

REVIEW ARTICLE

Why sex matters: the biological mechanisms of cardiovascular disease

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Cardiovascular disease (CVD) is the leading determinant of mortality and morbidity in women. However, a full understanding of the basic and clinical aspects of CVD in women is far from being accomplished. Sexual dimorphism in CVD has been reported both in humans and experimental animals. Menopause is a risk factor for CVD due to the reduction of endogenous estrogen, although the mechanisms underlying are poorly understood. Estrogens act through binding to vascular estrogen receptors and by non-genomic mechanisms. Advances in this field are essential to improve CVD diagnostic and clinical strategies in women, and to develop sex-specific prevention plans as much as female-oriented treatment algorithms. This paper reviews pathophysiology of CVD in women and its potential clinical implications. Particular emphasis is given to biochemical markers and to indicators of cardiovascular dysfunction and damage. Estimation of these parameters, central to cardiovascular pathophysiology, could represent a particularly relevant tool in female patients. More research is needed to identify women who will profit most of early intervention.

Keywords: Cardiovascular risk, sex, gender, menopause, steroid hormones

Introduction

Cardiovascular disease (CVD) is the leading cause of death and morbidity in the developed countries. There are marked gender differences in its pathogenesis, symptoms, responses to therapies and, ultimate outcomes. Within the past few years, a number of International Organizations have initiated extensive research and educational programs on CVD in women, aimed to facilitate the development of specific management strategies for their health [1,2]. At the basis of this ongoing effort is the need to understand the gender-related characteristics in the pathophysiology and pharmacology of CVD [3,4].

This review focuses on the pathophysiology of CVD in women. Particular emphasis is given to biochemical markers, and to indicators of cardiovascular dysfunction and damage. Estimation of these parameters, central to cardiovascular pathophysiology, could represent a particularly relevant tool in female patients.

Steroid hormones and the cardiovascular system

Steroid hormones intimately regulate the fundamental cardiovascular functions including blood pressure, blood flow, vasodilatation and vasoconstriction, vascular inflammation and remodeling

atherosclerosis [5]. Vascular endothelial cells, vascular smooth muscle cells as well as cardiomyocytes are all well equipped with sex steroid hormone receptors as well as with the steroid metabolizing enzyme aromatase [6]. One obvious difference between genders is represented by the remarkably complex changes in sex steroids observed in women between puberty and menopause. The equilibrium of estrogen or progesterone concentrations changes is associated with significant modifications of cardiovascular function in women.

The mechanistic actions of sex steroid hormones in cultured cardiovascular cells have been investigated in detail and are thought to explain a large part of the gender specificities in the cardiovascular system [7]. Sex steroids act by binding deputed receptors. This leads to structural changes in the tertiary and quaternary structure of the receptor, favoring (or decreasing) the interaction with scaffolds and other interacting proteins, known as co-activators or co-repressors, and facilitating the nuclear localization of these modified complexes. Sex steroid receptors are herein able to identify specific DNA response elements on regulatory areas of regulated genes, and by doing so they guide the regulatory elements they interact with to the final targets. This modifies the expression of a large set of proteins, enzymes and receptors, thus changing the functional status of the cell [8].

On top of this, sex steroid receptors also regulate vascular cells through rapid actions elicited at the membrane or within the cytoplasm. These effects require interactions between steroid receptors and signaling intermediates, such as G proteins or small guanosine triphosphate (GTP)-ases and lead to rapid changes of intracellular ion (particularly potassium and calcium) concentrations and of the phosphorylation status of kinases (such as mitogen-activate protein kinases) [9]. These extra-nuclear mechanisms of actions turn into faster changes of functional status of cells as compared to the nuclear ones, and are particularly important in the vascular system. Whenever changes in circulating estrogen or progesterone ensue, such as after menopause, modifications in the activity of all the above-mentioned signaling activities can be identified in the cardiovascular system, and these changes relate to discrete functional modifications.

Human arteries and veins express aromatase, which locally converts testosterone (T) into estradiol. In men, aromatase deficiency accelerates atherogenesis [10]. In a large population of women, T (total and free), dehydroepiandrosterone sulfate (DHEAS), and androstenedione (A) levels decrease with aging [11]. Although androgens appear to be correlated with different cardiovascular risk factors, an inverse relationship has been observed between T and A and carotid atherosclerosis in both

premenopausal and postmenopausal women [12,13]. Thus, the relationship between androgens and CVD in women remains controversial.

Endothelial cells

Endothelium represents an elective cellular target for estrogens. Chronic estrogen administration enhances endothelial function in a number of vascular beds. Conversely, women exhibit an age-related impairment of flow-mediated vasodilation as men, but this reduction is particularly marked after menopause, suggesting a protective role of estrogens. Estrogen receptors (ERs) are expressed in endothelial cells and have an atheroprotective effect. Through the recruitment of ERs, estradiol increases endothelial nitric oxide (NO) and prostacyclin synthesis, thus slowing early atheroma formation. Estradiol also decreases synthesis of pro-inflammatory cytokines by circulating or resident immune cells. In addition, estradiol facilitates endothelial vascular healing and neo-angiogenesis. While many of these effects are regulated by either ER- α or ER- β , ER- α is found to be dominant at the vascular level [14] and in animal models this receptor sub-type mediates protection of coronary endothelial dysfunction after ischemia [15]. Emerging evidence suggests that estradiol also exerts vascular actions through other receptors, and particularly through the recently-identified G-protein-coupled receptor dubbed GPR30. GPR30 immunoreactivity has been demonstrated in endothelial and vascular smooth muscle cells of carotid arteries from both rat genders. In addition, GPR30 agonists elicit endothelial-derived NO-dependent relaxation of the carotid artery in male and female rats [16].

Protective effects exerted by estrogens on endothelium include multiple cellular mechanisms, as evidenced by a number of experimental and clinical data. Estrogen has been demonstrated to activate calcium-dependent potassium channels and induce a rapid increase in NO release [17]. These non-genomic effects of estrogens on NO production are paralleled by their genomic actions exerted by activation of endothelial NO synthase (eNOS) through a receptor-mediated system [18]. Estrogen has anti-oxidant and anti-inflammatory properties, acting through multiple effects. Among them, estrogen may upregulate prostacyclin synthase and the expression of vascular endothelial growth factor. Conversely, it inhibits endothelin-1 release, and modulates adhesion-molecule and tumor necrosis factor- α (TNF- α) expression and endothelial cell apoptosis [19]. Moreover, estrogen can act by upregulating superoxide dismutase in the vascular district, which contributes to increased superoxide ion clearance [20]. In addition to this genomic effect, estrogen can detoxify superoxide ions through binding with the proton in the hydroxyl group of its aromatic ring [21].

Most of experimental studies suggest the protective role of estrogen in terms of the oxidative stress status that may improve the oxidative balance in the vascular sites, improving local NO bioavailability and consequently enhancing endothelium-dependent dilatation. Estrogen may also influence the redox balance through modulation of mitochondrial enzyme activity. Thus, the antioxidant effects are regarded as one of the main mechanisms by which hormones protect women during their fertile life, when they are at lower risk of cardiovascular events respect to men [22]. In fact, oxidative stress is generally higher in men compared to premenopausal women [23]. After the menopause, when hormonal levels markedly fall, the risk of experiencing cardiovascular events rapidly rise in women, in parallel to a rapidly increase of oxidative stress biomarker levels [24]. However, although some studies evidenced that women have higher levels of oxidative

stress than do men, other authors found higher oxidative stress biomarkers in men, and others no gender-related differences [23,25,26]. Nonetheless, in old populations of healthy and coronary artery disease subjects, women appear more susceptible to oxidative damage [24]. Thus, the estimation of oxidative stress could represent a promising biomarker for cardiovascular risk estimation particularly relevant in female patients. Moreover, it is likely that women might benefit more than men from antioxidant vitamin supplementation.

Low levels of androgen are associated with endothelial dysfunction, adverse lipid profiles, and inflammatory responses. In recently postmenopausal women, T levels have proatherogenic effects, including an association of T with C-reactive protein (CRP) and endothelin-1, waist circumference and blood pressure [27]. Physiological levels of T and dihydrotestosterone (DHT) increase endothelial synthesis of NO, whereas at supra-physiological doses of T or DHT the induction of NO synthesis is lost. Testosterone may activate both androgen and estrogen receptors (by local aromatase conversion to estradiol) in cardiovascular tissues, thus providing important atheroprotective effects through estrogen-dependent mechanisms [13,28]. Therefore, T and DHT act on endothelial cells through androgen receptors (ARs) or via conversion to estradiol. In fact, conversion of T to estrogen by aromatase may help maintain normal vascular tone as is supported by data in healthy human males showing that aromatase inhibitors negatively affect vascular relaxation, while aromatase knockout mice demonstrate abnormal vascular relaxation [24]. In cell culture studies, T inhibits expression of adhesion molecules, such as vascular cell adhesion molecule (VCAM) 1, and this effect, being reversed by an aromatase inhibitor, seems essentially due to conversion of T into estrogen [29].

DHEAS is an acute pig coronary artery vasodilator, although less potent than T. Its action might be mediated through androgen receptors and may involve ATP-sensible potassium channels [19]. T does not affect vascular proliferation, whereas DHEA, in particular, increases endothelial proliferation and angiogenesis, probably by increasing endothelial NO production [30]. Accordingly, DHEA is metabolized in the endothelial cell to other biologically active steroids, including estradiol. However, DHEA also increases endothelin-1 secretion, exerting vasoconstrictive effects [13].

Progesterone and progestin effects on adhesion molecule and endothelial cells are even more unclear, depending of progestin type and concentration [31]. However, progesterone has inhibitory effects on different endothelial cell types [6]. In general, there is evidence that the molecular actions of progesterone receptor (PR) agonists in endothelial cells can be quite different, likely due to the induction of specific tertiary structures upon binding to the ligand binding pocket of the receptor [32]. The progestin drospirenone is a unique compound that acts on both mineralcorticoid and progesterone receptors. Drospirenone induces rapid activation and expression of eNOS, in addition, it also antagonizes the detrimental effect of aldosterone on NO secretion [33]. Aldosterone antagonism is limited to drospirenone, since progesterone or medroxyprogesterone do not produce this effect [34].

Vascular smooth muscle cells

Female vascular smooth muscle cells (VSMCs) have greater expression of ER- α and ER- β than males, while having similar quantity of GPR30. The lower contractile activity of female VSMCs has been related to this gender-related difference in the expression of ER- α and ER- β [35]. An important effect of estrogen on VSMC related to atherosclerosis is the inhibition of their proliferation following vascular injury, an opposite effect

respect to the induction of proliferation in endothelial cells. The divergence of the estrogen effects on different cellular types may depend upon the modulation of transcriptional factors involved in the regulation of the proliferative mediators. Specifically, treatment with estradiol increased both insulin growth factor (IGF) I and cyclooxygenase-2 mRNA expression in human umbilical venous endothelial cells, with an opposite effect on the transcription of these genes in human aortic smooth muscle cells [36].

In cultured VSMCs from young rats pre-treated with estradiol, induced premature senescence was suppressed in a dose-dependent manner, and these senescent-inhibiting effects of estradiol could be blocked by the estrogen receptor antagonist ICI 182,780. On the contrary, in VSMCs from old rats the senescent-inhibiting effect of estradiol is not present and even some senescent-promoting effect was demonstrated [37]. These experimental findings support the “time window theory” of menopause hormone treatment. In addition, it seems that estradiol may have opposite actions, under some circumstances it has proliferative actions and under others has pro-senescent vascular effects.

Most available data suggest that progesterone and progestins in general inhibit, while androgens increase VSMC proliferation [6]. Progesterone also increases oxidative stress in VSMC and antagonizes the vasoprotective effect of estrogen on antioxidant enzyme expression and function [38]. Recent experimental results using rat aortic VSMC cultures have shown that progesterone enhances cell proliferation, migration and apoptosis [39]. In experimental setting, acute administration of T induces arterial vasodilatation, through an endothelium-independent mechanism, rather involving ATP sensitive potassium channels on smooth muscle cells [40]. Accordingly, ATP-sensitive K⁺ channels appear to play a role in the vasodilatory effect of T in an *in vivo* model [41].

Cardiomyocytes

Sex differences exist in normal heart function, as cardiac contractility results greater in women than in men, and myocardial mass appears better preserved in women with aging [42]. These effects appear due to multiple mechanisms including sex differences in mRNA expression of functional and structural cardiac proteins. Sex differences also exist in cardiac electrophysiological function and in characteristics of both inherited and acquired forms of heart disease. In particular, specific familial hypertrophic cardiomyopathies result more severe in male than in female subjects, while women with aortic stenosis retain hypertrophic hearts with a better contractile function respect to those of men with the same disease [43].

Treatment with T may improve exercise capacity, ventilator function, muscle strength, and insulin sensitivity in men [44]. Since postmenopausal women have relative low T levels, androgen supplementation could produce similar effects in women to those reported in men. A recent study has reported that T supplementation improves functional capacity, insulin sensitivity and muscle strength in a small number of older women with congestive heart failure, and the mechanism of action seems to be peripheral without action on the left ventricular function [45].

Natriuretic peptides

In healthy subjects, brain natriuretic peptide has been found to vary by age and sex, with higher levels seen in women than in men [46]. On the other hand, in cycling women estrogens exert a stimulating effect on cardiac endocrine function by increasing the secretion of natriuretic peptides from cardiomyocytes,

whereas hormone replacement therapy also induces a rise in the levels of cardiac natriuretic hormones, suggesting that atrial natriuretic peptides may play an important role in mediating the cardioprotective effects of female steroid sex hormones throughout female life. Cardiac natriuretic hormones have several important physiological actions, in terms of decreasing blood pressure, increasing natriuresis and diuresis, inhibiting the sympathetic nervous system and releasing several hormones, including aldosterone, angiotensin II, endothelins, renin, and vasopressin [47]. Conversely, in subjects with heart failure brain natriuretic peptide levels rise, paradoxically, to a lesser degree in women than in men, even in cases of comparable functional impairment [48].

Vascular inflammation and oxidative stress

Vascular inflammation and oxidative stress represent crucial basic mechanisms which take part in all the steps from the onset, to development and progression of CVD. The complexity of effects exerted by estrogen is evidenced by controversial data obtained in this field, being the estrogen either anti- or pro-inflammatory depending on diverse factors such as the target cell type and organ, timing and levels of estrogen. The pro-inflammatory role of estrogens and their impact in the modulation of B-cell and T-cell immunity seems underlined by sex-related differences in the occurrence of inflammatory immunological diseases, including rheumatoid arthritis and systemic lupus erythematosus, and from the estrogenic modulation of their symptoms associated with the different phases of female life. As puberty, pregnancy and menopause [49].

Estrogen effects on myogenic- and shear stress-dependent mechanisms of arterioles significantly contribute to the control of local blood flow and peripheral resistance, also through modulation of endothelial mediators, such as NO and prostaglandins [50]. Consequently, postmenopausal women presented higher systolic and diastolic blood pressure values than premenopausal women and even higher than in men of the same age [10].

Accordingly, elevated levels of pro-inflammatory cytokines and increased expression of iNOS are induced by chronic administration of estradiol in animal studies, whereas estradiol increases pro-inflammatory cytokine production from activated peritoneal macrophages, all these effects through mediated ER- α . Conversely, estrogens reduced levels of pro-inflammatory cytokines, such as TNF- α , interleukin (IL)-1 β , IL-6, monocyte chemoattractant protein-1, and metalloproteinases, and increase those of anti-inflammatory cytokines, as IL-4, IL-10, transforming growth factor (TGF) β and tissue inhibitor of metalloproteinases, again mediated by ER- α [6]. In further support of the anti-inflammatory effects of estrogen, women with higher estrogen status have significantly lower levels of monocyte chemo-attractant protein-1 (a chemokine responsible for the recruitment of monocytes to sites of inflammation) than those with lower estrogen [51]. Conversely, pro-inflammatory cytokines and expression of cellular surface adhesion molecules (E-selectin, P-selectin, VCAM-1 and intercellular adhesion molecule-1) increase in parallel to the decrease of estrogens after the menopause, and replacement hormonal therapy is able to restore levels to those of pre-menopause [6].

Among cytokines, the IL-6, that stimulates hepatocytes to synthesize acute phase response proteins such as CRP and fibrinogen, has been the most studied in relation to cardiovascular risk. Data from the British Women's Heart Study evidenced the association of IL-6 levels and coronary heart disease (CHD) risk factors. However, after adjusting for components of the metabolic

syndrome, the association lost significance [52]. Conversely, a previous analysis of 17 studies found an association between IL-6 and CHD, which remained significant also after adjustment for established CHD risk factors [53].

Endothelial microparticles and thrombogenicity

Vascular injury induces a progressive endothelial loss of function and integrity, until the detachment of whole endothelial cells or endothelial microvesicles or microparticles (MP) derived from activated or apoptotic cells [54]. These endothelial MPs are important in the CVD pathophysiology and transport specific biochemical messengers which, in turn, may initiate or accelerate inflammatory response and vascular dysfunction in other territories [6]. They disturb coagulation, angiogenesis and vascular homeostasis contributing to atherosclerosis [55]. In vitro study of isolated arteries exposed to endothelial MP from patients diagnosed with metabolic syndrome, acute coronary syndrome, severe renal failure or preeclampsia produced endothelial dysfunction as compared to endothelial MP from healthy individuals [56,57].

Healthy women show menstrual cycle-specific differences in platelet-cell-derived and endothelial MP which differ significantly from age-matched men. Number of these cells and their thrombogenic capacity were significantly greater in recent postmenopausal women with high coronary artery calcium who would not be evidenced by considering traditional screening cardiovascular risk parameters, such as BMI, lipid profile and glucose [58]. Moreover, estrogen regulates cell-specific endothelial MP, being the number of endothelium-, platelet-, monocyte- and granulocyte-derived MP greater when estrogen is low [59].

Endothelial progenitor cells

Endothelial progenitor cells (EPCs) are bone marrow-derived cells actively involved in cardiovascular homeostasis, providing a circulating pool of cells that repair endothelial damage in physiological conditions. These cells are mobilized from bone marrow and other organs to peripheral blood towards the damaged sites by ischemic insult to promote angiogenesis [60]. EPCs repair endothelial structure and enhance activity of eNOS, restoring the functional status of the endothelium. Contrary to mature endothelial cells which have limited regenerative capacity, EPCs reside at sites of endothelial injury and ischemia proliferating and integrating into the endothelium. In addition, they produce vascular growth factors [60,61]. EPCs are higher in fertile women than men, their number fell after menopause, and their cyclic EPCs mobilization changes with menstrual/hormonal cycle and may be related to endometrial regeneration [62]. EPC subpopulations in premenopausal women may partially explain the lower prevalence of cardiovascular events in menstruating women as compared to men [63,64]. EPC number is reduced in castrated mice and enhanced in hyperstimulated ovaries from women included in in vitro fertilization protocols [65,66]. These cell number and functions are also influenced by lifestyle, adiposity, weight changes and exercise [60]. Despite this, it seems that EPC are better predictive factor for vascular health than conventional risk factors, such as lipid profile, hypertension and diabetes. The evaluation of EPC number and function might reflect the degree of cardiovascular protection of the female population. However, improvement of available methods for EPC quantification is expected, in order to allow a better characterization of different cellular phenotypes and functional properties.

Final remarks

Available evidence clearly indicates that being male or female is a variable that may profoundly affect the course of CVD. The burden of CVD in middle-aged women relative to men is increasing [67]. Sex represents an important modifier of the cardiovascular system and should be recognized as an important factor in both basic science and clinical research involved in the pathophysiology of CVD. Currently, research on sex-related differences in pathophysiology of CVD has begun, but sex in all its complexity is only starting to be recognized as a scientific category in the medical practice. Until now, no risk factor has been recognized as acting on one sex but not on the other. This finding implies that general mechanisms involved in the pathogenesis of CHD are evidently very similar for men and women. Thus, it is unlikely to image specific biomarkers or additional testing only targeted for women. However, the influence of sexual hormones is critical in determining the weight of cardiovascular risk factors between men and women. In this sense, the effect of the menopause on both cardiovascular risk factors and CHD is unique for women, which represent a distinct subpopulation within CVD patients.

New tests are needed to define risk and guide treatment according to sex issues also in laboratory medicine, because sex-related differences may have a great impact on patient stratification and outcome. These aspects are not generally taken into account when using biochemical biomarkers as discriminating parameters in clinical studies. Consequently, a significant part of the commercially available assays lack these data. Thus, it would be desirable the availability of gender-related reference values for relevant biochemical parameters, and even differential for the pre or post-menopausal status.

The areas related to recently proposed biomarkers, such as inflammatory and oxidative stress parameters as well as EPC, endothelial MP and genetic factors appear promising in providing new basic information and development in the clinical practice, also from a sex point of view. However, we yet need to understand the entity of sex-related differences in CVD and whether multiple biomarkers contribute to improved CHD risk prediction when compared with assessment using traditional risk factors in post-menopausal women. This knowledge will allow to identify more important sex-associated biomarkers or panels to optimize diagnostic and therapeutic strategies targeted for men and women when appropriate.

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