromatic ring present in estrogen, which is absent in progestins and androgens, may function as an anti-oxidant moiety [76]. Indeed, the superoxide anion levels are increased in arteries after ovariectomy and normalized following hormone replacement with 17β-estradiol (17β-E_2) [19, 31, 113]. Also the correlations between ADMA and age in women has been attributed to differences in estrogen levels, since a significant increase in ADMA concentration was observed after onset of the menopause [100].

Estrogen also inhibits constrictions to ET-l in coronary microvessels isolated from male and female dogs [61], reduces ET-1 expression in human EC and inhibits its basal and thrombin-stimulated production of ET-1 [9]. Long-term blockade of ET_A receptors reduces blood pressure in post-menopausal SHR to a level found in young females [120], and expression of preproendothelin-1 is significantly up-regulated in ovarectomised (OVX) pigs [112]. Estrogen is also associated with a decreased plasma ET-1 level in OVX rabbits [124] and in post-menopausal women [8]. After OVX, ET-1 mRNA expression is enhanced in mesenteric arteries of hypertensive and normotensive rats, whereas ET_B receptor mRNA expression is enhanced only in hypertensive animals and 17β-E_2 reverses all these effects [22].

The role of estrogen on PGI_2 production is less conclusive, since both positive [34, 53, 86] and negative [46, 118] results have been reported in different experimental setups including isolated artery systems or cultured EC.

Estrogen may also modulate EDHF-mediated relaxation [47, 66, 109], and this has been suggested to serve as an additional mechanism for cardiovascular protection by HRT [67, 98]. Isolated small arteries from OVX animals demonstrate a severely impaired EDHF-mediated hyperpolarization, which is reversed by 17β-E_2 replacement therapy [67]. ACh induced EDHF-mediated relaxation is also greater in mesenteric arteries from male compared to OVX rats. A possible explanation for this gender difference in EDHF-mediated response has been attributed to the difference in circulating estrogen levels, since levels of 17β-E_2 are higher in male rats than in OVX females due to a metabolism of testosterone by aromatase in the adipose tissue [98]. Furthermore, EDHF-mediated response to ACh is suppressed in arteries from diestrus females, although its extent is less severe than in OVX rats, suggesting that even short-term estrogen deficiency could explicitly diminish EDHF-mediated reactivity [66].

Prolonged administration of 17β-E_2 or isoflavone daidzein to male rats resultes in an enhanced contribution of EDHF to endothelium-dependent relaxation in isolated aorta, in which ACh-mediated dilatation is usually entirely mediated by NO. The level of EDHF-mediated relaxation is up to 30%, and CYP450 metabolite of AA and K^+ ions *per se* have been introduced as potential mechanisms for the EDHF response [118].

Estrogen modulates EDHF contribution to flow-induce shear stress when NO activity is compromised, as reflected by studies on skeletal muscle arterioles. Flow response in L-NAME-treated male and OVX female rats is solely mediated by prostaglandins, whereas 17β-E_2 replacement switches that to an EDHF-mediated response, recovering the similar profile observed in NO-deficient arterioles obtained from intact female rats [47]. EDHF is also involved in the acute 17β-E_2 -mediated-relaxation in coronary arteries from both female and male rats, and presents a constitutive component of 17β-E_2 mediated response as reflected by inhibition of CYP450 pathway [99].

The mechanisms behind the beneficial effects of estrogen on EDHF-mediated responses are associated with functional and reversible alterations in the membrane composition, ion channels, signal transduction or receptors [66]. Indeed,17β-E_2 has been shown to acutely enhance the activity of the Ca^{2+}-dependent K^+-channels in ECs of the rabbit aorta [97] and in human coronary arteries [115]. Chronic treatment with 17β-E_2 prevents impairment of EDHF-mediated relaxation in hypercholesterolemic rabbit carotid arteries through activation of both Ca^{2+}-dependent and ATP-sensitive K^+-channels [35]. The K_{IR} channels are also of interest, as K^+-induced cerebral vasodilatation *in vivo* is greater in female than in male rats, and this vasodilatation is reduced after OVX and restored by 17β-E_2 [15].

The MEGJ pathway is also pertinent for 17β-E_2 mediated up-regulation of EDHF-responses. The number of gap junctions, at least in myometrial cells, depends on hormonal milieu and their number increases with increasing estrogen but reduced progesterone levels [70]. A significant reduction in the expression of Cx 43 protein occurs in mesenteric arteries from OVX rats, while supplementation with 17β-E_2 completely prevents the reduction to a level

similar in control animals [67]. However, additional studies on mechanisms by which estrogen influence EDHF-typed responses in small arteries under different hormonal environment are highly warranted.

The role of estrogen receptors subtypes

Two known estrogen receptors (ERs) ERα and ERβ are expressed in ECs and SMCs of both genders [43]. ERα can be considered as a principal receptor found in association with estrogen-induced modulation of endothelial NO synthesis [20, 81, 108]. The basal release of endothelium-derived NO is decreased in male ERα-knockout (ERαKO) mice compared with wild-type (WT) mice [96]. An important role of ERs (presumably ERα) in human vasculature in males is supported by observation in a young man with disruptive mutation in the ER gene (presumably ERα). This subject was found to have a severe endothelial dysfunction and premature coronary atherosclerosis at age of 31 years [106].

Estrogen increases urinary excretion of PGI_2 metabolites in ERβ- but not ERα-deficient mice [27]. ERβ is also indicated to play an important part in cardiovascular response to estrogen. Endothelial denudation leads to an increased levels of ERβ in male rat aorta, while ERα levels remain unchanged [65]. Recent studies indicate that ER subtypes may control or counteract activity of each other in the vasculature [12, 17, 96, 125], as, for example, ERβ offsets the rapid ERα-mediated NO release in small arteries from mice or healthy male [16] or estrogen-stimulated induction of iNOS mediated by ERβ is antagonized by ERα [125].

Mice double deficient in eNOS and apoprotein-E are hypertensive and atherosclerotic, however gonadectomy reduces blood pressure and atherosclerosis in animals of both genders [44]. Levin [62] suggested that ERα might mediate the hypertension-promoting actions of estrogen when ERβ or eNOS is perturbed. Akishita et al. [2] demonstrated that the inhibitory effect of estrogen on ET-1 production and its mRNA expression are blocked by estrogen receptor antagonist, ICI 182,780, suggesting an ER-dependent pathway. The ERβ has been implicated, since 17β-E_2, the ERβ agonist DPN (diarylpropionitrile), but not the ERα agonist PPT, attenuated the ET-1-induced constriction of the aorta from male rats [4]. It is important to note, however, that over expression of ERα in ECs dramatically decreases the ET-1 secretion [3].

Absence of numerous ERβ-regulated gene products has been associated with abnormal vascular contraction, ion channel function and hypertension in ERβ knockout (ERβKO) mice [125]. The study reported that prior to the aging period (i.e. 6 month) ERβKO mice develop hypertension in both genders with blood pressure in males being significantly

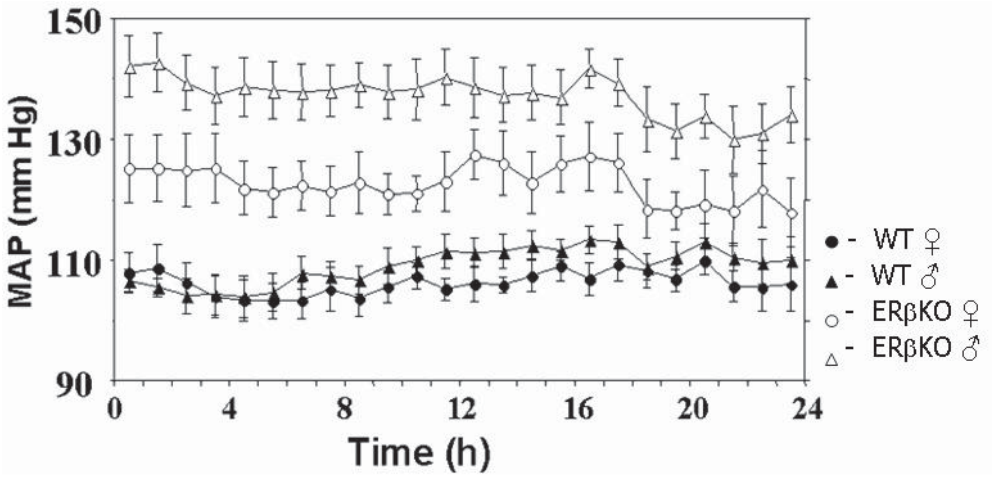

Fig. 2. Mean arterial pressure (MAP) in conscious female and male WT and ERβKO mice at 6 months of age. MAP in male ERβKOs (137 ± 4.6 mm Hg, n = 9) was significantly higher ($p < 0.01$) than that in WT males (109 ± 2.7 mmHg, n=10), or female ERβKOs (123 ± 4.9 mmHg, n = 10). MAP in female ERβKOs was significantly increased ($p < 0.01$) compared to that of WT females (106 ± 2.9 mmHg, n=10) [125]. (Modified and reprinted with permission from *Prof. Michael E. Mendelsohn*)

higher than in ERβKO females without any difference in WT animals (Fig. 2). An increased basal NO release, but decreased sensitivity to ACh has been observed in aortic rings from OVX ERβKO and WT mice after 17β-E_2 supplementation. This effect was not observed in ERαKO mice, suggesting that ERα, but not ERβ, could be involved in the modulation of basal NO production by estrogen [20].

We have recently shown that ERβ confers gender differences in endothelium-dependent relaxation in small femoral arteries from mice by reducing the contribution of EDHF-induced relaxation through gap junction communications in male arteries. The up-regulation of the EDHF pathway in ERβKO males with no change in female arteries, does not support the suggestion that an alteration in endothelial function may predispose these animals to the development of hypertension as they age [69], however ERβ modulated enhancement of adrenergic stimulation and alterations in structural properties of vascular wall in male arteries, as demonstrated in our separate studies [24, 68], could be of interest.

Hormone replacement therapy and CVD

A large body of experimental and epidemiological evidence in postmenopausal women with and without HRT has demonstrated the beneficial cardiovascular action of estrogen. Our own group has recently described functional and morphological abnormalities present at the level of resistance vasculature in healthy postmenopausal women, and that 17β-estradiol improved endothelium-dependent dilatation *via* NO pathway [60]. However, data from controlled, prospective randomized clinical trials for primary [94] and secondary prevention [49] by HRT have produced an equivocal result towards harm characterized by increased risk for cancer [92, 119] and by adverse effect on the cardiovascular system through activation of pro-coagulant and plaque-destabilizing systems [38]. The outcomes of these trials have been comprehensively dissected in several excellent reviews, and major features that may account for the apparent lack of cardiovascular benefit of HRT have been identified [36, 41, 87].

The above issues have motivated basic scientists and clinicians to re-evaluate the benefits and risks of combine HRT at special regime and in a define group of women at certain age, including those with CVD, and at the time when menopause commences ("the timing hypothesis"). There is now significant evidence supporting the concept that age or time since menopause may influence the benefit/risk ratio associated with HRT, particularly in respect to cardiovascular outcomes [45, 71]. Thus, although the evidence suggests that older women and those with subclinical or overt CHD should not take HRT, the effect of estrogen on the development of CHD in perimenopausal women deserves further investigation and two prospective clinical studies have directly addressed the question if the potential reduction in development of CHD by HRT occurs in perimenopausal women. The KEEPS (Kronos Early Estrogen Protection Study) has enrolled 720 perimenopausal women between the ages of 40 and 55 years, and will report after 5 years in 2010 [28]. To test the importance of route of administration, women in this study will receive either oral or transdermal estrogen. Women will also receive oral progesterone (for 12 days of the month). The end point for this study is the intima-media thickness of the carotid and coronary arteries. The ELITE trial is designed to investigate the differences between peri- and postmenopausal women. It will also run for 5 years, reporting in 2010. Women were considered perimenopausal if they were menopausal for < 6 years compared with postmenopausal women who were menopausal for > 10 years. Women will receive oral 17β-estradiol, and those with an intact uterus will also receive progesterone vaginal gel for the last 10 days of every month. The end point of this study, as in KEEPS, is the intima-media thickness of the carotid and coronary arteries. The results of these trials may help in targeting of HRT to a more appropriate population, however it should be stressed that further research is needed to answer the question how targeted HRT effects the vascular function with focus on endothelium-dependent dilatation in combination with other vascular surrogate markers.

Pharmacogenomics (determining susceptibility to drug treatment based on an understanding of an individual's genetic profile) may also have an impact on the future use of HRT. Moreover, the development of pure agonists or antagonists for ER subtypes might provide benefits of estrogen replacement therapy with reduced side effects. Indeed, selective activation of ERα and ERβ, both of which differ in tissue-specific expression and biological function, appears as a novel

pharmacological principle to improve the safety and efficiency of ER-selective ligands in the prevention of CVD [117], although it can't be excluded that these compounds may have other non-cardiovascular actions. It is well known that the physiological effects of estrogen on gene transcription are determined by the distinctive combination of co-activator and co-repressor proteins with which the ERs interact in any given cell [74]. Manipulation of these interactions may provide a rationale of specifically targeting the cardiovascular system. Selective estrogen receptor modulators (SERMs) are drugs that exhibit some tissue specificity in their effects. For example, raloxifene has similar properties to estrogen in the cardiovascular system, such as reducing serum LDL cholesterol concentrations and improving endothelial function, although it is antagonistic to estrogen's proliferative effects in the uterus and breast. These properties of raloxifene provided the basis for the RUTH (Raloxifene Use for The Heart) randomized, controlled trial that studied its ability to decrease fatal myocardial infarction, fatal coronary disease and hospitalization for acute coronary syndrome. The RUTH trial commenced in 2001, with an average 5-year follow-up, and included 10,000 postmenopausal women > 55 years of age, and the study reported a reduced risk of invasive breast cancer, but a no effect of raloxifene on primary coronary events. Moreover, there was an increased risk of venous thromboembolism and fatal stroke [6].

The role of sex hormones in males

In general, the role of hormonal environment in the male cardiovascular system needs to be further explored. Estrogen deficiency in males has been associated with increased cardiovascular risk [59, 107], and significant associations exist between ERα gene polymorphism/s and susceptibility and development of myocardial infarction, angina pectoris and atherosclerosis in males [102, 103]. Number of studies also reported the vasodilator properties of testosterone in different species [122], including humans [123], however some of them seem to be endothelium-independent involving activation of K^+-channels [123] and/or inhibition of Ca^{2+} channels [29].

Recently, an impaired endothelial-dependent vasodilation due to reduced NO availability has been shown in hypogonadal patients, but testosterone administration further impaired NO availability in these subjects [7]. Other study has reported that increased endothelium-dependent dilatation is significantly associated with low serum testosterone levels but not with cholesterol levels or with a past history of malignancy, suggesting that the withdrawal of male sex hormones may be associated with enhanced endothelial function in adult men [40]. In contrast, intracoronary infusion of testosterone induces relaxation of coronary arteries and augments blood flow in men with diagnosed coronary artery disease (CAD) [114]. In line, acute administration of high-dose testosterone enhanced endothelium-dependent flow-mediated vascular reactivity of brachial artery in men with CAD [84]. This study concurs with observation that low-dose of oral testosterone augments both endothelium-dependent (flow-mediated) and endothelium-independent vascular reactivity of brachial artery in men with CAD [54], but contrast to demonstration that transdermal testosterone treatment in men with low testosterone levels did not affect endothelium-dependent dilatation [56]. The controversy about testosterone treatment and vascular reactivity in man could be related to pre-existing cardiovascular condition, duration of treatment, age or vasculature investigated.

The male mice lacking a functional aromatase enzyme demonstrate blunted responses to ACh in aortic rings [57]. It has been also shown that aromatase is expressed in SMCs [37], and therefore local changes in estrogen concentrations within the vasculature could play an important role in the modification of vascular tone in response to vasoactive substances. Estrogen can affect the male cardiovascular system with a potential for clinical benefit. Acute administration of estradiol to young men, at concentrations comparable to that obtained in pre-menopausal women, induces enhancement of endothelium-dependent dilatation to ACh in the skin circulation [58]. Longer-term estrogen supplementation in male-to-female transsexuals enhances flow-mediated responses [82]. Our group has also recently reported that phytoestrogens *ex vivo* at concentrations achievable *in vivo* with moderate red wine or soy-derived products consumption in daily diet evoke an acute relaxation in small subcutaneous arteries from men with coronary heart disease or healthy controls (Fig. 3) [18].

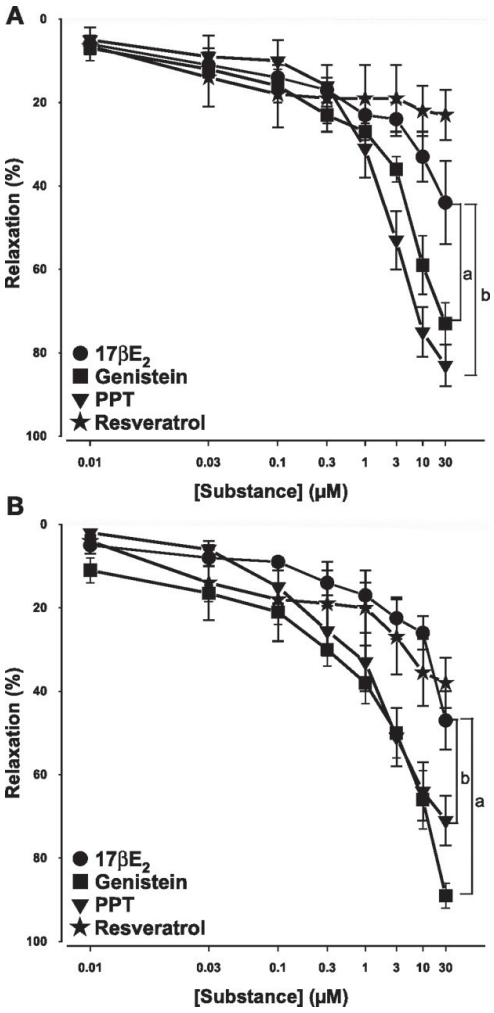

Fig. 3. Concentration-response curves to propyl-[1H]-pyrazole-1,3,5-triyl-trisphenol (PPT; n = 6 patients), genistein (n = 6), resveratrol (n = 5), and 17β-estradiol (17βE_2; n = 7) in arteries from patients with coronary heart disease (CHD) (**A**) and concentration-response curves to PPT (n = 7), genistein (n = 6), resveratrol (n = 6) and 17βE_2 (n = 5) in arteries from healthy male volunteers (**B**). (Reprinted with permission from American Journal of Physiology – Heart and Circulatory Physiology [18])

Summary

The vascular endothelium seems to be an important target for the action of sex hormones, and endothelial dysfunction is one of the key risk factors for development of CVD in both women and men. The current evidence highlights that the gender differences in endothelial function is associated not only with the quantitative production of endothelium-derived vasodilators, but also in the relative contribution of NO *vs.* EDHF. In this respect, the investigations on the pooled to one group arteries from women and men, as frequently utilized approach in research of human vascular function *in vitro*, should be considered with caution and not appreciated in the modern physiology.

The future of hormonal therapy will depend on an improved understanding of vascular cell-specific actions of sex hormones. An additional knowledge about the alterations in cardiovascular maintenance *per se* that occurs at menopausal transition and exploration of alternatives that would be beneficial for cardiovascular protection without inducing negative risks are highly desired [18].

Acknowledgments:
We acknowledge the support of Swedish Heart and Lung foundation and the Centre of Gender Medicine at Karolinska Institutet, Stockholm, Sweden

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Received:
December 4, 2007; in revised form: December 20, 2007.