JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY VOL. 75, NO. 9, 2020

PUBLISHED BY ELSEVIER

JACC REVIEW TOPIC OF THE WEEK

Menopause-Related Estrogen Decrease and the Pathogenesis of HFpEF

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ABSTRACT

Heart failure (HF) is a complex condition affecting >40 million people worldwide. It is defined by failure of the heart to pump (HF with reduced ejection fraction) or by the failure of the heart to relax, resulting in reduced filling but with preserved ejection fraction (HFpEF). HFpEF affects approximately 50% of patients with HF, most of whom are women. Given that the annual mortality ranges from 10% to 30% and as there are no treatments specifically directed for HFpEF, there is a need for better understanding of the underlying mechanisms of this condition. We put forward the hypothesis that the decline of estrogen at menopause might contribute to the pathogenesis of HFpEF and we highlight potential underlying mechanisms of estrogen action, which may attenuate the development of HFpEF. We also discuss areas in which additional research is needed to develop new approaches for prevention and treatment of HFpEF.
(J Am Coll Cardiol 2020;75:1074–82) © 2020 by the American College of Cardiology Foundation.

Heart failure (HF) is a complex syndrome characterized by structural and functional impairment of ventricular filling or ejection of the blood (1). HF with an ejection fraction of ≥50% is classified as HF with preserved ejection fraction (HFpEF), where left ventricular (LV) diastolic dysfunction is evident from slow LV relaxation and increased LV stiffness (2). In this process, stiffening may alter the forces during systole, resulting in reduced early diastolic filling. Approximately 50% of patients with HF present with HFpEF and the annual mortality ranges from 10% to 30% (1).

In HF, the heart adapts differently between men and women. In response to hemodynamic stress, LV hypertrophy develops, with women presenting more frequently with smaller LV diameter, higher LV performance indices, and preservation of ejection parameters compared with men (3–5). Studies with experimental animals also show that females maintain better indices of systolic function and have smaller LV dimensions (5). Importantly, clinical and experimental studies have demonstrated that the lack of estrogen enhances LV hypertrophy, whereas menopausal hormone therapy (HT) or treatment with 17β-estradiol (E2) prevents the development of LV hypertrophy in postmenopausal women and ovariectomized animals, respectively (**Figure 1A**). In particular, HT in postmenopausal women resulted in a 20% decrease in LV mass compared with postmenopausal women not taking HT (6), and the LV mass index was significantly reduced in hypertensive women taking HT (7). Treatment with E2 also improved LV diastolic function in ovariectomized animals and it was suggested that the cardioprotective effects of E2 in diastolic function are mediated via estrogen receptors in vascular endothelial cells (8–11). However, estrogen receptors are present in cardiac cells, including cardiac myocytes and fibroblasts. Along this line, there are direct

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The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

Manuscript received October 12, 2019; revised manuscript received December 13, 2019, accepted December 13, 2019.

ISSN 0735-1097/$36.00 https://doi.org/10.1016/j.jacc.2019.12.049

HIGHLIGHTS

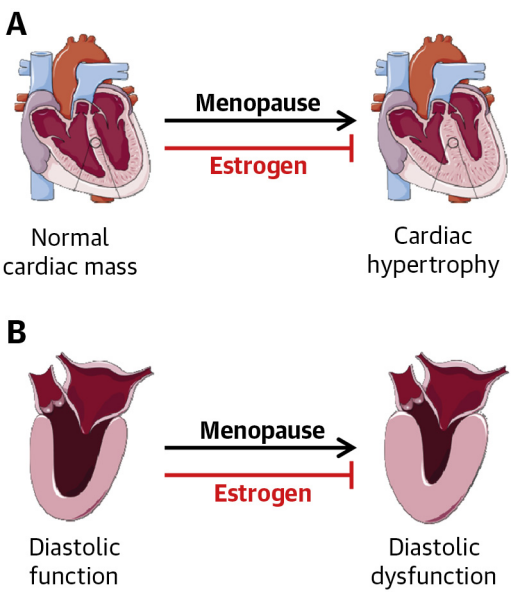
- HFpEF is a condition predominantly of postmenopausal women.
- Menopause-related E2 decline might contribute to HFpEF onset.
- E2 regulates several pathways involved in the pathogenesis of HFpEF.
- Targets for the prevention and therapy of HFpEF are necessary.

effects of E2 in the myocardium (12–19), affecting cardiac gene and protein regulation, which may influence the development of HF.

Given that patients with HFpEF are more likely to be older and female (20), and as postmenopausal women are susceptible to a higher incidence of LV diastolic dysfunction than men of the same age, a close link between diastolic dysfunction and E2 deficiency had been previously suggested (21,22). To this extent, age-related studies demonstrated progression of LV diastolic dysfunction in women (23). Along this line, postmenopausal women without HT presented with parameters of diastolic dysfunction, which appeared to be attenuated with use of HT (21,22), suggesting a role of E2 in the maintenance of diastolic function in postmenopausal women (Figure 1B).

Currently, the pathophysiology of HFpEF is not completely understood, particularly at the myocardial tissue level, and there is a general belief that there is substantial pathophysiologic heterogeneity among affected patients. Nevertheless, various studies have shown that at the cellular and molecular levels, the pathogenesis of HFpEF is characterized by changes in the renin-angiotensin-aldosterone system (RAAS) and natriuretic peptides, extracellular matrix (ECM), oxidative stress and endothelial dysfunction, and inflammation. In the present review, we discuss the role of E2 in these processes. We put forward the overall hypothesis that the decline of E2 at menopause might contribute to the pathogenesis of HFpEF (Figure 2), and we highlight potential underlying mechanisms of E2 action, which may attenuate the development of HFpEF.

FIGURE 1 Menopause and Estrogen Effects on Cardiac Structure and Function



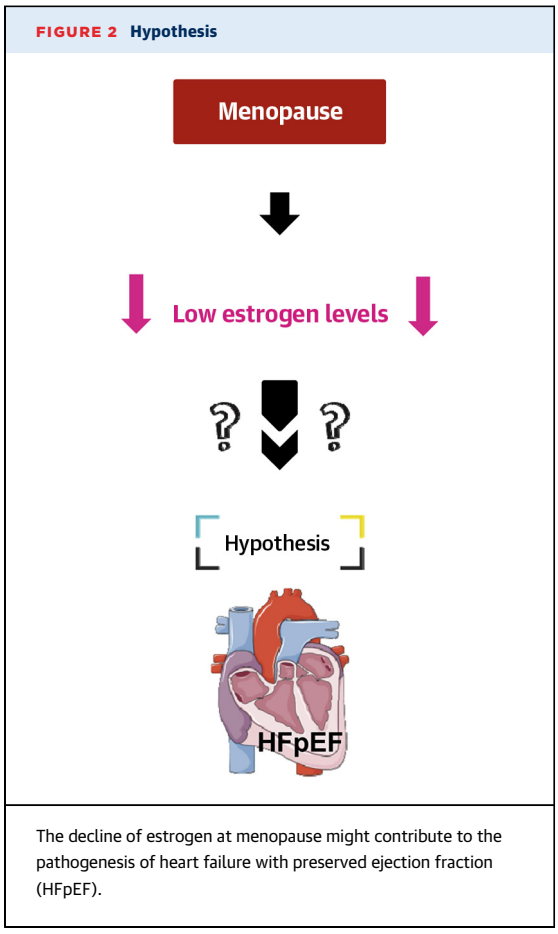
(A) Menopause-related estrogen deficiency is associated with increased cardiac mass, whereas estrogen administration prevents the development of cardiac hypertrophy. **(B)** Menopause-related estrogen deficiency is associated with diastolic dysfunction, whereas estrogen administration appears to maintain diastolic function.

ABBREVIATIONS AND ACRONYMS

ANG = angiotensin
ANP = atrial natriuretic peptide
AT1 = angiotensin II receptor type 1
BNP = B-type natriuretic peptide
E2 = 17β-estradiol
ECM = extracellular matrix
eNOs = endothelial NO synthase
ER = estrogen receptor
HF = heart failure
HFpEF = heart failure with preserved ejection fraction
HT = hormone therapy
LV = left ventricular
NFkB = nuclear factor kappa-light-chain-enhancer of activated B cells
NO = nitric oxide
RAAS = renin-angiotensin-aldosterone system
ROS = reactive oxygen species
TNF-α = tumor necrosis factor alpha

RAAS AND NATRIURETIC PEPTIDES

The RAAS, controlling water and electrolyte homeostasis, has been involved in hypertension, cardiac hypertrophy, impaired cardiomyocyte relaxation, and cardiac fibrosis, thereby contributing to the pathogenesis of HFpEF. The vasoconstrictive properties of RAAS include the activation of angiotensin (ANG) II, a potent vasoconstrictor also involved in cell proliferation, hypertrophy, generation of oxidative radicals and inflammation, and aldosterone, which is involved in hypertension and inflammation. Epidemiological and experimental studies have shown that components of the circulating (and tissue-based) RAAS are markedly affected in the premenopausal to postmenopausal transition, thereby suggesting that decreases in E2 may modulate these changes. To this extent, E2 reduced the activity of the angiotensin-converting enzyme, ANG II levels, tissue responsiveness to ANG II, and angiotensin II receptor type 1 (AT1) density in ANG II-target tissues (24–26). In addition, exposure of endothelial cells to E2 significantly increased ANG-(1-7) production, an angiotensin with antioxidant and anti-inflammatory



The decline of estrogen at menopause might contribute to the pathogenesis of heart failure with preserved ejection fraction (HFpEF).

effects (27). Furthermore, E2 inhibited production of reactive oxygen species (ROS) induced by RAAS and attenuated angiotensin-induced increased leukocyte recruitment via nitric oxide (NO) (28). However, E2 also augments both tissue and circulating levels of angiotensinogen (29,30). Angiotensinogen can exist in several forms (e.g., varying redox state, or bound to other proteins), thereby affecting the ability of renin to cleave ANG I from angiotensinogen (31). It has been put forward that the bi-directional actions of E2 on RAAS lead to a net balance promoting antihypertensive effects (32). Collectively, E2 appears to inhibit the activity of RAAS overall, which could be crucial in attenuating the development of HFpEF. It must be noted that this might be distinct to situations in which HFpEF has already developed and clinical trials targeting the RAAS indicated a modest positive trend only for secondary outcomes or retrospectively defined subgroups (33–35). In addition, potential sex differences in treatment responses have not been fully assessed. A recent secondary analysis of spironolactone therapy in HFpEF revealed a nonsignificant interaction between spironolactone and sex for the primary outcome (36). However, there was a reduction in all-cause mortality associated with spironolactone therapy in women, thereby highlighting the need to evaluate prospectively whether spironolactone therapy may be effective for treatment of HFpEF in women (36).

Natriuretic peptides are hormones secreted mainly by the heart, but also the vasculature, kidney, and central nervous system. In failing hearts, atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are synthesized and released from atrial and ventricular myocardium. Sex differences have been described in the levels of natriuretic peptides. In particular, ANP levels were reported significantly higher in younger women compared with younger men, but there was no sex difference in older individuals, where ANP levels were similar to those in younger women (37). Administration of E2 in postmenopausal hysterectomized women resulted in elevated plasma levels of N-terminal pro-ANP (38). It was also shown that men have lower levels of N-terminal pro-BNP compared with women, independent of exogenous E2 administration and of age (39). Therefore, in the more recent studies assessing the effects of exogenous E2, there was a positive association between E2 and the levels of natriuretic peptides (38,39), which may exert diuretic, natriuretic, and vasodilatory effects, as well as antifibrotic, antihypertrophic, and anti-inflammatory effects in the heart. Along this line, E2 has been previously shown to stimulate production and secretion of ANP and BNP in cultured cardiomyocytes (40) and to upregulate the ANP gene, thereby attenuating phenylephrine-induced cardiomyocyte hypertrophy (41). In addition, E2-mediated stimulation of ANP production and secretion was associated with the inhibition of ANG II-dependent protein kinase C and extracellular signal-regulated kinase signaling in cardiomyocyte hypertrophy (40). Together, these data indicate that decreased levels of natriuretic peptides associated with decreased levels of E2 may contribute to the pathogenesis of HFpEF at the postmenopausal stage. However, the modulatory effects of ANP and BNP in HF, in general, and in HFpEF, in particular, warrant further research. Notably, the latest data show that inhibition of the angiotensin receptor and neprilysin did not result in a significantly lower rate of total hospitalizations for HF and death from cardiovascular causes among patients with HFpEF (42). Nevertheless, in the pre-specified group of women, there was a suggestion of benefit (42), indicating that the effects of this combination drug might differ between male and female patients.

EXTRACELLULAR MATRIX

LV remodeling and progression of cardiac dysfunction are associated with alterations and disruptions of homeostasis of the ECM. Increased deposition and cross-linking of ECM components in the myocardium lead to myocardial stiffness, thereby being crucial in the pathogenesis of HFpEF (43). Decreased levels of circulatory E2 might contribute to observed increases in cardiac ECM components in postmenopausal women (44). In particular, the levels of collagens, a tissue inhibitor of metalloproteinase, and members of the SMAD family were higher in LV samples of older women compared with those of younger women (44). LV stiffness has been widely associated with fibrillar collagen and cross-linking. E2 is involved in the regulation of the synthesis of collagen. For example, treatment of female cardiac fibroblasts with E2 led to decreased gene expression of collagen types I and III compared with untreated cells (45), which was mediated by estrogen receptor (ER) alpha (46). Therefore, this regulation of ECM turnover by E2 may attenuate the development of HFpEF.

Renal dysfunction also contributes to development of HFpEF. E2 alters renal morphology, particularly through the inhibition of mesangial ECM accumulation, common in the development of glomerular sclerosis (47,48). At menopause, the effect is reduced, but it is restored with E2 administration (47). Also, in the kidney, E2 suppresses collagen synthesis in the glomerular mesangial cells by modulating mitogen-activated protein kinase activity and the expression of the transcription factor AP-1 (48–50), suggesting that E2 may limit the progression of glomerulosclerosis. E2 also inhibits collagen synthesis induced by ANG II and transforming growth factor-β (48), which are associated with progressive renal injury in kidney disease models. Thus, in both the heart and the kidney, E2 may modulate the pathogenesis of HFpEF through the regulation of ECM components. However, how E2 regulates several components of the ECM is not clear and requires further research.

OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION

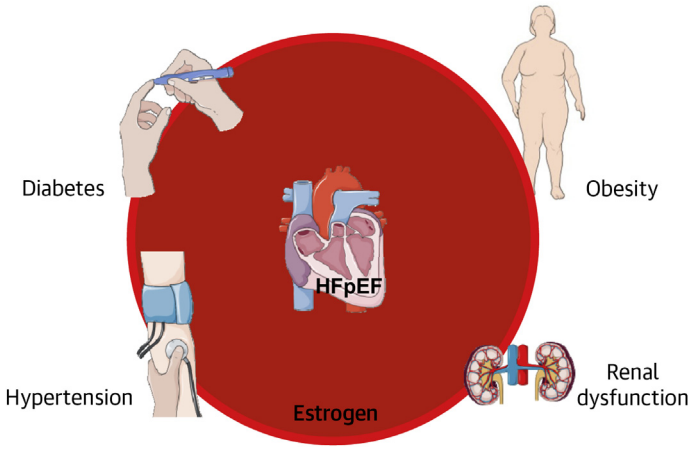
Oxidative stress occurs when the rate of ROS formation exceeds the rate of the antioxidant defense system. In pathological conditions, ROS levels can increase dramatically and may result in significant damage to cellular structures. Endothelial dysfunction is associated with increased systemic oxidative stress and vascular inflammation, characterized by reduced vasodilators modulating vascular tone, such as NO. Premenopausal women appear to have lower levels of oxidative stress (51), resulting in part from the antioxidant properties of E2 scavenging free radicals (52) than menopausal women, in whom circulating levels of E2 are reduced. E2 exerts numerous effects on mitochondria, which are important regulators of ROS production (53). In particular, E2 deficiency increased stress-induced ROS production of cardiac mitochondria (54), which may be mediated by a reduction in mitochondrial proteins involved in oxidative stress (55). As expected, administration of E2 inhibited mitochondrial ROS formation (56).

Compared with premenopausal women, production of NO is reduced in postmenopausal women, but this is restored following HT (57). E2 regulates the production of NO through tetrahydrobiopterin, an essential cofactor for the production of NO by endothelial NO synthase (eNOs), whose activity is a hallmark in LV remodeling and diastolic dysfunction (58). E2 deprivation was associated with a deficiency in cardiac tetrahydrobiopterin, thereby resulting in increased cardiac superoxide production, decreased cardiac NO release, LV remodeling, and diastolic dysfunction (59). The antioxidant properties of E2 enhancing the bioavailability of NO are also mediated directly through the stimulation of eNOs and release of NO from endothelial cells, thereby promoting vasodilation at supraphysiological concentrations in human coronary arteries (60) and at a physiological (nanomolar) range of concentrations in porcine coronary arteries (61), as well as by upregulation of eNOs messenger RNA and protein levels (62,63), which in turn mediate the effects of E2 on endothelial progenitor cell recruitment (64,65). Furthermore, E2 may lead to downregulation of vascular AT1, whereas E2 deficiency resulted in AT1 upregulation (25), thereby leading to increased oxidative stress and endothelial dysfunction. Interestingly, E2 was shown to prevent oxidative stress-induced endothelial cell apoptosis (66). In addition, decreases in E2 were associated with increased tumor necrosis factor alpha (TNF-α) levels, resulting in oxidative stress and inflammation, whereas E2 supplementation was shown to reduce TNF-α levels (67). Collectively, these regulatory actions of E2 on endothelial vasodilation function and oxidative stress regulation would also reduce progression toward HFpEF.

INFLAMMATION

Inflammation contributes to the development of cardiovascular disease in part through alterations in production of NO and ROS from the vascular endothelium and other cells, including cardiac myocytes. The resulting changes would manifest as

FIGURE 3 Comorbidities in HFpEF Influenced by Estrogen



Menopause-related estrogen decline is associated with increased risk for diabetes, hypertension, obesity, and renal dysfunction, which are part of the mosaic of comorbidities in heart failure with preserved ejection fraction (HFpEF).

cardiomyocyte stiffness, myocardial hypertrophy, and fibrosis, thereby leading to the development of HFpEF (68).

E2 influences inflammatory processes through modulation of endothelium-derived vasoactive factors and cellular pathways associated with circulating leukocytes. Along this line, HT led to a reduction of inflammatory processes, lowering circulatory cytokine levels, including interleukin-6, as well as inflammation soluble intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin (69). In addition to modulation of NO, decreases in E2 were associated with increased endothelin-1, a potent vasoconstrictor and proinflammatory peptide with important roles in inflammation, whereas E2 supplementation reduced its levels (70). However, the regulation of innate immunity and inflammatory responses by E2 are complex and may result in varying effects depending on age, species, tissue, and disease model (71–73).

E2 also appears to exert regulatory effects on nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB), a central regulator of a variety of proinflammatory genes involved in inflammatory pathways and protective cellular responses. In particular, NFkB modulates expression of target genes, such as TNF-α and interleukin-6, and E2 can repress NFkB activity by blocking its binding to the promoter region of target genes, thereby preventing their transcription (74). Moreover, the E2/ER axis represses several inflammatory pathways in the heart, including leukocyte transendothelial migration (15).

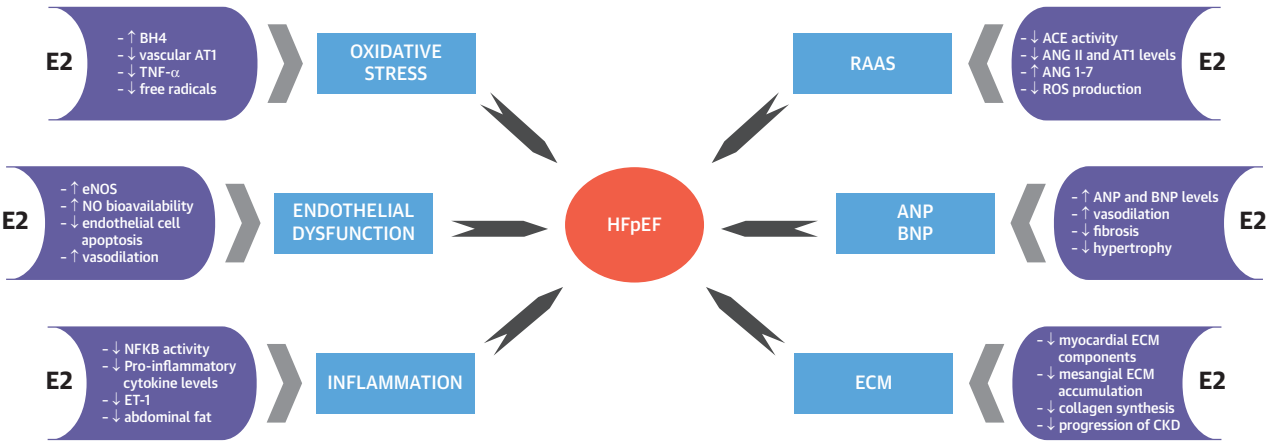
Adipose tissue is also a major source of inflammatory signaling in obesity-associated metabolic alterations. Postmenopausal women are 3 times more likely to develop obesity and metabolic syndrome than premenopausal women (75). A meta-analysis of >100 pooled randomized trials of HT in postmenopausal women showed an increase in lean body mass, reduced abdominal fat, improved insulin resistance, and decreased blood pressure (76). Consequently, the increased adipose fat accumulation and the lack of E2-based cellular modulation in postmenopausal women may be important sources of inflammatory mediators (contributing to the development of HFpEF), which were improved after HT in postmenopausal women (77).

Adipokines, such as leptin and adiponectin, pro- and anti-inflammatory hormones, are cytokines released by the adipose tissue. Higher levels of adipose tissue associated with higher levels of adipokines highlight their potential contribution to obesity-related cardiovascular disease (78,79). In fact, a recent hypothesis put forward that excess intra-abdominal fat is pivotal to the pathogenesis of HFpEF, contributing to systemic inflammation, reduced insulin sensitivity, and hypertension (80–82). To this extent, E2 may increase leptin sensitivity (83). In addition, E2 regulates female fat distribution, as it directly increases the number of α-2A-adrenergic receptors, which are antilipolytic, in subcutaneous adipocytes but not in adipocytes from the intra-abdominal fat depot (84). E2 also increases the expression of β-3-adrenergic receptor, which is the predominant receptor mediating lipolysis (85). These findings provide a mechanistic insight for the effects of E2 on energy balance opposing excessive body fat accumulation with an increased use of lipids as an energy source, which may partially promote fat reduction in abdominal fat. However, any effects of E2 on epicardial adipose tissue are incompletely understood and whether these, in turn, would affect function. Together, these data suggest that E2 may regulate systemic and localized persistent inflammation.

ESTROGEN IN THE MANAGEMENT OF HFpEF AND FUTURE DIRECTIONS

HFpEF is a mosaic of comorbidities (86–88). These include obesity, hypertension, diabetes, and renal dysfunction (**Figure 3**). The decline in E2 levels following menopause is associated with significant changes in body fat, blood pressure, lipid levels, and other risk factors, which influenced also by aging can account for cardiovascular diseases in postmenopausal women (89,90). Therefore, strategies

CENTRAL ILLUSTRATION **Potential Mechanisms of 17β-Estradiol Action Attenuating the Development of Heart Failure With Preserved Ejection Fraction**



Sabbatini, A.R. and Kararigas, G. J Am Coll Cardiol. 2020;75(9):1074–82.

This schematic representation shows regulatory mechanisms of estrogen (17β-estradiol [E2]) on biological processes contributing to the development of heart failure with preserved ejection fraction (HFpEF). The decline of E2 at menopause might contribute to the pathogenesis of HFpEF. ACE = angiotensin-converting enzyme; ANG = angiotensin; ANP = atrial natriuretic peptide; AT1 = angiotensin II receptor type 1; BH4 = tetrahydrobiopterin; BNP = B-type natriuretic peptide; ECM = extracellular matrix; eNOs = endothelial NO synthase; LV = left ventricular; NFkB = nuclear factor kappa-light-chain-enhancer of activated B cells; NO = nitric oxide; RAAS = renin-angiotensin-aldosterone system; ROS = reactive oxygen species; TNF = tumor necrosis factor.

targeting E2 could be beneficial in risk factor modification. However, the risks of HT differ depending on type, dose, duration of use, route of administration, and timing of initiation (91). Certainly, a better understanding of the effects of E2 and the underlying mechanisms is critically needed, particularly in the cardiovascular system. This holds the potential to identify novel targets for the development of new preventive and therapeutic strategies in postmenopausal women, which could be beneficial also to men.

Overall, clinical trials with different therapeutic strategies have been attempting to treat HFpEF that has already developed. However, pre-existing (cardiovascular) disease may influence the effects of an agent. Furthermore, the association between menopause-related E2 decline and the development of HFpEF has not been systematically assessed. On the basis of the concepts and the potential mechanisms of action discussed in this review, we postulate that E2 may protect against the development of HFpEF. Accordingly, we highlight the need for preventive strategies and prospective clinical trials to provide outcome data supporting this notion. In such future studies, it also will be useful to take into account genetic variation (92,93) and ER levels (94) to avert potentially unwanted effects or abnormal hormone-receptor interactions. The incorporation of artificial intelligence by means of in silico models (95,96) also will help predict responses in large scales.

CONCLUSIONS

Several clinical and experimental studies demonstrate the importance of E2 in biological processes that appear to be critical in the onset of HFpEF. The E2-regulated processes discussed here provide a mechanistic overview for potentially increased susceptibility to the development of HFpEF in women after menopause (**Central Illustration**). Considering that HFpEF is more prevalent in women than men, a better understanding of the role of E2 in the pathophysiology of HFpEF may help us to delineate future studies that would guide improvements in the knowledge and treatment of this cardiovascular disorder. In fact, elucidation of the underlying mechanisms may lead to the identification of potential therapeutic targets and the development of more efficient treatments according to individual needs. As the "timing hypothesis" about the interval between menopause and HT initiation is gaining more momentum and newer formulations and versions of HT are expected to be tested, future clinical trials and preclinical studies are needed to investigate the effects of E2 on the development of HFpEF. Ultimately, such studies would pave the way for appropriate

targeting of the E2/ER axis that might prove useful for the prevention of this disorder.

ACKNOWLEDGMENT Figures were drawn using Servier Medical Art.

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KEY WORDS 17β-estradiol, cardiac function, heart failure, hormone therapy, molecular mechanisms, postmenopausal