Mechanisms underlying the influence of oestrogen on cardiovascular physiology in women

Susana Novella, Daniel Pérez-Cremades, Ana Mompeón and Carlos Hermenegildo

Department of Physiology, Faculty of Medicine and Dentistry, University of Valencia, and INCLIVA Biomedical Research Institute, Valencia, Spain

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Abstract  Women show a lower incidence of cardiovascular diseases than age-matched men, but this benefit disappears after menopause. Oestrogen-mediated vascular actions are mainly attributed to oestradiol and exerted by oestrogen receptors (ERα, ERβ and G protein-coupled oestrogen receptor), through rapid and/or genomic mechanisms, but these effects depend on ageing and inflammation. A cardiovascular approach in women’s health has arisen due to...
controversy regarding oestrogen’s beneficial impact as reported in experimental and observational studies and large randomized trials. These can be explained, in part, by two mutually non-exclusive hypotheses. On the one hand, the timing hypothesis, which states that oestrogen-mediated benefits may occur before the detrimental effects of ageing are established in the vasculature; on the other hand, ageing and/or hormonal-associated changes in ER expression that could lead to a deleterious imbalance in favour of ERβ over ERα, generally associated with higher inflammation and endothelial dysfunction. In experimental studies, oestradiol acting on ERα promotes the release of vasoactive compounds such as nitric oxide (NO) and prostacyclin, and shifts the angiotensin axis towards angiotensin 1–7 production. Mechanisms underlying oestradiol vascular function also include anti-inflammatory and epigenetic modifications. 17β-Oestradiol changes the transcriptional profile of endothelial cells, and the involvement of miRNA in the regulatory pathways of vascular function reinforces assumptions regarding the vascular actions of oestrogen. Thus, the present Symposium Review aims to postulate the role of ERα in oestrogen modulation of endothelium-derived mediators and vascular physiology, as well as its relationship with miRNA and inflammation, and elucidate how physiological changes in postmenopausal women counteract the observed effects.

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Corresponding author S. Novella: Universitat de València, Department of Physiology, Av/Blasco Ibáñez, 15, Valencia, E46010, Spain. Email: susana.novella@uv.es

Abstract figure legend The beneficial effects mediated by oestrogen involve different intracellular signalling pathways, such as nitric oxide (NO), prostanoids and the renin–angiotensin system (RAS), towards a vasoprotective profile involving oestrogen receptors, mainly ERα. Physiological changes as ageing and menopause and in epigenomics affect the cardiovascular effects of oestrogen. (Created by Biorender.com.)

In-depth study of cardiovascular diseases (CVD) has led to a fuller understanding of sex differences in cardiovascular physiology. CVD is currently the leading cause of death in women from developed countries (WHO, 2016) although statistical data reveal that women develop CVD 10 years later than men (Burns & Korach, 2012), and incidence increases from menopause on (Deroo & Korach, 2006; Burns & Korach, 2012; Vrtacnik et al. 2014). Women’s time-related advantage regarding CVD development has been attributed to hormonal status, and both clinical and experimental data have demonstrated the beneficial effects of oestrogen at the cardiovascular level (Hayward et al. 2000; Mendelsohn & Karas, 2005). However, hormonal replacement therapies (HRT) have been used in postmenopausal women with controversial findings (Mendelsohn & Karas, 1999; Mikkola et al. 2013). While the largest randomized controlled trial, Women’s Health Initiative (WHI), initially reported no protective role against coronary heart disease risk (Rossouw et al. 2002), a reanalysis by age and years since menopause (Rossouw et al. 2007) demonstrated a significant benefit in healthy women initiating oestrogen therapy soon after menopause onset (Manson et al. 2003; Rossouw et al. 2007; Novella et al. 2012). In fact, age and years since menopause are important variables affecting the benefit/risk profile of HRT (Sood et al. 2014). The so-called timing hypothesis postulates that the beneficial impact of hormonal replacement in CVD prevention can occur only when HRT is initiated before the detrimental effects of ageing on the cardiovascular system have become established (Clarkson et al. 2013). In this regard, it has been reported that age moderates oestrogen’s vasodilatory (Sherwood et al. 2007) and anti-inflammatory (Novella et al. 2012) effect on vascular tissue in postmenopausal women. The current consensus on HRT is that the cardiovascular protective role of oestrogen depends on the timing of treatment after menopause (Lobo, 2017).

Since the publication of the WHI results in 2002, much has been learned, yet much controversy remains. The 2017 position statement of the North American Menopause Society (NAMS), which evaluates new literature and reaches consensus on recommendations for the use of HRT for the treatment of menopause-related symptoms, identified future research needs as the risks of HRT differ depending not only on timing of initiation but also on type, dose, duration of use, route of administration and whether a progestogen is needed (Hormone Therapy Position Statement Advisory Panel, 2017). In agreement with the timing hypothesis, the position statement of NAMS assessed that for women aged younger than 60 years or who are within 10 years of menopause onset, HRT appears favourable for treatment of some menopausal symptoms, but for those who initiate HRT more than 10 or 20 years from menopause onset or when aged
60 years or older, the benefit–risk ratio appears less favourable than for younger women, with greater absolute risks of coronary heart disease, stroke, venous thromboembolism and dementia.

Oestrogen receptors in the cardiovascular system

The most abundant form of circulating oestrogen is oestradiol, also termed 17β-oestradiol, which is predominantly synthesized and secreted by the ovaries during a woman’s reproductive years. Vascular tissues, particularly endothelial cells, vascular smooth muscle cells (VSMCs) and cardiomyocytes, are oestriol targets as they express different types of oestrogen receptors (ERs) (Khalil, 2013). This expression is also shared by monocytes, macrophages and dendritic cells, suggesting a modulatory role for oestradiol in inflammatory processes, a key event in onset and development of CVD (Harkonen & Vaananen, 2006; Kovats, 2015). Oestradiol binds to classical ERs including both ERα and ERβ in cytoplasm, to create homo- or heterodimers. They then bind to specific DNA motifs called oestrogen response elements (EREs) in the promoter region of oestrogen-responsive genes to regulate transcription (Klinge, 2001) and induce changes in gene expression. ERα and ERβ have different distributions, and selective activation of either the ERα or the ERβ isoform may involve contrasting biological effects, having opposing gene-expression regulatory effects (Lindberg et al. 2003; Tsutsumi et al. 2008) or alternatively having redundant mediatory roles (Arias-Loza et al. 2007; Lahm et al. 2008). Oestrogen signalling is thus selectively regulated by the relative balance between ERα and ERβ expression in target organs (Murphy & Steenbergen, 2014), although studies using ERα and ERβ knockout mice revealed that the beneficial effects oestrogen has on the vascular system are mainly mediated by ERα (Pare et al. 2002; Arnal et al. 2017).

Besides this classical genomic action, oestradiol also binds to membrane-bound ERα and ERβ receptors as well as to G protein-coupled ER (GPER) (Levin, 2009), rapidly activating nuclear transcription factors and triggering faster responses (within minutes). Many of the effects of oestrogen seen in human and animal models, such as reduced myocardial pro-inflammatory cytokine expression, inhibition of VSMC proliferation, and nitric oxide (NO)-dependent vasodilatation (Prossnitz & Barton, 2011), have recently been attributed to GPER expression in the cardiovascular system (Revankar et al. 2005).

The present overview is a Symposium Review presented in Europhysiology 2018, partially based on our own results, and aims to highlight the role of ERα in oestrogen modulation of endothelial-derived mediators and vascular physiology, and how physiological changes in postmenopausal women counteract the observed effects.

Vascular protective effects of oestrogen through ERα

The vascular-protective impact of oestrogen has also been attributed to its effects on the vascular wall, in both endothelium and smooth muscle, releasing vasoactive-mediators which promote arterial vasodilatation, modulate inflammatory processes and regulate systemic lipid metabolism and oxidative-stress balance (Kondo et al. 2009; Barton, 2013; Usselman et al. 2016). Figure 1 summarizes the role of ERα in endothelium-derived mediator and vascular smooth muscle cell function. Next the effects on these mediators, in particular NO, prostacyclin and angiotensin 1–7 pathways, will be discussed (some of the actions are summarized in Table 1).

Oestrogen and nitric oxide. In endothelial cells, which form the luminal cell monolayer of the vascular wall, oestradiol modulates the release of multiple vasoactive substances via both genomic and non-genomic action. Oestradiol increases NO bioavailability by either directly increasing NO generation or decreasing NO inactivation. Oestradiol increases NO bioavailability by mechanisms such as increasing endothelial NO synthase (eNOS) gene expression at the transcriptional level (Sumi & Ignarro, 2003); non-genomic and rapid activation of enzyme activity via cascades that activate kinases c-Src (Haynes et al. 2003), extracellular signal-regulated kinase (ERK) (Chen et al. 2004), phosphoinositide 3-kinase (PI3K) (Simoncini et al. 2003), and Akt, which leads to eNOS activation through phosphorylation at residue Ser1177 (Haynes et al. 2000; Meyer et al. 2009); increasing intracellular free Ca2+ concentration in endothelial cells (Rubio-Gayosso et al. 2000); regulating endogenous inhibitors and cellular location (Chambliss & Shaul, 2002; Monsalve et al. 2007; Novella et al. 2013); and attenuating superoxide anion (O2−) concentration, thereby decreasing O2−-mediated NO inactivation (Wassmann et al. 2001; Dantas et al. 2002; Ospina et al. 2002). Some of these rapid effects of oestradiol on the NO signalling pathway require no changes in gene expression and are mediated by different plasma membrane-associated ER subtypes. In addition to full-length ERα (ER66), an N-terminal truncated ERα isoform, ER46, plays a key role in these endothelial responses to oestradiol (Kim et al. 2014). Besides this, recent findings reveal that a GPER-mediated cascade acts as an alternative pathway in oestradiol-induced endothelium-dependent vasodilatation and NO formation via c-Src/PI3K signalling pathways (Fredette et al. 2018). Thus, in physiological conditions oestradiol stimulates vascular NO formation via GPER and mainly through ERα, which acts at a vascular level as a potent vasodilator, but also conveys vasoprotection through antithrombotic mechanisms and modifies proliferation and migration of the underlying...
VSMC (Förstermann & Sessa, 2012), thereby controlling the vascular tone.

In processes related to vascular injury associated with inflammation, ERβ expression in endothelium increases the expression of superoxide dismutase (SOD2) and eNOS, which altogether also raise NO bioavailability, ameliorating ischaemia–reperfusion-mediated vascular injury and minimizing reactive oxygen species generation (Zhan et al. 2016). On the other hand, pharmacological activation of the ERβ increases the expression of cytokine-driven inducible NO synthase (iNOS) in rat vascular smooth muscle (Panic et al. 2018), raising the hypothesis that ERβ can be induced by injuries and contributes to inflammation (Sartoretto et al. 2019). In non-vascular cells, ERβ activation also increases levels of phosphorylated neuronal NO synthase (nNOS) and NO production through a Src/PI3K/Akt-dependent pathway in hypothalamic neurons (Gingerich & Krukoff, 2008).

The effect of oestradiol on the NO pathway observed in cultured cells has been confirmed in a large number of isolated blood vessel preparations including the rat aorta (Freay et al. 1997), rat femoral artery and rat portal vein (Kitazawa et al. 1997), rabbit coronary artery (Jiang et al. 1991) and porcine coronary artery (Teoh et al. 1999). Although oestradiol’s mechanism of action differs according to the vascular bed and species studied, in general ERα, ERβ and GPER all seem to contribute. Generally, oestradiol exposure in women increases vascular relaxation and endothelial-dependent vasodilatation, increasing blood flow in numerous vascular beds. In studies performed in healthy young women, oestradiol is also associated with increases in

**Figure 1. Role of ERα on endothelium-derived mediators and vascular smooth muscle cell function**

Oestradiol (E2) binds to oestrogen receptor α (ERα) triggering both genomic and cytoplasmic response. E2–ERα complex is translocated to the nucleus and induces transcription of specific genes by binding to oestrogen response elements (ERE) in their promoter region. Endothelial nitric oxide synthase (eNOS), cyclooxygenase 1 (COX1), prostacyclin synthase (PGIS) and angiotensin converting enzymes (ECA) are regulated transcriptionally by ERα. In addition to its genomic effect, E2–ERα also regulate eNOS activity by inducing phosphorylation through different kinase signalling pathways (PI3K/AKT, SCR, ERK), and reducing the endogenous inhibitor asymmetric dimethylarginine (ADMA) by regulating dimethylarginine dimethylaminohydrolase (DDAH). Moreover, E2–ERα enhances activity of angiotensin converting enzymes (ECA), increasing the production of angiotensin 1–7, and plays a role in NO-dependent vasodilatation through a mechanism that involves Mas receptor. As a result, NO diffuses into the vascular smooth muscle cells and binds to guanylate cyclase (GC), increasing cGMP that in turn cause relaxation. E2 also increase prostacyclin (PGI2) production through the COX1–PGIS pathway. PGI2 is released from endothelial cells and binds to specific receptors located in the membrane of smooth muscle cells, which leads to an increment of cAMP by adenylate cyclase (AC) and muscle relaxation. E2 can also interfere with different signalling pathways, such as protein kinase C (PKC) and Rho-kinase and membrane ion channel activity through non-genomic actions. Altogether, these mechanisms lead to oestrogen-mediated vascular relaxation. (Created by BioRender.com.)
Table 1. Vascular protective factors mediated by oestradiol

<table>
<thead>
<tr>
<th>Vasoactive-mediator production</th>
<th>Mechanism</th>
<th>Experimental model</th>
<th>Reference</th>
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<tbody>
<tr>
<td>↑ NO bioavailability</td>
<td>↑ eNOS activation</td>
<td>EA.hy926 cells</td>
<td>Haynes et al. (2003)</td>
</tr>
<tr>
<td></td>
<td>↑ eNOS expression</td>
<td>COS-7 cells and bovine pulmonary artery endothelial cells</td>
<td>Sumi &amp; Ignarro, (2003)</td>
</tr>
<tr>
<td></td>
<td>↑ NO production</td>
<td>Human endothelial cells, TIVE cells</td>
<td>Fredette et al. (2018)</td>
</tr>
<tr>
<td>↓ O₂−-mediated NO inactivation</td>
<td>↓ the endogenous L-arginine analogue</td>
<td>Mesenteric arteries from SHR</td>
<td>Dantas et al. (2002)</td>
</tr>
<tr>
<td>↑ DDAH activity and ↓ the endogenous L-arginine analogue</td>
<td>ADMA</td>
<td>HUVECs</td>
<td>Monsalve et al. (2007)</td>
</tr>
<tr>
<td>↑ PGI2</td>
<td>↑ COX-1 and PGIS expression</td>
<td>HUVECs</td>
<td>Sobrino et al. (2010)</td>
</tr>
<tr>
<td>↓ ET-1</td>
<td>↓ ET-1 expression and secretion</td>
<td>Mesenteric arteries from DOCA hypertensive rats</td>
<td>David et al. (2001)</td>
</tr>
<tr>
<td></td>
<td>↓ ET-1 expression and secretion</td>
<td>Porcine coronary artery endothelial cells</td>
<td>Dubey et al. (2001)</td>
</tr>
<tr>
<td>↑ Ang-(1–7)</td>
<td>↑ ACE2 activity and expression</td>
<td>Renal wrap model of hypertension</td>
<td>Ji et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>↑ ACE2 activity and expression</td>
<td>HUVECs</td>
<td>Mompeón et al. (2016)</td>
</tr>
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Oestradiol mediates the release of vasoactive mediators mainly from endothelium, some of which effects are summarized in the following table along with the oestrogen-receptor involved. ACE2, angiotensin-converting enzyme 2; ADMA, asymmetric dimethylarginine; Ang-(1–7), angiotensin 1–7; COX-1, cyclooxygenase 1; DDAH, dimethylarginine dimethylaminohydrolase; DOCA, deoxycorticosterone acetate; ER, oestrogen receptor; ET-1, endothelin-1; GPER, G protein-coupled oestrogen receptor; HUAEC, human umbilical artery endothelial cell; HUVEC, human umbilical vein endothelial cell; PGI2, prostacyclin; PGIS, PGI2 synthase; SHR, spontaneously hypertensive rat; TIVE, telomerase-immortalized human umbilical vein endothelial.

**Oestrogen and cyclooxygenases.** Cyclooxygenase (COX)-derived factors are particularly important in regulating vascular tone as they can induce both vascular relaxation (through prostacyclin (PGI2) production) and contraction (through thromboxane A₂ (TXA₂) and prostaglandin H₂ (PGH₂) production). Oestradiol has been implicated in the modulation of peripheral vascular synthesis of vasodilatory mediators, including prostanoids through COX, as the rate-limiting step in the formation of vasoactive prostanoids (Sobrino et al. 2009). In human endothelial cells, oestradiol, acting through ERα, induces stimulation of the vasodilator and antiaggregatory PGI₂ production by up-regulating COX-1 and PGI₂ synthase (PGIS) expression without altering vasoconstrictor TXA₂ production (Sobrino et al. 2010). This mechanism supports the hypothesis that oestradiol is able to maintain vascular health and protect endothelial cells against vascular disorders (Mikkola et al. 2013). The beneficial effects of oestrogen on the endothelium can also be partially explained by an inhibitory effect on flow-mediated dilatation (FMD), a measure of conduit artery endothelial function mediated primarily by NO (Adler et al. 2018). When oestrogen levels declines in post-menopausal women, oestradiol administration improves also endothelial function (Hurtado et al. 2016) but the magnitude of improvement depends on the timing of when this treatment is initiated. The interactions of oestrogens on multiple pathways regulating vascular function, which also are involved in the ageing process, are complex, multifactorial and not completely understood. For example, the expression of ERα and eNOS in endothelial cells harvested from peripheral veins of women are lower in postmenopausal women than in young women (Gavin et al. 2009). Thus, not only does oestradiol decline with ageing, ERα receptor expression also declines, and an increase in oxidative stress is produced as well. How female and male sex hormones interact with the cardiovascular system, and in age-associated endothelial dysfunction in healthy woman and men has been recently reviewed in depth (Stanhewicz et al. 2018).
on production of the COX-derived vasoconstrictor agents PGH\(_2\) and TXA\(_2\) (Davidge & Zhang, 1998; Dantas et al. 1999; Vidal-Gómez et al. 2016), and endothelin-1 (David et al. 2001; Dubey et al. 2001), tipping prostanoid balance toward increased PGI\(_2\) production. However, in the absence of oestrogen, arachidonic acid is actively converted to a COX-1-dependent constrictor, indicating that oestrogen-mediated elevation in COX-1 and PGI\(_2\) synthase appears to shift the balance of prostanoid products from constrictor to dilator (Ospina et al. 2002). These effects observed in cultured endothelial cells have been also observed in cerebral blood vessels from ovariectomized rats, where oestradiol increases protein levels in both COX-1 and PGIS and up-regulates the production of PGI\(_2\), promoting increased cerebral perfusion and conferring resistance against thrombotic events (Ospina et al. 2002). Apart from this, ER\(\beta\) has also been associated with COX-2 expression and both PGI\(_2\) and TXA\(_2\) concentrations at basal state, which suggests the possibility of a ligand-independent regulation of COX-2 activity and PGH\(_2\) substrate availability (Su et al. 2009).

GPER also mediates oestrogen-dependent inhibition of endothelium-derived vasoconstrictor prostanoid production and activity under pro-inflammatory conditions, providing evidence for a novel mechanism through which GPER could inhibit vascular tone and inflammation (Meyer et al. 2015).

In addition to regulating endothelium-derived factors, oestradiol directly regulates the smooth muscle layer by inhibiting VSMC proliferation, migration and vascular contraction (Suzuki et al. 1996). Indeed, oestrogen-mediated relaxation can also occur in endothelium-denuded segments (Mugge et al. 1993). Several mechanisms, involving among others ion channels and kinase cascades, have been proposed to explain this vasorelaxant effect. Oestrogen can interfere with ion channels through non-genomic actions and decrease smooth muscle constriction by interfering with Ca\(^{2+}\) mobilization and Ca\(^{2+}\) entry responses (Crews & Khalil, 1999) and activating K\(^+\) channels (White et al. 2002), leading to membrane hyperpolarization and vascular relaxation. The role of ER has been studied in female rat mesenteric microvessels, where ER subtypes mediate distinct vasodilatation and decreased intracellular Ca\(^{2+}\) (mainly through ER\(\alpha\), with both ER\(\beta\) and GPER being also implicated) through endothelium- and K\(^+\) channel-independent inhibition of Ca\(^{2+}\) entry mechanisms of VSMC contraction (Mazzuca et al. 2015). Direct interaction of oestradiol with voltage-gated Maxi-K channel subunit \(\beta\), which confers higher Ca\(^{2+}\) sensitivity, may modulate vascular smooth muscle (Valverde et al. 1999). Oestrogen can also modulate vasoconstriction by interfering with protein kinase C (Kanashiro & Khalil, 2001) and Rho-kinase signalling in VSMC (Hiroki et al. 2005).

### Oestrogen and angiotensin 1–7.

Additionally, oestradiol is able to modulate the renin–angiotensin system (RAS) (Farhat et al. 1996; Alvarez et al. 2002), which plays a pivotal role in physiological regulation of blood volume and blood pressure and is involved in controlling vascular contractibility. Renin released from the kidney converts angiotensinogen from the liver to the decapeptide angiotensin-I (Ang 1), which undergoes proteolytic cleavage, through activating angiotensin-converting enzyme (ACE) to generate angiotensin-II (Ang II). The discovery of angiotensin-converting enzyme 2 (ACE2), which cleaves COOH-terminal residues from Ang I and II, producing primarily vasoprotective angiotensin 1–7 (Ang-(1–7)), suggested that RAS involves two axes: (1) Ang II, which mediates vasoconstriction and remodelling effects through receptor type 1 (AT1R) while exert opposing effects through Ang II receptor type 2 (AT2R), and (2) Ang-(1–7), which acts as a protective and vasodilator pathway acting on the Mas receptor. Changes in Ang II/Ang-(1–7) balance are therefore essential to maintain cardiovascular homeostasis (Jiang et al. 2014).

Evidence indicates that components of the RAS are markedly affected by oestrogen (Sullivan, 2008; Hilliard et al. 2013b) shifting the balance towards the ACE2/Ang-(1–7)/Mas and AT2R pathways in females. In general, oestrogen increases the synthesis of circulating angiotensinogen, while decreasing the synthesis of the RAS enzymes renin and ACE (Fischer et al. 2002; Komukai et al. 2010). Accordingly, sex differences in vascular RAS mechanisms have commonly been assumed to play a role in the relative protection against CVD in premenopausal women. Circulating plasma Ang-(1–7) concentrations have been reported to be higher in healthy premenopausal women than in healthy men of a similar age (Sullivan et al. 2015), and its relationship with oestrogen is underscored in studies showing an increase of urinary Ang 1–7 levels among pregnant women (Valdes et al. 2001). Vascular AT1R expression in ovariectomized rats treated with oestradiol is down-regulated (Niikenig et al. 1998; Rogers et al. 2007). There is also evidence that AT2R plays a protective role by regulating blood pressure in female mice (Armando et al. 2002; Brown et al. 2012), rats (Sampson et al. 2012) and women due to its up-regulation by oestrogen (Hilliard et al. 2013a). Furthermore, there is evidence that ER\(\alpha\) is involved in oestradiol-mediated effects on RAS as primarily responsible for oestrogen regulation of kidney ACE2, AT1R and AT2R genes in ovariectomized mice (Brosnihan et al. 2008), which reinforces the central role of ER\(\alpha\) in oestrogen’s beneficial impact on cardiovascular physiology.

Aside from the classical circulating RAS pathway, the intracellular RAS described as the ‘non-classical’ RAS pathway has gained attention for its ability to antagonize classical RAS signalling. RAS components are also expressed in the heart and vascular wall, and control...
vascular tone and arterial structure (Nguyen Dinh Cat & Touyz, 2011). Oestrogen also regulates tissue RAS, in that oestradiol diminishes cardiac ACE expression in human atrial tissue, while simultaneously inducing ACE2, which counteracts the classical RAS activity towards the vasodilator pathway. This ACE2 induction is prevented by the ERα antagonist, suggesting a role for ERα in mediating the cardio-vascular protective effects of oestrogen (Bukowska et al. 2017). Enhanced ACE2 activity and expression have also been reported in the kidney and uterus of experimental animals during pregnancy (Joyner et al. 2007; Neves et al. 2008) and in different models of hypertensive rats, where both ACE (Gallagher et al. 1999; Dean et al. 2005) and ACE2 tissue expression are decreased by ovariectomy and restored by oestrogen replacement (Ji et al. 2008; Shenoy et al. 2009). In endothelial cells we reported that oestradiol stimulates the production of Ang-(1–7) via ERα by increasing ACE and ACE2 expression and activity (Mompeón et al. 2016), and demonstrated that the Mas receptor plays an essential role in NO-dependent vasodilatation mediated by oestradiol (Sobrino et al. 2017). In this regard, the blockade of Mas receptor is equivalent to ER blockade in preventing the effects of oestradiol, indicating crosstalk between oestradiol and the Ang-(1–7)–Mas axis (Sobrino et al. 2017). Thus, the loss of cardiovascular protection observed in postmenopausal women could also partly result from the change from the ACE2–RAS protective axis to the classic ACE–RAS pathway (Komukai et al. 2010; Hilliard et al. 2013b; Stanheuwicz et al. 2018).

### Oestrogen-regulated miRNA

Besides oestrogen-mediated regulation of important cardiovascular pathways through a direct gene transcription mechanism, oestrogen has recently been posited as a modulator of cardiovascular physiology by modifying another group of important gene expression regulators based on epigenetic mechanisms. Among them, miRNAs are small non-coding RNAs that can inhibit gene expression post-transcriptionally via sequence-specific interactions with target genes. In addition, circulating miRNAs found in the blood stream have been proposed as non-invasive biomarkers in CVD (Fichtlscherer et al. 2011) and changes in the circulating miRNA profile have been linked to oestrogen levels in women (Perez-Cremades et al. 2018a).

Changes in miRNA levels induced by sex hormones, sex chromosome expression and regulation of key components of miRNA biosynthesis machinery have been described as possible underlying mechanisms of miRNA-mediated regulation of cardiovascular function in women. In this regard, ERs have an important role in the regulation of the miRNA-mediated oestrogen effects. First, they act as a transcription factor, as demonstrated by differences in miRNA profile between ER+ and ER− breast cancer cells (Bailey et al. 2015; Cizeron-Clairac et al. 2015). Indeed, down-regulated miRNAs in ER− breast cancer compared to ER+ lose their ER binding sites in the promoter region near the miRNA sequence (Bailey et al. 2015). At a cardiovascular level, ER binding sites near oestrogen–regulated miRNAs have also been found in VSMC (Zhao et al. 2013; Deng et al. 2015) and endothelial cells (Vidal-Gomez et al. 2018). We recently demonstrated the involvement of different ERs in the expression of oestriadiol–regulated miRNAs by using specific ER agonists and antagonists. Although most of the analysed miRNA were regulated by ERα, ERβ and GPER were also found to be involved in oestriadiol–regulated miRNA expression (Vidal-Gomez et al. 2018). In addition, oestradiol can regulate miRNA expression by acting directly on its biosynthesis machinery (Gupta et al. 2012). Although oestriadiol regulation of miRNA biosynthesis components has been reported mainly in reproductive tissues, for example in differences observed between ER+ and ER− breast cancer cells (Cheng et al. 2009; Cizeron-Clairac et al. 2015), transcriptomic data in human endothelial cells treated with physiological concentrations of oestriadiol highlight the changes in the expression of specific genes involved in miRNA synthesis (Perez-Cremades et al. 2018b).

The role of specific miRNAs in the regulatory mechanisms of oestrogen in cardiovascular function has recently been reviewed in depth elsewhere (Perez-Cremades et al. 2018a). However, the role of ERs in this effect has been addressed only for certain miRNAs (Fig. 2). For example, miR-203 is one of the dysregulated miRNAs in cultured VSMCs after oestradiol exposure (Zhao et al. 2013). It is up-regulated by an ERα-dependent mechanism, but not by ERβ, through a transcription activation mechanism mediated by the transcription factors activator protein 1 (AP-1) and Zinc finger E-box-binding homeobox 1 (Zeb-1). Moreover, the authors demonstrated the role of miR-203 in regulating VSMC proliferation, showing that inhibition of miR-203 expression cancelled out the oestriadiol-mediated effect on VSMC proliferation through targeting Abl and p63 (Zhao et al. 2013). These results suggest that this miRNA is involved in the antiproliferative action of oestrogens on VSMCs and could explain oestrogen-induced inhibition of neointimal formation after vascular damage (Mori et al. 2000; Xing et al. 2009). In addition, the ER-regulated miRNA, miR-22, contributes to the antioxidant effect of oestradiol on cardiovascular tissues (Wang et al. 2015). miR-22 activity is related to cardiac remodelling and hypertrophy (Huang & Wang, 2014). However, oestradiol treatment decreases miR-22 expression in in vitro cardiomyocytes and in vivo myocardium of ovariecctomized mice via ERα-mediated mechanisms. miR-22 down-regulation increases the expression of its
target SP1, a transcription factor that regulates the cytoprotective enzyme cystathionine γ-lyase, as well as H₂O₂ production and antioxidant defence (Wang et al. 2015). Taken together, these results may explain in part the female cardioprotection against oxidative stress (Wang et al. 2010). Furthermore, miR-22 inhibits oestrogen signalling by targeting ERα, suggesting a reciprocal regulation (Pandey & Picard, 2009). Finally, miR-21 is regulated by oestradiol via ERβ-dependent mechanisms in female cardiac tissue (Queiros et al. 2013). This miRNA has been implicated in myocardial hypertrophy by regulating mitogen-activated protein kinase (MAPK) signalling in fibroblasts (Thum et al. 2008). In this regard, miR-21 is down-regulated in female cardiac fibroblasts exposed to oestradiol and to a specific ERβ agonist, and its expression is up-regulated in the left ventricle of ERβ knockout female mice (Queiros et al. 2013). Oestradiol regulates MAPK signalling through targeting three specific negative regulators of this pro-fibrotic pathway, the negative regulator sprouty homologue 1 (Spry1), RAS p21 protein activator 1/GTPase activating protein 1 (Rasa1) and Gap1m/RAS p21 protein activator 2 (Rasa2), results that may explain the mechanisms underlying the protective effect of oestrogen on cardiac remodelling.

**Effects of oestrogen on inflammation**

Female cardiovascular health is a complex issue, since at menopause women face a decrease in oestrogen levels together with an active vascular ageing process. Coupled with known risk factors, findings from epidemiological and experimental studies have closely linked inflammatory processes with vascular ageing (Seals et al. 2011). Predominant features of the ageing process are chronic progressive increase in pro-inflammatory status (Najjar et al. 2005) and development of a more adhesive endothelium (Csiszar et al. 2008), and the process per se is known to have a positive association with levels of inflammation biomarkers and increased risk of CVD (Singh & Newman, 2011).

Data regarding the effects of oestrogen on the inflammatory process are contradictory, with both...
anti-inflammatory (Straub, 2007) and pro-inflammatory (Cutolo et al. 2006) effects reported. The inflammatory pathway is downstream of many vascular signalling mechanisms that are affected by sex and ageing, further obscuring distinct effects of sex hormones on inflammation. On one hand, oestrogen has been reported to suppress vascular inflammation by down-regulation of pro-inflammatory molecules, including cytokines and adhesion molecules (Stork et al. 2002; Kip et al. 2005). On the other hand, several clinical studies have described oestrogen as a pro-inflammatory modulator in autoimmune diseases (Cutolo et al. 2006). Numerous experimental studies report that oestradiol down-regulates tumor necrosis factor α and interleukin 1β in different cell types and suggest an anti-inflammatory and vasoprotective action for oestrogens (Straub, 2007; Novella et al. 2012). Whether the conversion of vasoprotective/anti-inflammatory effects of oestrogen to vasotoxic/pro-inflammatory effects in ageing subjects is a function of prolonged oestrogen deficiency per se or is related to the ageing process and/or the development of vascular disease remains unresolved.

As previously expressed, ERα mediates a great number of oestriadiol effects that can be beneficial to cardiovascular physiology: it produces vasodilatation and prevents vasoconstrictive and proaggregating factors, reduces VSMC proliferation and induces a beneficial lipid profile. ERβ (and as far as is known, GPER) exerts different effects and, in some conditions, counteracts the beneficial profile of oestradiol through ERα. The balance, or imbalance, between ERα and ERβ is therefore an important factor when analysing the cardiovascular effects of oestradiol. In fact, ERα activation has been shown to attenuate injury-induced vascular remodelling (Brouchet et al. 2001), but in vitro studies have also shown that ERβ also plays a protective role in injured arteries (Xing et al. 2007), leading us to posit that both ER subtypes contribute to vasoprotection.

Note that ERβ is more highly expressed than ERα in oxidative stress, hypoxia and inflammation (Rider et al. 2006). In these cases, ERβ modulation can be important in regulating pathophysiological ERα-stimulated processes. This link between ERs seems to be more evident in the vascular response to oestrogen, which appears to change during ageing and depend on years since menopause. In previous studies, we observed a gradual increase in ERβ expression in uterine arteries of postmenopausal women in line with age, even 10 years after menopause onset, while there was only a slight increase in ERα expression (Novella et al. 2012). This age-related increase in ERβ expression was positively associated with a pro-inflammatory profile of oestradiol. Likewise, in an experimental murine model of menopause, an increased ratio of ERβ/ERα in both vascular endothelium and smooth muscle in aged female mice caused a reversal of the antioxidant effect of oestrogen to a pro-oxidant profile responsible for increased oxidative stress during ageing (Novenssa et al. 2011); also, in bone marrow-derived macrophages, ERα expression is greatly diminished with age (Bowling et al. 2014). Thus, evidence suggests that vasoprotective effects of oestradiol are age-dependent and this could explain the high cardiovascular risk of HRT seen in clinical trials in postmenopausal women. While the role of ERα has been extensively studied, the actions of ERβ on the cardiovascular system and the age- and menopause-related changes of vascular ERβ actions remain unclear.

Conclusion

The beneficial effects conferred by oestrogen involve a precise balance of different intracellular signalling pathways, such as NO, prostanoids and RAS, towards a vasodilator and vasoprotective profile involving oestrogen receptors, mainly ERα. Changes in vascular oestrogen receptor expression, age- and menopause-related endothelial injury and epigenomics could also affect the cardiovascular effects of oestrogen. More research is therefore warranted to elucidate these important topics that are probably closely related to the sex differences observed in cardiovascular physiology and pathophysiology.

Future perspectives

Recent studies have provided compelling evidence that the sex of the endothelial cells will influence the responses to not just the sex hormones, but the host of vasoactive agents (Hermenegildo et al. 2013; Addis et al. 2014; Cattaneo et al. 2017). Even more, it is important to note that not only the sex of the subject but the location of the endothelial cells in the body have profound influence (Huxley et al. 2018).

The majority of the studies performed so far, including those reviewed in the present article and Table 1, do not take into account those factors. Sex is as a fundamental variable that should be considered when designing and analysing basic and clinical research. Cells of males and females have many basic biochemical differences, and many of these stem from genetic and also hormonal differences. Thus, including female subjects or female-derived specimens in research would lead to a better understanding of cardiovascular physiology in both women and men.

References


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Additional information

Competing Interests

None of the authors has any conflicts of interests.

Author Contributions

All authors worked together to conceive the topic of the review, and contributed to writing, editing and revising the text and figures. All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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