*J Physiol* 597.19 (2019) pp 4873–4886



The Journal of Physiology

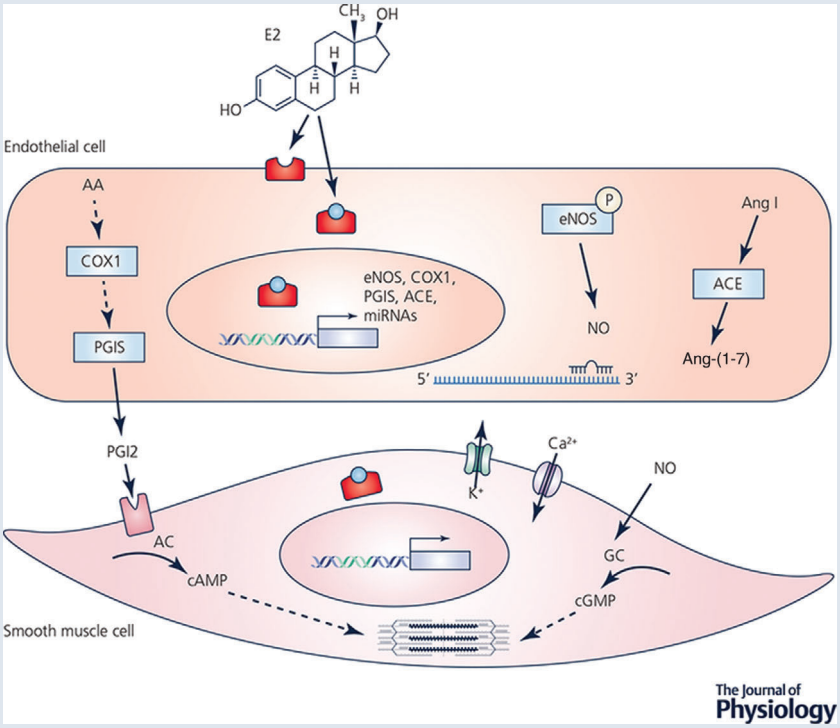
SYMPOSIUM REVIEW

# 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*

Edited by: Ole Petersen & Ylva Hellsten




**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 (ER$\alpha$, ER$\beta$ 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

**Susana Novella's** research focuses on the study of the vascular effects of oestrogens. She performs studies on cultured vascular cells and on vascular reactivity in a mouse model of experimental menopause to determine the mechanisms of endothelial dysfunction associated to ageing, specially related to nitric oxide and prostanoid signalling pathways. Another research line includes the involvement of miRNA in ageing and cardiovascular diseases. She holds a degree in Pharmacy and PhD in Physiology from University Complutense of Madrid. She is Professor of Physiology at the Faculty of Medicine of University of Valencia, and a Researcher at INCLIVA Biomedical Research Institute.

This review was presented at the Europhysiology 2018 symposium 'Estrogen, exercise and vascular function', which took place at QEII Centre, London, UK, 14 September 2018.



DOI: 10.1113/JP278063

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 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 ER$\beta$ over ER$\alpha$, generally associated with higher inflammation and endothelial dysfunction. In experimental studies, oestradiol acting on ER$\alpha$ 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. 17$\beta$-Oestradiol changes the transcriptomic 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 ER$\alpha$ 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.

(Resubmitted 30 April 2019; accepted after revision 25 June 2019; first published online 2 August 2019)
**Corresponding author** S. Novella: Universitat de València, Department of Physiology, Av/Blasco Ibañez, 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 ER$\alpha$. 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 17$\beta$-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 oestradiol 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 ER$\alpha$ and ER$\beta$ 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. ER$\alpha$ and ER$\beta$ have different distributions, and selective activation of either the ER$\alpha$ or the ER$\beta$ 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 ER$\alpha$ and ER$\beta$ expression in target organs (Murphy & Steenbergen, 2014), although studies using ER$\alpha$ and ER$\beta$ knockout mice revealed that the beneficial effects oestrogen has on the vascular system are mainly mediated by ER$\alpha$ (Pare *et al.* 2002; Arnal *et al.* 2017).

Besides this classical genomic action, oestradiol also binds to membrane-bound ER$\alpha$ and ER$\beta$ 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 ER$\alpha$ 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 ER$\alpha$

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 ER$\alpha$ 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. Oestrogen 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 $Ca^{2+}$ 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 ($O_2^-$) concentration, thereby decreasing $O_2^-$-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 ER$\alpha$ (ER66), an N-terminal truncated ER$\alpha$ 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 ER$\alpha$, 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, ER$\beta$ 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 ER$\beta$ increases the expression of cytokine-driven inducible NO synthase (iNOS) in rat vascular smooth muscle (Panic *et al.* 2018), raising the hypothesis that ER$\beta$ can be induced by injuries and contributes to inflammation (Sartoretto *et al.* 2019). In non-vascular cells, ER$\beta$ 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 ER$\alpha$, ER$\beta$ 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

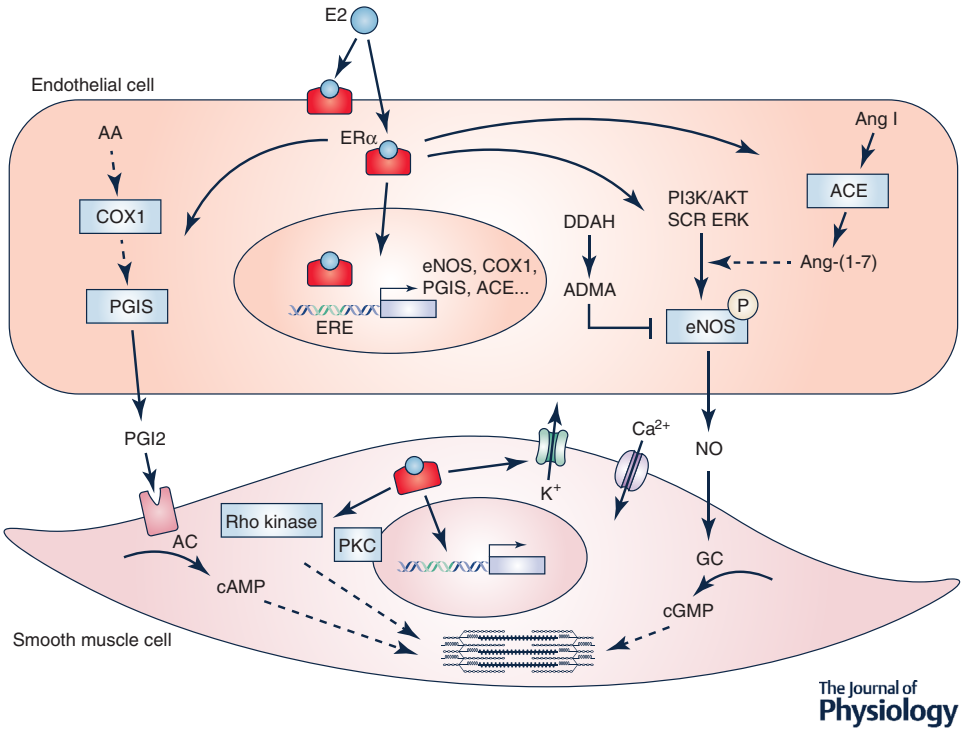



**Figure 1. Role of ER$\alpha$ on endothelium-derived mediators and vascular smooth muscle cell function**
Oestradiol (E2) binds to oestrogen receptor $\alpha$ (ER$\alpha$) triggering both genomic and cytoplasmic response. E2–ER complex is translocated to the nucleus and induces transcription of specific 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 ER$\alpha$. In addition to its genomic effect, E2–ER$\alpha$ 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–ER$\alpha$ 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 ($PGI_2$) production through the COX1–PGIS pathway. $PGI_2$ 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**

| Vasoactive-mediator production | Mechanism | Experimental model | Reference |
|---|---|---|---|
| ↑ NO bioavailability | ↑ eNOS activation<br>ER46 | EA.hy926 cells | Haynes *et al.* (2003) |
| | ↑ eNOS expression<br>Oestrogen-related receptor $\alpha$1 (ERR$\alpha$1) | COS-7 cells and bovine pulmonary artery endothelial cells | Sumi & Ignarro, (2003) |
| | ↑ NO production<br>GPER | Human endothelial cells, TIVE cells | Fredette *et al.* (2018) |
| | ↓ $O_2^-$-mediated NO inactivation | Mesenteric arteries from SHR | Dantas *et al.* (2002) |
| | ↑ DDAH expression and ↓ the endogenous L-arginine analogue ADMA<br>ER$\alpha$ | HUVECs | Monsalve *et al.* (2007) |
| | ↑ DDAH activity and ↓ the endogenous L-arginine analogue ADMA<br>ER$\alpha$ | HUAECs | Novella *et al.* (2013) |
| ↑ $PGI_2$ | ↑ COX-1 and PGIS expression<br>ER$\alpha$ | HUVECs | Sobrino *et al.* (2010) |
| ↓ ET-1 | ↓ ET-1 expression and secretion<br>ER$\alpha$ | Mesenteric arteries from DOCA hypertensive rats | David *et al.* (2001) |
| | ↓ ET-1 expression and secretion<br>ER-independent mechanism | Porcine coronary artery endothelial cells | Dubey *et al.* (2001) |
| ↑ Ang-(1–7) | ↑ ACE2 activity and expression | Renal wrap model of hypertension | Ji *et al.* (2008) |
| | ↑ ACE2 activity and expression<br>ER$\alpha$ | HUVECs | Mompeón *et al.* (2016) |

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; $PGI_2$, prostacyclin; PGIS, $PGI_2$ synthase; SHR, spontaneously hypertensive rat; TIVE, telomerase-immortalized human umbilical vein endothelial.

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 ER$\alpha$ 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, ER$\alpha$ 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).

**Oestrogen and cyclooxygenases.** Cyclooxygenase (COX)-derived factors are particularly important in regulating vascular tone as they can induce both vascular relaxation (through prostacyclin ($PGI_2$) production) and contraction (through thromboxane $A_2$ ($TXA_2$) and prostaglandin $H_2$ ($PGH_2$) 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 ER$\alpha$, induces stimulation of the vasodilator and antiaggregatory $PGI_2$ production by up-regulating COX-1 and $PGI_2$ synthase (PGIS) expression without altering vasoconstrictor $TXA_2$ 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 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 I), 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.* 2013*b*) 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 (Nickenig *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.* 2013*a*). 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 ER$\alpha$ antagonist, suggesting a role for ER$\alpha$ in mediating the cardiovascular 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 ER$\alpha$ 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.* 2013*b*; Stanhewicz *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 (Peréz-Cremades *et al.* 2018*a*).

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 oestradiol-regulated miRNAs by using specific ER agonists and antagonists. Although most of the analysed miRNA were regulated by ER$\alpha$, ER$\beta$ and GPER were also found to be involved in oestradiol-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 oestradiol 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 oestradiol highlight the changes in the expression of specific genes involved in miRNA synthesis (Pérez-Cremades *et al.* 2018*b*).

The role of specific miRNAs in the regulatory mechanisms of oestrogen in cardiovascular function has recently been reviewed in depth elsewhere (Pérez-Cremades *et al.* 2018*a*). 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 ER$\alpha$-dependent mechanism, but not by ER$\beta$, 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 oestradiol-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 oestrogen 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 ovariectomized mice via ER$\alpha$-mediated mechanisms. miR-22 down-regulation increases the expression of its

target SP1, a transcription factor that regulates the cytoprotective enzyme cystathionine $\gamma$-lyase, as well as $H_2O$ 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 ER$\alpha$, suggesting a reciprocal regulation (Pandey & Picard, 2009). Finally, miR-21 is regulated by oestradiol via ER$\beta$-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 ER$\beta$ agonist, and its expression is up-regulated in the left ventricle of ER$\beta$ 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

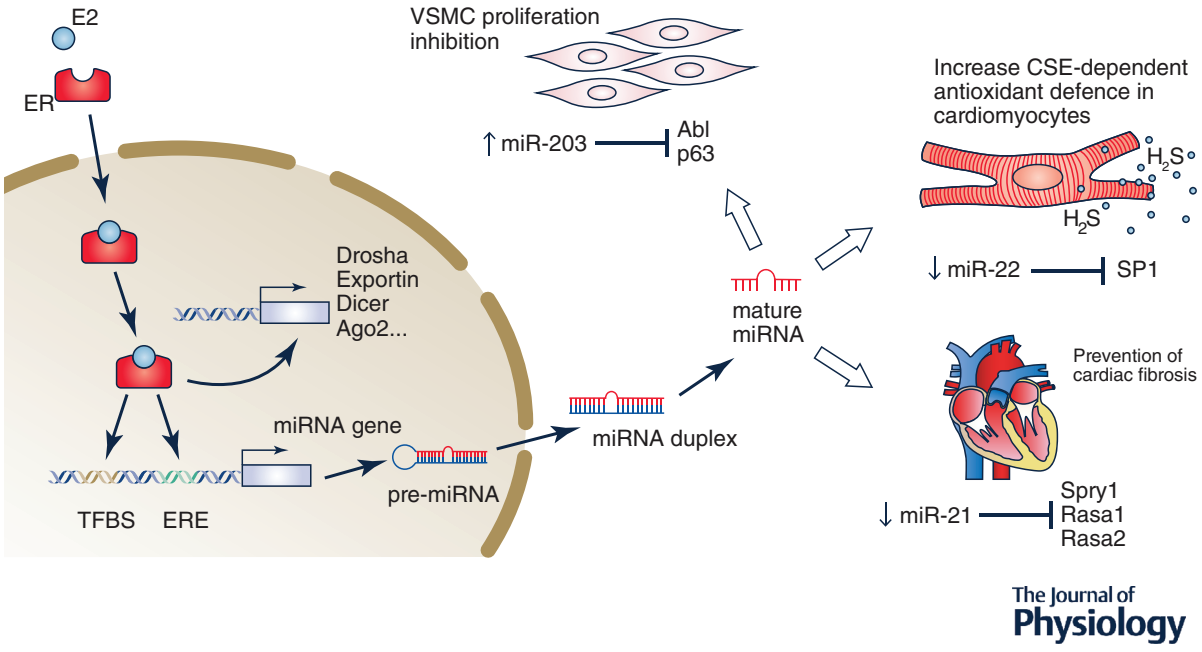



**Figure 2. Role of oestrogen receptors in miRNA-dependent regulation of cardiovascular physiology in women**
Oestradiol (E2) binds to oestrogen receptor (ER) and regulates miRNA expression directly by binding to specific oestrogen responsive elements (ERE) in the promoter region of the miRNA genes, or indirectly by recruiting different transcription factors that regulate miRNA expression via their transcription factor binding sites (TFBS). E2 can also impact miRNA expression by regulating transcription activity of miRNA biosynthesis components. The role of ER in E2-dependent miRNA regulation in cardiovascular physiology has been described for different miRNA: E2-dependent up-regulation of miR-203 is implicated in vascular smooth muscle cell (VSMC) proliferation through targeting Abl and p63 (Zhao *et al.* 2013); miR-22 down-regulation mediated by E2 in cardiomyocytes induces increased expression of SP1 transcription factor that increase cystathionine $\gamma$-lysase (CSE)-dependent $H_2S$ production and antioxidant defence (Wang *et al.* 2015); miR-21 is regulated by E2 via ER$\beta$ in cardiac tissue, preventing cardiac fibrosis through direct inhibition of three negative regulators of the MAPK signalling pathway (Spry1, Rasa1, Rasa2) (Queiros *et al.* 2013). (Created by BioRender.com.)

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 $\alpha$ and interleukin 1$\beta$ in different cell types and suggest an anti-inflammatory and vasculoprotective 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, ER$\alpha$ mediates a great number of oestradiol 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. ER$\beta$ (and as far as is known, GPER) exerts different effects and, in some conditions, counteracts the beneficial profile of oestradiol through ER$\alpha$. The balance, or imbalance, between ER$\alpha$ and ER$\beta$ is therefore an important factor when analysing the cardiovascular effects of oestradiol. In fact, ER$\alpha$ activation has been shown to attenuate injury-induced vascular remodelling (Brouchet *et al.* 2001), but *in vitro* studies have also shown that ER$\beta$ 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 ER$\beta$ is more highly expressed than ER$\alpha$ in oxidative stress, hypoxia and inflammation (Rider *et al.* 2006). In these cases, ER$\beta$ modulation can be important in regulating pathophysiological ER$\alpha$-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 ER$\beta$ 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 ER$\alpha$ expression (Novella *et al.* 2012). This age-related increase in ER$\beta$ expression was positively associated with a pro-inflammatory profile of oestradiol. Likewise, in an experimental murine model of menopause, an increased ratio of ER$\beta$/ER$\alpha$ 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 (Novensa *et al.* 2011); also, in bone marrow-derived macrophages, ER$\alpha$ 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 ER$\alpha$ has been extensively studied, the actions of ER$\beta$ on the cardiovascular system and the age- and menopause-related changes of vascular ER$\beta$ 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 ER$\alpha$. 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

Addis R, Campesi I, Fois M, Capobianco G, Dessole S, Fenu G, Montella A, Cattaneo MG, Vicentini LM & Franconi F (2014). Human umbilical endothelial cells (HUVECs) have a sex: characterisation of the phenotype of male and female cells. *Biol Sex Differ* **5**, 18.

Adler TE, Usselman CW, Takamata A & Stachenfeld NS (2018). Blood pressure predicts endothelial function and the effects of ethinyl estradiol exposure in young women. *Am J Physiol Heart Circ Physiol* **315**, H925–H933.

Alvarez A, Hermenegildo C, Issekutz AC, Esplugues JV & Sanz MJ (2002). Estrogens inhibit angiotensin II-induced leukocyte-endothelial cell interactions in vivo via rapid endothelial nitric oxide synthase and cyclooxygenase activation. *Circ Res* **91**, 1142–1150.

Arias-Loza PA, Hu K, Dienesch C, Mehlich AM, Konig S, Jazbutyte V, Neyses L, Hegele-Hartung C, Heinrich Fritzemeier K & Pelzer T (2007). Both estrogen receptor subtypes, $\alpha$ and $\beta$, attenuate cardiovascular remodeling in aldosterone salt-treated rats. *Hypertension* **50**, 432–438.

Armando I, Jezova M, Juorio AV, Terron JA, Falcon-Neri A, Semino-Mora C, Imboden H & Saavedra JM (2002). Estrogen upregulates renal angiotensin II $AT_2$ receptors. *Am J Physiol Renal Physiol* **283**, F934–F943.

Arnal JF, Lenfant F, Metivier R, Flouriot G, Henrion D, Adlanmerini M, Fontaine C, Gourdy P, Chambon P, Katzenellenbogen B & Katzenellenbogen J (2017). Membrane and nuclear estrogen receptor $\alpha$ actions: from tissue specificity to medical implications. *Physiol Rev* **97**, 1045–1087.

Bailey ST, Westerling T & Brown M (2015). Loss of estrogen-regulated microRNA expression increases HER2 signaling and is prognostic of poor outcome in luminal breast cancer. *Cancer Res* **75**, 436–445.

Barton M (2013). Cholesterol and atherosclerosis: modulation by oestrogen. *Curr Opin Lipidol* **24**, 214–220.

Bowling MR, Xing D, Kapadia A, Chen Y-F, Szalai AJ, Oparil S & Hage FG (2014). Estrogen effects on vascular inflammation are age dependent: Role of estrogen receptors. *Arterioscler Thromb Vasc Biol* **34**, 1477–1485.

Brosnihan KB, Hodgin JB, Smithies O, Maeda N & Gallagher P (2008). Tissue-specific regulation of ACE/ACE2 and AT1/AT2 receptor gene expression by oestrogen in apolipoprotein E/oestrogen receptor-$\alpha$ knock-out mice. *Exp Physiol* **93**, 658–664.

Brouchet L, Krust A, Dupont S, Chambon P, Bayard F & Arnal JF (2001). Estradiol accelerates reendothelialization in mouse carotid artery through estrogen receptor-$\alpha$ but not estrogen receptor-$\beta$. *Circulation* **103**, 423–428.

Brown RD, Hilliard LM, Head GA, Jones ES, Widdop RE & Denton KM (2012). Sex differences in the pressor and tubuloglomerular feedback response to angiotensin II. *Hypertension* **59**, 129–135.

Bukowska A, Spiller L, Wolke C, Lendeckel U, Weinert S, Hoffmann J, Bornfleth P, Kutschka I, Gardemann A, Isermann B & Goette A (2017). Protective regulation of the ACE2/ACE gene expression by estrogen in human atrial tissue from elderly men. *Exp Biol Med (Maywood)* **242**, 1412–1423.

Burns KA & Korach KS (2012). Estrogen receptors and human disease: an update. *Arch Toxicol* **86**, 1491–1504.

Cattaneo MG, Vanetti C, Decimo I, Di Chio M, Martano G, Garrone G, Bifari F & Vicentini LM (2017). Sex-specific eNOS activity and function in human endothelial cells. *Sci Rep* **7**, 9612.

Cizeron-Clairac G, Lallemand F, Vacher S, Lidereau R, Bieche I & Callens C (2015). MiR-190b, the highest up-regulated miRNA in ER$\alpha$-positive compared to ER$\alpha$-negative breast tumors, a new biomarker in breast cancers? *BMC Cancer* **15**, 499.

Clarkson TB, Melendez GC & Appt SE (2013). Timing hypothesis for postmenopausal hormone therapy: its origin, current status, and future. *Menopause* **20**, 342–353.

Crews JK & Khalil RA (1999). Antagonistic effects of 17$\beta$-estradiol, progesterone, and testosterone on $Ca^{2+}$ entry mechanisms of coronary vasoconstriction. *Arterioscler Thromb Vasc Biol* **19**, 1034–1040.

Csiszar A, Wang M, Lakatta EG & Ungvari Z (2008). Inflammation and endothelial dysfunction during aging: role of NF-$\kappa$B. *J Appl Physiol (1985)* **105**, 1333–1341.

Cutolo M, Capellino S, Sulli A, Serioli B, Secchi ME, Villaggio B & Straub RH (2006). Estrogens and autoimmune diseases. *Ann N Y Acad Sci* **1089**, 538–547.

Chambliss KL & Shaul PW (2002). Estrogen modulation of endothelial nitric oxide synthase. *Endocr Rev* **23**, 665–686.

Chen DB, Bird IM, Zheng J & Magness RR (2004). Membrane estrogen receptor-dependent extracellular signal-regulated kinase pathway mediates acute activation of endothelial nitric oxide synthase by estrogen in uterine artery endothelial cells. *Endocrinology* **145**, 113–125.

Cheng C, Fu X, Alves P & Gerstein M (2009). mRNA expression profiles show differential regulatory effects of microRNAs between estrogen receptor-positive and estrogen receptor-negative breast cancer. *Genome Biol* **10**, R90.

Dantas AP, Scivoletto R, Fortes ZB, Nigro D & Carvalho MH (1999). Influence of female sex hormones on endothelium-derived vasoconstrictor prostanoid generation in microvessels of spontaneously hypertensive rats. *Hypertension* **34**, 914–919.

Dantas AP, Tostes RC, Fortes ZB, Costa SG, Nigro D & Carvalho MH (2002). In vivo evidence for antioxidant potential of estrogen in microvessels of female spontaneously hypertensive rats. *Hypertension* **39**, 405–411.

David FL, Carvalho MH, Cobra AL, Nigro D, Fortes ZB, Reboucas NA & Tostes RC (2001). Ovarian hormones modulate endothelin-1 vascular reactivity and mRNA expression in DOCA-salt hypertensive rats. *Hypertension* **38**, 692–696.

Davidge ST & Zhang Y (1998). Estrogen replacement suppresses a prostaglandin H synthase-dependent vasoconstrictor in rat mesenteric arteries. *Circ Res* **83**, 388–395.

Dean SA, Tan J, O'Brien ER & Leenen FH (2005). 17$\beta$-Estradiol downregulates tissue angiotensin-converting enzyme and ANG II type 1 receptor in female rats. *Am J Physiol Regul Integr Comp Physiol* **288**, R759–R766.

Deng L, Blanco FJ, Stevens H, Lu R, Caudrillier A, McBride M, McClure JD, Grant J, Thomas M, Frid M, Stenmark K, White K, Seto AG, Morrell NW, Bradshaw AC, MacLean MR & Baker AH (2015). MicroRNA-143 activation regulates smooth muscle and endothelial cell crosstalk in pulmonary arterial hypertension. *Circ Res* **117**, 870–883.

Deroo BJ & Korach KS (2006). Estrogen receptors and human disease. *J Clin Invest* **116**, 561–570.

Dubey RK, Jackson EK, Keller PJ, Imthurn B & Rosselli M (2001). Estradiol metabolites inhibit endothelin synthesis by an estrogen receptor-independent mechanism. *Hypertension* **37**, 640–644.

Farhat MY, Lavigne MC & Ramwell PW (1996). The vascular protective effects of estrogen. *FASEB J* **10**, 615–624.

Fichtlscherer S, Zeiher AM & Dimmeler S (2011). Circulating microRNAs: biomarkers or mediators of cardiovascular diseases? *Arterioscler Thromb Vasc Biol* **31**, 2383–2390.

Fischer M, Baessler A & Schunkert H (2002). Renin angiotensin system and gender differences in the cardiovascular system. *Cardiovasc Res* **53**, 672–677.

Förstermann U & Sessa WC (2012). Nitric oxide synthases: regulation and function. *Eur Heart J* **33**, 829–837, 837a–837d.

Freay AD, Curtis SW, Korach KS & Rubanyi GM (1997). Mechanism of vascular smooth muscle relaxation by estrogen in depolarized rat and mouse aorta. Role of nuclear estrogen receptor and $Ca^{2+}$ uptake. *Circ Res* **81**, 242–248.

Fredette NC, Meyer MR & Prossnitz ER (2018). Role of GPER in estrogen-dependent nitric oxide formation and vasodilation. *J Steroid Biochem Mol Biol* **176**, 65–72.

Gallagher PE, Li P, Lenhart JR, Chappell MC & Brosnihan KB (1999). Estrogen regulation of angiotensin-converting enzyme mRNA. *Hypertension* **33**, 323–328.

Gavin KM, Seals DR, Silver AE & Moreau KL (2009). Vascular endothelial estrogen receptor $\alpha$ is modulated by estrogen status and related to endothelial function and endothelial nitric oxide synthase in healthy women. *Mol Endocrinol* **23**, 1307.

Gingerich S & Krukoff TL (2008). Activation of ER$\beta$ increases levels of phosphorylated nNOS and NO production through a Src/PI3K/Akt-dependent pathway in hypothalamic neurons. *Neuropharmacology* **55**, 878–885.

Gupta A, Caffrey E, Callagy G & Gupta S (2012). Oestrogen-dependent regulation of miRNA biogenesis: many ways to skin the cat. *Biochem Soc Trans* **40**, 752–758.

Harkonen PL & Vaananen HK (2006). Monocyte-macrophage system as a target for estrogen and selective estrogen receptor modulators. *Ann N Y Acad Sci* **1089**, 218–227.

Haynes MP, Li L, Sinha D, Russell KS, Hisamoto K, Baron R, Collinge M, Sessa WC & Bender JR (2003). Src kinase mediates phosphatidylinositol 3-kinase/Akt-dependent rapid endothelial nitric-oxide synthase activation by estrogen. *J Biol Chem* **278**, 2118–2123.

Haynes MP, Sinha D, Russell KS, Collinge M, Fulton D, Morales-Ruiz M, Sessa WC & Bender JR (2000). Membrane estrogen receptor engagement activates endothelial nitric oxide synthase via the PI3-kinase-Akt pathway in human endothelial cells. *Circ Res* **87**, 677–682.

Hayward CS, Kelly RP & Collins P (2000). The roles of gender, the menopause and hormone replacement on cardiovascular function. *Cardiovasc Res* **46**, 28–49.

Hermenegildo C, Lazaro-Franco M, Mompeon A, Perez-Cremades D, Vidal-Gomez X, Bueno-Beti C & Novella S (2013). Gender differences in nitric oxide production induced by estradiol in human endothelial cells. *J Hypertens* **31**, e357.

Hilliard LM, Mirabito KM & Denton KM (2013*a*). Unmasking the potential of the angiotensin AT2 receptor as a therapeutic target in hypertension in men and women: what we know and what we still need to find out. *Clin Exp Pharmacol Physiol* **40**, 542–550.

Hilliard LM, Sampson AK, Brown RD & Denton KM (2013*b*). The "his and hers" of the renin-angiotensin system. *Curr Hypertens Rep* **15**, 71–79.

Hiroki J, Shimokawa H, Mukai Y, Ichiki T & Takeshita A (2005). Divergent effects of estrogen and nicotine on Rho-kinase expression in human coronary vascular smooth muscle cells. *Biochem Biophys Res Commun* **326**, 154–159.

Huang ZP & Wang DZ (2014). miR-22 in cardiac remodeling and disease. *Trends Cardiovasc Med* **24**, 267–272.

Hurtado R, Celani M & Geber S (2016). Effect of short-term estrogen therapy on endothelial function: a double-blinded, randomized, controlled trial. *Climacteric* **19**, 448–451.

Huxley VH, Kemp SS, Schramm C, Sieveking S, Bingaman S, Yu Y, Zaniletti I, Stockard K & Wang J (2018). Sex differences influencing micro- and macrovascular endothelial phenotype in vitro. *J Physiol* **596**, 3929–3949.

Ji H, Menini S, Zheng W, Pesce C, Wu X & Sandberg K (2008). Role of angiotensin-converting enzyme 2 and angiotensin(1-7) in 17$\beta$-oestradiol regulation of renal pathology in renal wrap hypertension in rats. *Exp Physiol* **93**, 648–657.

Jiang CW, Sarrel PM, Lindsay DC, Poole-Wilson PA & Collins P (1991). Endothelium-independent relaxation of rabbit coronary artery by 17$\beta$-oestradiol in vitro. *Br J Pharmacol* **104**, 1033–1037.

Jiang F, Yang J, Zhang Y, Dong M, Wang S, Zhang Q, Liu FF, Zhang K & Zhang C (2014). Angiotensin-converting enzyme 2 and angiotensin 1–7: novel therapeutic targets. *Nat Rev Cardiol* **11**, 413–426.

Joyner J, Neves LA, Granger JP, Alexander BT, Merrill DC, Chappell MC, Ferrario CM, Davis WP & Brosnihan KB (2007). Temporal-spatial expression of ANG-(1-7) and angiotensin-converting enzyme 2 in the kidney of normal and hypertensive pregnant rats. *Am J Physiol Regul Integr Comp Physiol* **293**, R169–R177.

Kanashiro CA & Khalil RA (2001). Gender-related distinctions in protein kinase C activity in rat vascular smooth muscle. *Am J Physiol Cell Physiol* **280**, C34–C45.

Khalil RA (2013). Estrogen, vascular estrogen receptor and hormone therapy in postmenopausal vascular disease. *Biochem Pharmacol* **86**, 1627–1642.

Kim KH, Young BD & Bender JR (2014). Endothelial estrogen receptor isoforms and cardiovascular disease. *Mol Cell Endocrinol* **389**, 65–70.

Kip KE, Marroquin OC, Shaw LJ, Arant CB, Wessel TR, Olson MB, Johnson BD, Mulukutla S, Sopko G, Merz CN & Reis SE (2005). Global inflammation predicts cardiovascular risk in women: a report from the Women's Ischemia Syndrome Evaluation (WISE) study. *Am Heart J* **150**, 900–906.

Kitazawa T, Hamada E, Kitazawa K & Gaznabi AK (1997). Non-genomic mechanism of 17$\beta$-oestradiol-induced inhibition of contraction in mammalian vascular smooth muscle. *J Physiol* **499**, 497–511.

Klinge CM (2001). Estrogen receptor interaction with estrogen response elements. *Nucleic Acids Res* **29**, 2905–2919.

Komukai K, Mochizuki S & Yoshimura M (2010). Gender and the renin-angiotensin-aldosterone system. *Fundam Clin Pharmacol* **24**, 687–698.

Kondo T, Hirose M & Kageyama K (2009). Roles of oxidative stress and redox regulation in atherosclerosis. *J Atheroscler Thromb* **16**, 532–538.

Kovats S (2015). Estrogen receptors regulate innate immune cells and signaling pathways. *Cell Immunol* **294**, 63–69.

Lahm T, Crisostomo PR, Markel TA, Wang M, Wang Y, Tan J & Meldrum DR (2008). Selective estrogen receptor-$\alpha$ and estrogen receptor-$\beta$ agonists rapidly decrease pulmonary artery vasoconstriction by a nitric oxide-dependent mechanism. *Am J Physiol Regul Integr Comp Physiol* **295**, R1486–R1493.

Levin ER (2009). Plasma membrane estrogen receptors. *Trends Endocrinol Metab* **20**, 477–482.

Lindberg MK, Moverare S, Skrtic S, Gao H, Dahlman-Wright K, Gustafsson JA & Ohlsson C (2003). Estrogen receptor (ER)-$\beta$ reduces ER$\alpha$-regulated gene transcription, supporting a "ying yang" relationship between ER$\alpha$ and ER$\beta$ in mice. *Mol Endocrinol* **17**, 203–208.

Lobo RA (2017). Hormone-replacement therapy: current thinking. *Nat Rev Endocrinol* **13**, 220–231.

Manson JE, Hsia J, Johnson KC, Rossouw JE, Assaf AR, Lasser NL, Trevisan M, Black HR, Heckbert SR, Detrano R, Strickland OL, Wong ND, Crouse JR, Stein E, Cushman M; Women's Health Initiative Investigators (2003). Estrogen plus progestin and the risk of coronary heart disease. *N Engl J Med* **349**, 523–534.

Mazzuca MQ, Mata KM, Li W, Rangan SS & Khalil RA (2015). Estrogen receptor subtypes mediate distinct microvascular dilation and reduction in $[Ca^{2+}]_i$ in mesenteric microvessels of female rat. *J Pharmacol Exp Ther* **352**, 291.

Mendelsohn ME & Karas RH (1999). The protective effects of estrogen on the cardiovascular system. *N Engl J Med* **340**, 1801–1811.

Mendelsohn ME & Karas RH (2005). Molecular and cellular basis of cardiovascular gender differences. *Science* **308**, 1583–1587.

Meyer MR, Fredette NC, Barton M & Prossnitz ER (2015). G protein-coupled estrogen receptor inhibits vascular prostanoid production and activity. *J Endocrinol* **227**, 61–69.

Meyer MR, Haas E, Prossnitz ER & Barton M (2009). Non-genomic regulation of vascular cell function and growth by estrogen. *Mol Cell Endocrinol* **308**, 9–16.

Mikkola TS, Gissler M, Merikukka M, Tuomikoski P & Ylikorkala O (2013). Sex differences in age-related cardiovascular mortality. *PLoS One* **8**, e63347.

Mompeón A, Lázaro-Franco M, Bueno-Betí C, Pérez-Cremades D, Vidal-Gómez X, Monsalve E, Gironacci MM, Hermenegildo C & Novella S (2016). Estradiol, acting through ER$\alpha$, induces endothelial non-classic renin-angiotensin system increasing angiotensin 1–7 production. *Mol Cell Endocrinol* **422**, 1–8.

Monsalve E, Oviedo PJ, Garcia-Perez MA, Tarin JJ, Cano A & Hermenegildo C (2007). Estradiol counteracts oxidized LDL-induced asymmetric dimethylarginine production by cultured human endothelial cells. *Cardiovasc Res* **73**, 66–72.

Mori T, Durand J, Chen Y, Thompson JA, Bakir S & Oparil S (2000). Effects of short-term estrogen treatment on the neointimal response to balloon injury of rat carotid artery. *Am J Cardiol* **85**, 1276–1279.

Mugge A, Riedel M, Barton M, Kuhn M & Lichtlen PR (1993). Endothelium independent relaxation of human coronary arteries by 17$\beta$-oestradiol in vitro. *Cardiovasc Res* **27**, 1939–1942.

Murphy E & Steenbergen C (2014). Estrogen regulation of protein expression and signaling pathways in the heart. *Biol Sex Differ* **5**, 6.

Najjar SS, Scuteri A & Lakatta EG (2005). Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* **46**, 454–462.

Neves LA, Stovall K, Joyner J, Valdes G, Gallagher PE, Ferrario CM, Merrill DC & Brosnihan KB (2008). ACE2 and ANG-(1-7) in the rat uterus during early and late gestation. *Am J Physiol Regul Integr Comp Physiol* **294**, R151–R161.

Nguyen Dinh Cat A & Touyz RM (2011). A new look at the renin–angiotensin system—Focusing on the vascular system. *Peptides* **32**, 2141–2150.

Nickenig G, Baumer AT, Grohe C, Kahlert S, Strehlow K, Rosenkranz S, Stablein A, Beckers F, Smits JF, Daemen MJ, Vetter H & Bohm M (1998). Estrogen modulates AT1 receptor gene expression in vitro and in vivo. *Circulation* **97**, 2197–2201.

Novella S, Heras M, Hermenegildo C & Dantas AP (2012). Effects of estrogen on vascular inflammation: a matter of timing. *Arterioscler Thromb Vasc Biol* **32**, 2035–2042.

Novella S, Laguna-Fernandez A, Lazaro-Franco M, Sobrino A, Bueno-Beti C, Tarin JJ, Monsalve E, Sanchis J & Hermenegildo C (2013). Estradiol, acting through estrogen receptor $\alpha$, restores dimethylarginine dimethylaminohydrolase activity and nitric oxide production in oxLDL-treated human arterial endothelial cells. *Mol Cell Endocrinol* **365**, 11–16.

Novensa L, Novella S, Medina P, Segarra G, Castillo N, Heras M, Hermenegildo C & Dantas AP (2011). Aging negatively affects estrogens-mediated effects on nitric oxide bioavailability by shifting ER$\alpha$/ER$\beta$ balance in female mice. *PLoS One* **6**, e25335.

Ospina JA, Krause DN & Duckles SP (2002). 17$\beta$-Estradiol increases rat cerebrovascular prostacyclin synthesis by elevating cyclooxygenase-1 and prostacyclin synthase. *Stroke* **33**, 600–605.

Pandey DP & Picard D (2009). miR-22 inhibits estrogen signaling by directly targeting the estrogen receptor $\alpha$ mRNA. *Mol Cell Biol* **29**, 3783–3790.

Hormone Therapy Position Statement Advisory Panel (2017). The 2017 hormone therapy position statement of The North American Menopause Society. *Menopause* **24**, 728–753.

Panic A, Stanimirovic J, Obradovic M, Zafirovic S, Sudar-Milovanovic E, Petrovic N & Isenovic ER (2018). 17$\beta$-Estradiol inhibits hepatic iNOS via the activation of the estrogen receptor ER-$\alpha$ and inhibition of ERK1/2-miR-221 axis. *J Biol Regul Homeost Agents* **32**, 1369–1377.

Pare G, Krust A, Karas RH, Dupont S, Aronovitz M, Chambon P & Mendelsohn ME (2002). Estrogen receptor-$\alpha$ mediates the protective effects of estrogen against vascular injury. *Circ Res* **90**, 1087–1092.

Pérez-Cremades D, Mompeón A, Vidal-Gomez X, Hermenegildo C & Novella S (2018*a*). Role of miRNA in the regulatory mechanisms of estrogens in cardiovascular ageing. *Oxid Med Cell Longev* **2018**, 6082387.

Pérez-Cremades D, Mompeón A, Vidal-Gómez X, Hermenegildo C & Novella S (2018*b*). miRNA as a new regulatory mechanism of estrogen vascular action. *Int J Mol Sci* **19**, 473.

Prossnitz ER & Barton M (2011). The G-protein-coupled estrogen receptor GPER in health and disease. *Nat Rev Endocrinol* **7**, 715–726.

Queiros AM, Eschen C, Fliegner D, Kararigas G, Dworatzek E, Westphal C, Sanchez Ruderisch H & Regitz-Zagrosek V (2013). Sex- and estrogen-dependent regulation of a miRNA network in the healthy and hypertrophied heart. *Int J Cardiol* **169**, 331–338.

Revankar CM, Cimino DF, Sklar LA, Arterburn JB & Prossnitz ER (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science* **307**, 1625–1630.

Rider V, Li X, Peterson G, Dawson J, Kimler BF & Abdou NI (2006). Differential expression of estrogen receptors in women with systemic lupus erythematosus. *J Rheumatol* **33**, 1093–1101.

Rogers JL, Mitchell AR, Maric C, Sandberg K, Myers A & Mulroney SE (2007). Effect of sex hormones on renal estrogen and angiotensin type 1 receptors in female and male rats. *Am J Physiol Regul Integr Comp Physiol* **292**, R794–R799.

Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM & Ockene J (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA* **288**, 321–333.

Rossouw JE, Prentice RL, Manson JE, Wu L, Barad D, Barnabei VM, Ko M, LaCroix AZ, Margolis KL & Stefanick ML (2007). Postmenopausal hormone therapy and risk of cardiovascular disease by age and years since menopause. *JAMA* **297**, 1465–1477.

Rubio-Gayosso I, Sierra-Ramirez A, Garcia-Vazquez A, Martinez-Martinez A, Munoz-Garcia O, Morato T & Ceballos-Reyes G (2000). 17$\beta$-Estradiol increases intracellular calcium concentration through a short-term and nongenomic mechanism in rat vascular endothelium in culture. *J Cardiovasc Pharmacol* **36**, 196–202.

Sampson AK, Moritz KM & Denton KM (2012). Postnatal ontogeny of angiotensin receptors and ACE2 in male and female rats. *Gend Med* **9**, 21–32.

Sartoretto SM, Santos FF, Costa BP, Ceravolo GS, Santos-Eichler R, Carvalho MHC, Fortes ZB & Akamine EH (2019). Involvement of inducible nitric oxide synthase and estrogen receptor ESR2 (ER$\beta$) in the vascular dysfunction in female type 1 diabetic rats. *Life Sci* **216**, 279–286.

Seals DR, Jablonski KL & Donato AJ (2011). Aging and vascular endothelial function in humans. *Clin Sci (Lond)* **120**, 357–375.

Shenoy V, Grobe JL, Qi Y, Ferreira AJ, Fraga-Silva RA, Collamat G, Bruce E & Katovich MJ (2009). 17$\beta$-Estradiol modulates local cardiac renin-angiotensin system to prevent cardiac remodeling in the DOCA-salt model of hypertension in rats. *Peptides* **30**, 2309–2315.

Sherwood A, Bower JK, Fetridge-Durdle J, Blumenthal JA, Newby LK & Hinderliter AL (2007). Age moderates the short-term effects of transdermal 17$\beta$-estradiol on endothelium-dependent vascular function in postmenopausal women. *Arterioscler Thromb Vasc Biol* **27**, 1782–1787.

Simoncini T, Rabkin E & Liao JK (2003). Molecular basis of cell membrane estrogen receptor interaction with phosphatidylinositol 3-kinase in endothelial cells. *Arterioscler Thromb Vasc Biol* **23**, 198–203.

Singh T & Newman AB (2011). Inflammatory markers in population studies of aging. *Ageing Res Rev* **10**, 319–329.

Sobrino A, Mata M, Laguna-Fernandez A, Novella S, Oviedo PJ, Garcia-Perez MA, Tarin JJ, Cano A & Hermenegildo C (2009). Estradiol stimulates vasodilatory and metabolic pathways in cultured human endothelial cells. *PLoS One* **4**, e8242.

Sobrino A, Oviedo PJ, Novella S, Laguna-Fernandez A, Bueno C, Garcia-Perez MA, Tarin JJ, Cano A & Hermenegildo C (2010). Estradiol selectively stimulates endothelial prostacyclin production through estrogen receptor $\alpha$. *J Mol Endocrinol* **44**, 237–246.

Sobrino A, Vallejo S, Novella S, Lázaro-Franco M, Mompeón A, Bueno-Betí C, Walther T, Sánchez-Ferrer C, Peiró C & Hermenegildo C (2017). Mas receptor is involved in the estrogen-receptor induced nitric oxide-dependent vasorelaxation. *Biochem Pharmacol* **129**, 67–72.

Sood R, Faubion SS, Kuhle CL, Thielen JM & Shuster LT (2014). Prescribing menopausal hormone therapy: an evidence-based approach. *Int J Womens Health* **6**, 47–57.

Stanhewicz AE, Wenner MM & Stachenfeld NS (2018). Sex differences in endothelial function important to vascular health and overall cardiovascular disease risk across the lifespan. *Am J Physiol Heart Circ Physiol* **315**, H1569–H1588.

Stork S, von Schacky C & Angerer P (2002). The effect of 17$\beta$-estradiol on endothelial and inflammatory markers in postmenopausal women: a randomized, controlled trial. *Atherosclerosis* **165**, 301–307.

Straub RH (2007). The complex role of estrogens in inflammation. *Endocr Rev* **28**, 521–574.

Su EJ, Lin ZH, Zeine R, Yin P, Reierstad S, Innes JE & Bulun SE (2009). Estrogen receptor-$\beta$ mediates cyclooxygenase-2 expression and vascular prostanoid levels in human placental villous endothelial cells. *Am J Obstet Gynecol* **200**, 427–427.

Sullivan JC (2008). Sex and the renin-angiotensin system: inequality between the sexes in response to RAS stimulation and inhibition. *Am J Physiol Regul Integr Comp Physiol* **294**, R1220–R1226.

Sullivan JC, Rodriguez-Miguelez P, Zimmerman MA & Harris RA (2015). Differences in angiotensin (1-7) between men and women. *Am J Physiol Heart Circ Physiol* **308**, H1171–H1176.

Sumi D & Ignarro LJ (2003). Estrogen-related receptor $\alpha$1 up-regulates endothelial nitric oxide synthase expression. *Proc Natl Acad Sci U S A* **100**, 14451–14456.

Suzuki A, Mizuno K, Ino Y, Okada M, Kikkawa F, Mizutani S & Tomoda Y (1996). Effects of 17$\beta$-estradiol and progesterone on growth-factor-induced proliferation and migration in human female aortic smooth muscle cells in vitro. *Cardiovasc Res* **32**, 516–523.

Teoh H, Leung SW & Man RY (1999). Short-term exposure to physiological levels of 17$\beta$-estradiol enhances endothelium-independent relaxation in porcine coronary artery. *Cardiovasc Res* **42**, 224–231.

Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Koteliansky V, Rosenwald A, Basson MA, Licht JD, Pena JT, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J & Engelhardt S (2008). MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. *Nature* **456**, 980–984.

Tsutsumi S, Zhang X, Takata K, Takahashi K, Karas RH, Kurachi H & Mendelsohn ME (2008). Differential regulation of the inducible nitric oxide synthase gene by estrogen receptors 1 and 2. *J Endocrinol* **199**, 267–273.

Usselman CW, Stachenfeld NS & Bender JR (2016). The molecular actions of oestrogen in the regulation of vascular health. *Exp Physiol* **101**, 356–361.

Valdes G, Germain AM, Corthorn J, Berrios C, Foradori AC, Ferrario CM & Brosnihan KB (2001). Urinary vasodilator and vasoconstrictor angiotensins during menstrual cycle, pregnancy, and lactation. *Endocrine* **16**, 117–122.

Valverde MA, Rojas P, Amigo J, Cosmelli D, Orio P, Bahamonde MI, Mann GE, Vergara C & Latorre R (1999). Acute activation of Maxi-K channels (hSlo) by estradiol binding to the $\beta$ subunit. *Science* **285**, 1929–1931.

Vidal-Gómez X, Novella S, Pérez-Monzó I, Garabito M, Dantas AP, Segarra G, Hermenegildo C & Medina P (2016). Decreased bioavailability of nitric oxide in aorta from ovariectomized senescent mice. Role of cyclooxygenase. *Exp Gerontol* **76**, 1–8.

Vidal-Gomez X, Perez-Cremades D, Mompeon A, Dantas AP, Novella S & Hermenegildo C (2018). MicroRNA as crucial regulators of gene expression in estradiol-treated human endothelial cells. *Cell Physiol Biochem* **45**, 1878–1892.

Vrtacnik P, Ostanek B, Mencej-Bedrac S & Marc J (2014). The many faces of estrogen signaling. *Biochem Med (Zagreb)* **24**, 329–342.

Wang F, He Q, Sun Y, Dai X & Yang XP (2010). Female adult mouse cardiomyocytes are protected against oxidative stress. *Hypertension* **55**, 1172–1178.

Wang L, Tang ZP, Zhao W, Cong BH, Lu JQ, Tang XL, Li XH, Zhu XY & Ni X (2015). MiR-22/Sp-1 links estrogens with the up-regulation of cystathionine gamma-lyase in myocardium, which contributes to estrogenic cardioprotection against oxidative stress. *Endocrinology* **156**, 2124–2137.

Wassmann S, Baumer AT, Strehlow K, van Eickels M, Grohe C, Ahlbory K, Rosen R, Bohm M & Nickenig G (2001). Endothelial dysfunction and oxidative stress during estrogen deficiency in spontaneously hypertensive rats. *Circulation* **103**, 435–441.

White RE, Han G, Maunz M, Dimitropoulou C, El-Mowafy AM, Barlow RS, Catravas JD, Snead C, Carrier GO, Zhu S & Yu X (2002). Endothelium-independent effect of estrogen on $Ca^{2+}$-activated $K^+$ channels in human coronary artery smooth muscle cells. *Cardiovasc Res* **53**, 650–661.

WHO (2016). The top 10 causes of death. Global Health Observatory Data. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.

Xing D, Feng W, Miller AP, Weathington NM, Chen YF, Novak L, Blalock JE & Oparil S (2007). Estrogen modulates TNF-$\alpha$-induced inflammatory responses in rat aortic smooth muscle cells through estrogen receptor-$\beta$ activation. *Am J Physiol Heart Circ Physiol* **292**, H2607–H2612.

Xing D, Nozell S, Chen YF, Hage F & Oparil S (2009). Estrogen and mechanisms of vascular protection. *Arterioscler Thromb Vasc Biol* **29**, 289–295.

Zhan Y, Liu Z, Li M, Ding T, Zhang L, Lu Q, Liu X, Zhang Z, Vlessidis A, Aw TY, Liu Z & Yao D (2016). ER$\beta$ expression in the endothelium ameliorates ischemia/reperfusion-mediated oxidative burst and vascular injury. *Free Radic Biol Med* **96**, 223–233.

Zhao J, Imbrie GA, Baur WE, Iyer LK, Aronovitz MJ, Kershaw TB, Haselmann GM, Lu Q & Karas RH (2013). Estrogen receptor-mediated regulation of microRNA inhibits proliferation of vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* **33**, 257–265.

## 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.

### Funding

This work was supported by the Spanish Ministerio de Economía y Competitividad, Instituto de Salud Carlos III–FEDER-ERDF (grant number PI16/00229). D.P.-C. is funded by a Generalitat Valenciana/FSE (Fondo Social Europeo) fellowship (grant number APOSTD/2018/A/144).

### Keywords

estrogen receptors, endothelium, micro RNA