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Review

Endothelial maintenance in health and disease: Importance of sex differences

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ABSTRACT

The vascular endothelium has emerged as more than just an inert monolayer of cells lining the vascular bed. It represents the interface between the blood stream and vessel wall, and has a strategic role in regulating vascular homeostasis by the release of vasoactive substances. Endothelial dysfunction contributes to the development and progression of cardiovascular disease. Recognition of sex-specific factors implicated in endothelial cell biology is important for the identification of clinically relevant preventive and/or therapeutic strategies.

This review aims to give an overview of the recent advances in understanding the importance of sex specific observations in endothelial maintenance, both in healthy and diseased conditions. The female endothelium is highlighted in the context of polycystic ovary syndrome and pre-eclampsia. Furthermore, sex differences are explored in chronic kidney disease, which is currently appreciated as one of public health priorities.

Overall, this review endorses integration of sex analysis in experimental and patient-oriented research in the exciting field of vascular biology.

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1. Introduction

Sex and gender are factors associated with differences in cardiovascular (CV) morbidity and mortality. Both CV mortality rate and the prevalence of coronary heart disease (CHD) are higher in men than in women [1]. In both men and women, the risk of CHD increases with age, but shows a more prominent increase in women after the age of 50, i.e. around the menopause [2]. Age-adjusted mortality for CV disease (CVD) is currently declining, but to a lesser extent in women than in men [3].

Until the last decade, underestimation of CVD in women has been explained, not only by the lower prevalence in younger age, but also by a broad appreciation of CVD as a 'male disorder' [4]. It was anticipated that the knowledge based on studies on men is also applicable to women [4]. However, recent retrospective analyses suggest that there are clinically relevant differences between women and men in terms of prevalence, presentation, management and outcomes of CVD [5]. Thus, more insight into the influences of biological sex and gender on the development of CV disease is warranted in order to identify new preventive and therapeutic targets [5].

The endothelium has an important role in vascular homeostasis by the production and release of vasoactive substances in response to numerous stimuli. The 'healthy' endothelium promotes vasodilatation, inhibits platelet aggregation (and thus thrombus formation), and preserves vessel permeability.

It is widely appreciated that dysfunction of the endothelium is a hallmark of CVD [6]. Endothelial dysfunction (ED) is defined as an imbalance between endothelium-derived vasodilators such as nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF), and prostacyclin (PGI_2) on the one hand, and vasoconstrictors such as endothelin-1 (ET-1) and cyclooxygenase-derived prostanoids (thromboxane A_2 (TXA_2) and prostaglandin H_2 (PGH_2)) on the other hand. Endothelium-derived NO has an important role in dilatation of *conduit* arteries, and the combination of decreased NO bioavailability in the context of a pro-inflammatory and pro-oxidant milieu contributes to the development of atherosclerosis. EDHF primarily confers vasodilatation of the *resistance* vasculature, with an important role in peripheral resistance and blood pressure control. Moreover, EDHF production may increase when NO production is compromised or *vice versa* [7,8].

Thus, the NO signaling and/or EDHF system might represent a sex-specific target for preventive and therapeutic strategies. For example, altered EDHF responses may contribute to [9], or compensate for [10] ED in a sex-specific matter. It has been shown that EDHF contributes to sex-related differences in blood pressure control. Using specific gene-knockout technology to generate animals which lack both endothelial NO-synthase (eNOS) and cyclooxygenase (COX), i.e., the "EDHF mouse", Scotland et al. has directly assessed the sex-specific involvement of EDHF in endothelium-dependent relaxation of small arteries [11]. In this animal model, EDHF-mediated response compensated the absence of endothelial NO in females, but not in males. Indeed, in female mice, the deletion of eNOS and COX did not affect blood pressure, while males became hypertensive [11]. Accordingly, EDHF is considered to be more important for endothelium-dependent dilatation in females, while NO plays predominant role in males. Additional reports of mesenteric and tail arteries in different rodent models, further stressed the importance of NO in male vasculature [12,13].

Human studies on sex-specific differences in EDHF/NO contribution are rare. Sato el al. studied the influence of sex and menopausal status on endothelium-dependent dilatation induced by bradykinin (BK) of adipose arterioles. Small vessels from omental tissue and subcutaneous fat in premenopausal women were more sensitive to BK than those from postmenopausal women or men. The inhibition of eNOS erased those differences, suggesting that BK-induced dilation in peripheral small arteries from human fat tissue is predictably affected by both sex and hormonal status [14]. Although the influence of sex hormones is assumed, as reflected by majority of functional studies on changes in endothelium-dependent dilatation and altered NO contribution in animal models after ovariectomy and/or hormone supplementation therapy [15,16], endothelial cells themselves present intrinsic sex differences by means of observed sexual dimorphisms in gene- and protein- expression profiles as well as proliferative and migratory properties [17,18].

2. Maintenance of endothelial health

2.1. The role of endothelium-derived substances

The endothelium contributes to the maintenance of vascular homeostasis by releasing vasoactive substances and we will detail their action in following chapter. Importantly, we would like to stress that more detailed experimental studies in the field of vascular biology are needed to clarify the relative contribution of endothelium-derived factors in females and males. Also more experimental models assessing the intracellular pathways where sex is included as biological variable are required [19].

Nitric oxide (NO) is synthesized from L-arginine by the enzyme NO synthase (NOS). NO causes dilatation of vascular smooth muscle cells (VSMCs) through activation of soluble guanylyl cyclase receptor with generation of cyclic guanosine monophosphate [20]. Next to its vasodilatory effect, the molecule is also involved in regulation of cell growth and proliferation, and it affects transcription of certain genes implicated in the pathogenesis of atherosclerosis and hypertension [21]. Two constitutive isoforms of NOS play a vital role in NO production: endothelial NOS (eNOS) and to a lesser extent neuronal nNOS. [22]. The third isoform, inducible NOS (iNOS) is involved in inflammation [23,24]. The family of NOS enzymes (eNOS, iNOS and nNOS) all share a critical need for co-factors tetrahydrobiopterin (BH_4), nicotinamide adenine dinucleotide phosphate (NADPH) and the flavins and flavin mononycleotide, on top of the substrate L-arginine, to generate NO [25]. NO is released from endothelium under basal conditions, and in response to shear stress, circulating hormones and various autacoids (Fig. 1) [26].

The generation of eNOS uncoupling, oxidative stress and dysregulation of signal transduction are all involved [27–29] in the molecular base of ED characterized by a decreased bioavailability of NO. ROS generated from NADPH oxidase may reduce the bioavailability of NO *via* reaction of NO with O_2^- generating peroxynitrite, which oxidizes B H_4 with subsequent reduction of BH_4 and eNOS uncoupling (Fig. 2)[25].

Endothelium-derived hyperpolarizing factor (EDHF) is an intriguing substance released by the endothelium, whose identity has yet to be fully uncovered (for review, see [8]).

Early studies demonstrated hyperpolarization, in the presence of NO and PGI_2 blockade, monitoring membrane potentials in smooth muscle cells downstream from donor endothelial cells [30] further supporting observations that endothelium dependent relation involves the release of additional factor, which increases the membrane potential of VSMCs [31]. This additional relaxing factor has been named EDHF. EDHF-induced relaxation may be mediated simultaneously by several factors and/or pathways, depending on the species, type of vasculature bed or vessel size used, sex and physiological environment or disease status [8]. Thus, possible candidates for EDHF are epoxyeicosatrinoic acids, cannabinoids, potassium ions or myoendothelial gap junctions alone or in combination with H_2O_2 and/or cytochrome P450 2C9 (CYP2C9) products of arachidonic acid (AA) [8,32,33].