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Symposium Report

The molecular actions of oestrogen in the regulation of vascular health

Charlotte W. Usselman[1], Nina S. Stachenfeld[2,3,4] and Jeffrey R. Bender[5,6,7]

[1] *Faculty of Physical Education and Recreation, University of Alberta, Edmonton, AB, Canada*
[2] *The John B. Pierce Laboratory, Yale School of Medicine, New Haven, CT, USA*
[3] *Department of Obstetrics, Gynecology and Reproductive Sciences, Yale School of Medicine, New Haven, CT, USA*
[4] *Yale School of Public Health, Yale School of Medicine, New Haven, CT, USA*
[5] *Departments of Internal Medicine (Cardiovascular Medicine) and Immunobiology, Yale University School of Medicine, New Haven, CT, USA*
[6] *Yale Cardiovascular Research Center, Yale University School of Medicine, New Haven, CT, USA*
[7] *Raymond and Beverly Sackler Foundation Cardiovascular Laboratory, Yale University School of Medicine, New Haven, CT, USA*

New Findings

- **What is the topic of this review?**
  This review summarizes the beneficial actions of oestrogen on the vasculature, highlighting both molecular mechanisms and functional outcomes.
- **What advances does it highlight?**
  The net effect of oestrogen on the vascular health of women continues to be debated. Recent advances have provided strong evidence for the role of membrane-bound oestrogen receptors in the maintenance of normal endothelial function. On a broader scale, functional outcomes of oestrogen actions on the vasculature may mediate the reduced risk of cardiovascular disease in premenopausal women.

The conflicting implications of the large-scale clinical menopausal hormone therapy trials in humans *versus* the findings of studies on experimental animals underscore the limitations within our understanding of the molecular actions of oestrogen. However, recent research has provided improved insight into the actions of oestrogen on the endothelium and vascular smooth muscle. This review outlines the actions of oestrogen as it contributes to vascular structure, function and health.

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Corresponding author J. R. Bender: 300 George Street, Cardiovascular Research Center, 773 G, Yale University, New Haven, CT 06511, USA. Email: jeffrey.bender@yale.edu

Introduction

Premenopausal women benefit from a reduced incidence of cardiovascular disease relative to men of a similar age (Tunstall-Pedoe *et al.* 1994; Rosenthal & Oparil, 2000). A primary role for oestrogen in this 'cardioprotection' and a role in prevention of cardiovascular disease when supplemented following menopause had long been assumed, but large-scale clinical trials demonstrated that an increased understanding of the actions of oestrogen is required. For instance, both the Women's Health Initiative study and the Heart and Estrogen-Progestin Replacement Study indicated that the postmenopausal use of menopausal hormone therapy is associated with an increase in adverse cardiovascular events (Hulley *et al.* 1998; Grady *et al.* 2002; Rossouw *et al.* 2002; Anderson *et al.* 2004). The Kronos Early Estrogen Prevention Study, which examined postmenopausal women without cardiovascular risk factors, demonstrated no significant effect of hormone therapy on the progression of atherosclerosis (Harman *et al.* 2014; Kling *et al.* 2015), whereas the Early versus Late Intervention Trial with Estradiol showed beneficial effects of menopausal hormone therapy, but only in women with elevated lipids who were not taking statins (Karim *et al.* 2005). Taken together, the data suggest that the effects of hormone

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therapy are dependent on the timing of the initiation, dose and formulation of the treatment relative to menopause and the number or degree of pre-existing risk factors. An underlying implication of these data is that the cardiovascular actions of oestrogen are complex and incompletely understood.

Oestrogen receptors

The most well-established oestrogen receptors (ERs) are ERα and ERβ. The presence of both receptor subtypes has been documented in endothelial and vascular smooth muscle cells (Mendelsohn & Karas, 1999). While the distinct roles of each subtype continue to be elucidated, both ERα and ERβ have been shown to contribute to vascular function (Miller & Duckles, 2008). Receptor expression is modulated by circulating oestrogen levels (Ihionkhan *et al.* 2002), although ERα and ERβ appear to be regulated in a different manner by oestrogen concentrations (Okano *et al.* 2006; Miller & Duckles, 2008), and the effect of oestrogen on receptor densities is likely to be dependent on the tissue type (Haas *et al.* 2007; Miller & Duckles, 2008).

The classical view of ERα and ERβ is as ligand-activated transcription factors that reside in the cytosol. Within this context, they elicit genomic effects that require hours to days to become manifest. However, evidence has accumulated over the past 30 years to indicate that ERs and the signalling cascades initiated by ERs are more complex than previously appreciated. For example, cytosolic receptors can also mediate non-genomic responses; ER-mediated responses to oestrogen have been observed in the presence of transcriptional inhibitors (Caulin-Glaser *et al.* 1997). One of the most well-established vascular effects of oestrogen, the production of NO, appears to occur by both genomic and non-genomic mechanisms. On the one hand, long-term *in vitro* administration of oestrogen elicits increases in expression of the endothelial NO synthase (eNOS) mRNA and protein expression (Hishikawa *et al.* 1995; MacRitchie *et al.* 1997). On the other hand, activation of eNOS occurs rapidly, implicating non-genomic mechanisms as well (Caulin-Glaser *et al.* 1997; Haynes *et al.* 2000).

Another advance in oestrogen signalling has been the discovery of plasma membrane-bound ERs (Moriarty *et al.* 2006), the existence of which was debated for decades largely because of the lack of consensus regarding the molecular structure of the receptor (Hisamoto & Bender, 2005). This issue is made more complex by the presence of the various splice isoforms of the ERα, which are expressed in conditions of oestrogen deprivation (Li *et al.* 2003) and preserve oestrogen-mediated vascular responses. In exon 2-targeted female ERα knockout animals, the oestrogen-mediated protection from vascular injury is preserved (Iafrati *et al.* 1997; Karas *et al.* 1999; Pare *et al.* 2002) because of the retention of splice isoforms of ERα.

In humans, membrane-bound receptors appear to be important for the regulation of vascular function, because they have been identified on endothelial cells where the application of membrane-impermeant oestrogens results in the activation of eNOS within minutes (Russell *et al.* 2000). Importantly, cross-talk exists between oestrogen-mediated rapid signalling pathways and genomic pathways (Moriarty *et al.* 2006). Therefore, the vascular actions of oestrogen are likely to be mediated by a complex combination of membrane-associated and cytosolic ERs, as well as ER splice isoforms.

Endothelial effects of oestrogen

The endothelial layer is an important site of regulation of vascular function and plays a critical role in the determination of vascular health. Endothelial function is associated with oestrogen receptor levels such that male ER knockout mice are associated with reduced basal release of nitric oxide by the endothelium (Rubanyi *et al.* 1997). The endothelial effects of oestrogen are among the most well described and have been the topic of several reviews (Miller & Mulvagh, 2007; Kim & Bender, 2009; Arnal *et al.* 2010).

Oestrogen may affect endothelial function by increasing sensitivity to vasodilatory factors, such as acetylcholine, reducing the concentrations required to evoke similar vasodilatory responses to those observed in oestrogen-deprived animals (Gisclard *et al.* 1988; Miller & Mulvagh, 2007). Data from ovariectomized animals indicate that chronic oestrogen supplementation results in an upregulation of eNOS expression, and thereby, an increase in circulating NO (McNeill *et al.* 1999; Stirone *et al.* 2003; Okano *et al.* 2006). While this upregulation of eNOS is known to occur through genomic mechanisms (McNeill *et al.* 1999; Stirone *et al.* 2003), rapid, non-genomic pathways that lead to increases in eNOS function are activated upon oestrogen binding at membrane-associated ERs (Russell *et al.* 2000). The molecular pathways involved in oestrogen-mediated increases in endothelial NO include the rapid, oestrogen-induced activation of the tyrosine kinase c-Src, followed by sequential activation of phosphatidylinositol-3 kinase, Akt and eNOS (Haynes *et al.* 2000, 2003; Hisamoto *et al.* 2001). In some cellular preparations, the heterotrimeric G proteins $G\alpha_i$ and $G\beta\gamma$ are involved in membrane-initiated responses (Wyckoff *et al.* 2001). Plasma membrane ERs can be localized to caveolae, which are specialized lipid rafts abundant in endothelial cells (Chambliss *et al.* 2000; Li *et al.* 2003). In addition, ER46 can conform to a type I integral transmembrane protein with a ligand-binding ectodomain (Kim *et al.* 2011). The definition of these

various microdomains and components of ER-mediated signalling pathways that result in endothelial NO production provides a variety of therapeutic targets for promotion of vascular homeostasis and health.

The case study of a 31-year old man lacking ERα (Smith *et al.* 1994) has demonstrated that ERα plays a critical role in the maintenance of endothelial health. Alongside problems such as decreased bone mineral density and incomplete epiphyseal closure, the man had early-onset coronary atherosclerosis (Sudhir *et al.* 1997*a*) and lacked the flow-mediated vasodilatation (FMD) response (Sudhir *et al.* 1997*b*). Although these data also indicate that oestrogen is an important moderator of endothelial function in both men and women, sex differences in endothelial function have been observed. Whole-body production of nitric oxide, assessed over a 36 h period, is greater in women in the late follicular phase of the menstrual cycle relative to men (Forte *et al.* 1998). Additionally, when assessed in either the late follicular or the mid-luteal phase of the menstrual cycle, responses to FMD are potentiated in women relative to men (Hashimoto *et al.* 1995), pointing to an oestrogen-based increase in endothelial function in premenopausal women relative to men.

Flow-mediated dilatation presents a non-invasive means of assessing endothelial function. Flow-mediated dilatation responses have been shown to be predictive of adverse cardiovascular events (Inaba *et al.* 2010), and FMD is considered to be a valid clinical test of endothelial function (Thijssen *et al.* 2011). In support of a direct and functional effect of oestrogen on the endothelium, FMD responses are attenuated in the phase of the menstrual cycle when oestrogen levels are low (Hashimoto *et al.* 1995; Williams *et al.* 2001). Increases in FMD responses when oestrogen concentrations are elevated (Hashimoto *et al.* 1995) suggest that this effect is oestrogen dependent and not dependent on progesterone concentrations. The lack of an FMD response in the man lacking ERα likewise supports a strong role for oestrogen in the generation of the FMD response (Sudhir *et al.* 1997*b*).

Endothelial function declines markedly at menopause (Celermajer *et al.* 1994), and oestrogen administration in recently postmenopausal women has been shown to increase FMD responses (Lieberman *et al.* 1994). Likewise, postmenopausal women with vascular dysfunction experience an increase in acetylcholine-induced vasodilatation following an acute infusion of oestrogen (Gilligan *et al.* 1994). Improvements in endothelial responsiveness that occur with oestrogen administration in postmenopausal women are reduced with increasing age after menopause (Sherwood *et al.* 2007; Vitale *et al.* 2008), indicating that the prolonged absence of oestrogen elicits deleterious changes within the endothelium that cannot be restored by oestrogen treatment (Miller & Duckles, 2008).

Effects of oestrogen on vascular smooth muscle cells

Primary evidence for the effects of oestrogen on vascular smooth muscle cells has been derived from the study of endothelium-denuded arteries. In such *in vitro* preparations, oestrogen has been shown to inhibit vascular smooth muscle cell contraction, which may occur through the inhibition of calcium ion entry into the cell (Crews & Khalil, 1999*a*,*b*) and/or through the opening of potassium channels and subsequent cellular hyperpolarization (White *et al.* 1995; Wellman *et al.* 1996). In line with these findings, aortic vasoconstriction in response to phenylephrine infusions is reduced in female rats relative to male rats (Stallone *et al.* 1991; Kanashiro & Khalil, 2001). Likewise, in healthy young humans, the administration of noradrenaline elicits a greater vasoconstriction in men relative to women (Kneale *et al.* 2000).

Effects of oestrogen on atherosclerotic factors

One of the most important roles of oestrogen in the maintenance of vascular health may be its antiatherogenic properties (Rossouw, 1996). Oestrogen has been shown to affect each component of the atherosclerotic cascade (Hisamoto & Bender, 2005), including an effect on circulating lipids (Anon., 1995; Muesing *et al.* 1996) and the resultant inflammatory responses to the injury triggered by lipids and subsequent matrix deposition and intimal expansion (Beldekas *et al.* 1981). Oestrogen has also been shown to elicit a positive effect on endothelial cell growth (Krasinski *et al.* 1997), while exerting an inhibitory effect over the growth and proliferation of vascular smooth muscle cells (Kolodgie *et al.* 1996; Bhalla *et al.* 1997), both of which contribute to the antiatherosclerotic effects of oestrogen (Mendelsohn, 2000). Many of these effects may be NO mediated.

Conclusions and implications

The protective actions of oestrogen on the vasculature are multifaceted and profound. It is likely that the direct effects of oestrogen on the endothelium and vascular smooth muscle, through both rapid signalling pathways and genomic mechanisms, underlie much of the cardioprotection afforded to premenopausal women. Improved understanding of the molecular actions of oestrogen is required to optimize the development of hormonal treatments for postmenopausal women. One implication of such advances has been the development of selective ER modulators (SERMs), which have tissue-specific effects, functioning as ER agonists in some tissues and ER antagonists in others. With greater understanding of the molecular pathways affected by ER activation, a variety of SERMs are currently being engineered with the goal of maximizing

cardiovascular and other benefits (e.g. bone, vaginal) without adversely affecting breast or endometrial tissues (Khalil, 2013). Although the clinical benefits of current SERMs, primarily raloxifene, appear to be limited (Barrett-Connor *et al.* 2002, 2006), the development of novel SERMs remains a promising area of study for the preservation of cardiovascular health in postmenopausal women (Khalil, 2013) and underlines the importance of furthering our understanding of the molecular actions of oestrogen.

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Additional information

Competing interests

None declared.

Author contributions

All authors drafted and revised the work critically for important intellectual content. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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