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Estrogen Therapy, Independent of Timing, Improves Cardiac Structure and Function in Oophorectomized mRen2.Lewis Rats

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Abstract

Objective—mRen2.Lewis Rats exhibit exacerbated increases in blood pressure, left ventricular (LV) remodeling, and diastolic impairment following the loss of estrogens. In this same model, depletion of estrogens has marked effects on the cardiac biopterin profile concomitant with suppressed nitric oxide (NO) release. With respect to the establishment of overt systolic hypertension after oophorectomy (OVX), we assessed the effects of timing chronic 17 β -estradiol (E2) therapy on myocardial function, structure, and the cardiac NO system.

Methods—Oophorectomy (OVX; n=24) or sham-operation (Sham; n=13) was performed in 4-week-old, female mRen2.Lewis rats. Following randomization, OVX rats received E2 immediately (OVX + early E2; n=7), E2 at 11 weeks of age (OVX + late E2 N=8), or no E2 at all (OVX N=9).

Results—Early E2 was associated with lower body weight, less hypertension-related cardiac remodeling, and decreased LV filling pressure compared to OVX rats without E2 supplementation. Late E2 similarly attenuated the adverse effects of ovarian hormone loss on tissue-Doppler derived LV filling pressures and perivascular fibrosis, and significantly improved myocardial relaxation, or mitral annular velocity (e'). Early and late exposure to E2 decreased dihydrobiopterin, but only late E2 yielded significant increases in cardiac nitrite concentrations.

Conclusions—Although there were some similarities between early and late E2 treatment on preservation of diastolic function and cardiac structure after OVX, the lusitropic potential of E2

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was most consistent with late supplementation. The cardioprotective effects of late E2 were independent of blood pressure and may have occurred through regulation of cardiac biopterins and NO production.

Keywords

cardiac biopterins; diastolic dysfunction; estrogen timing; LV remodeling; menopause; nitrite

Introduction

Heart failure is a major health problem with significant morbidity and mortality. Heart failure is more frequently associated with left ventricular diastolic dysfunction (LVDD) in women than in men, due to increased cardiac stiffness for which there is no specific treatment.^{1–4} Diastolic heart failure accounts for 40–60% of chronic heart failure cases,⁵ and more than 75% of these cases occur in older women.^{6–8} Evidence suggests that the loss of estrogens in postmenopausal women contributes to the development of hypertension and cardiac hypertrophy,² which are risk factors for diastolic dysfunction. Women appear to be protected from LVDD prior to menopause, with a rapid increase in incidence after menopause.^{2,9} These observations underscore the importance of female sex hormones, particularly estrogen, in regulating blood pressure, diastolic function, and LV remodeling. However, the mechanisms by which estrogens exert cardiovascular protection remain poorly understood, and the appropriate timing for initiation of estrogen therapy continues to be controversial.^{10–12}

The results of the Women's Health Initiative (WHI) and Heart and Estrogen/progestin Replacement Study (HERS) studies stimulated new interest in the timing of initial estrogen therapy, in relation to cardiovascular differences between peri- and post-menopausal women.^{13,14} The HERS study found that therapy with estrogen plus progestin in postmenopausal women reduced risk for coronary heart disease during years 3–5 post-initiation of therapy but caused an unexpected 50% increase in risk during the 1st year of therapy, resulting in an overall null effect. Thus, timing may be the key to maximizing potential benefits and minimizing adverse effects of hormone therapy.^{15–17} It was hypothesized that the vessels and myocardium of older postmenopausal women undergo significant age-related remodeling and pathology, including systolic hypertension, so that "late" initiation of estrogen therapy (10 years or more after the loss of ovarian hormone production) may not reverse cardiovascular damage or prevent further disease progression.¹² While ongoing clinical trials, including the Early versus Late Intervention Trial with Estradiol (ELITE) (<http://clinicaltrials.gov/ct2/show/NCT00114517>) and Kronos Early Estrogen Prevention Study (KEEPS),¹⁸ are starting to provide insight into the validity of the timing hypothesis, animal models that replicate many of the critical cardiovascular features of aging women (hypertension, concentric LV hypertrophy, diastolic dysfunction) can help determine whether the timing of estrogen therapy affects the prevention, slowing, or reversal of the structural and functional cardiac defects that develop in postmenopausal women.

The female mRen2.Lewis rat is particularly well suited for testing the timing hypothesis. The mRen2.Lewis rat is a congenic rodent model of angiotensin II-dependent hypertension. The female, ovariectomized at the age 4–5 weeks, is a well-established animal model of sex-specific diastolic dysfunction that corresponds to the cardiovascular phenotype of postmenopausal women. Early bilateral oophorectomy (OVX) of this model consistently exacerbates increases in systolic blood pressure; LV remodeling, including collagen deposition and myocyte hypertrophy; and impairment of diastolic function.^{19–22} The present study mimicked early and late estrogen (E2) therapy and resulting clinical effects by determining the myocardial effectiveness of chronic E2 therapy before and after the

establishment of overt hypertension in OVX-mRen2.Lewis rats. The structural and functional LV phenotypes were compared for four groups of age-matched littermates: 1) untreated OVX rats; 2) rats that received “early” E2, initiated at the time of surgical OVX; 3) rats that received “late” E2, initiated 6 weeks after OVX, when systolic hypertension is well-established in this model; and 4) estrogen-intact rats that underwent sham operations. Previous research using this model showed that estrogen deprivation resulted in a reduction in BH4, a cardiac biopterin required for nNOS to produce nitric oxide rather than reactive oxygen species.^{20,21} Therefore, we also determined the effects of “early” and “late” E2 therapy on nitric oxide production and cardiac biopterins (BH2, BH4, and total biopterin).

Methods

Animals

Female mRen2.Lewis rats were obtained from the Hypertension and Vascular Research Center Congenic Colony at Wake Forest School of Medicine and all studies were approved by the institution’s Animal Care and Use Committee. All animal procedures conformed to the Guide to the Care and Use of Laboratory Animals published by the US National Institutes of Health. Rats were weaned at three weeks of age and allowed to acclimate to a temperature ($22 \pm 2^\circ\text{C}$) and light (12 h light/dark cycle)-controlled, Association for Assessment and Accreditation of Laboratory Animal Care-approved facility, with *ad libitum* access to food and water.

Experimental protocol

Rats were separated randomly into four groups: Sham (n=13), OVX (n=9), OVX + early E2 (n=7), OVX + late E2 (n=8) and housed individually throughout the experiment. At 4 weeks of age, rats underwent either bilateral oophorectomy or a sham procedure under anesthesia by inhalation of isoflurane, as described previously.^{20–22} Estrogen depletion, via OVX, in young mRen2.Lewis rats has consistently been found to have a marked stimulatory effect on the progression of increased systolic blood pressure, cardiac hypertrophy and impaired left ventricular relaxation.^{19–22,23} These characteristics were not as apparent when OVX was performed at 10 weeks of age (unpublished data). In fact, the kidneys of late OVX-mRen2.Lewis rats compared to ovary-intact littermates were protected from high salt intake, independent of blood pressure.^{24,25} Thus, the early, OVX-mRen2.Lewis female reliably exhibits features that emulate the cardiovascular phenotype of older postmenopausal women, making it an ideal model to study the role of estrogen therapy on the maintenance of diastolic function. Immediately at the time of oophorectomy, the early E2 rats were implanted subcutaneously in the posterior neck with 60-day-release 17 β -estradiol pellets (36 μg /pellet; Innovative Research of America, Sarasota, FL, USA). At 13 weeks of age, the pellets were replaced to maintain continual hormonal coverage for the 10-week treatment period. The late E2 rats received subcutaneous 17- β estradiol treatment via pellet from weeks 11 to 15. The achievement of physiological levels of circulating estrogens by subcutaneous estradiol therapy and the successful depletion of circulating estrogens by oophorectomy were confirmed using a serum estradiol assay (5 pg/mL detection limit; Plymedco, Cortlandt Manor; NY, USA) at the end of the experiment.

Weekly body weight and systolic blood pressures by the tail-cuff (NIBP-LE5001, Panlab, Barcelona, Spain) were monitored throughout the study. At 15 weeks of age, rats were euthanized via exsanguination by cardiac puncture while under ketamine/xylazine anesthesia (ketamine HCL 60 mg/kg and xylazine HCL 5 mg/kg), following echocardiographic evaluation. Whole hearts were isolated and further dissected to isolate the left ventricle, right ventricle, and atria. Tissue weights were measured with an analytical scale. The left ventricle was cut into pieces for biochemical and histological analyses.

Echocardiographic evaluation

Echocardiography of all animals was performed at the end of week 15 using a Philips 5500 echocardiograph (Philips Medical Systems, Andover, MA, USA) and a 5–12 MHz pediatric phased array probe (s12 Philips, Philips Medical Systems, Andover, MA, USA). All measurements were made in accordance with the conventions of the American Society of Echocardiography, and were conducted by the same investigator who was blinded to the experimental groups. For the procedure, animals were anesthetized with an intramuscular injection of ketamine HCL 60 mg/kg and xylazine HCL 5 mg/kg. Left ventricular end-diastolic and end-systolic dimensions (LVEDD and LVESD, respectively) and LV posterior and anterior wall thicknesses at the end of diastole (PWT and AWT, respectively) were measured from midpapillary, short-axis images obtained by M-mode echocardiography. The percentage of LV fractional shortening (%FS), an index of contractile function, was calculated as $FS (\%) = [(LVEDD - LVESD)/LVEDD] \times 100$. LV mass was calculated using a standard cube formula, which assumes a spherical LV geometry according to the formula: $LV \text{ mass (LV mass)} = 1.04 \times [(LVEDD + PWT + AWT)^3 - LVEDD^3]$, where 1.04 is the specific gravity of muscle. Relative wall thickness (RWT) was calculated as: $2 \times PWT / LVEDD$. Left ventricular diastolic function was assessed using conventional and tissue Doppler imaging. From an apical 4-chamber orientation, early (E_{max}) and late (A_{max}) transmitral filling velocities and early deceleration times (E_{dec}) were obtained with the Doppler sample volume placed at the mitral valve leaflet tips. The ratio of early-to-late filling, or E/A, was calculated. Using pulsed tissue Doppler imaging (DTI), early mitral annular descent velocity (e') and the ratio of early filling velocity-to-early mitral annular velocity (E/e') were obtained. All measurements were performed with an offline analysis system (Xcelera 3.1, Koninklijke Philips Electronics, Netherlands) by a masked investigator. An average of at least five consecutive cardiac cycles to minimize beat-to-beat variability was used for all measured and calculated systolic and diastolic indices.

Histopathologic evaluation

A horizontal short-axis section of the formalin-fixed heart was taken through the left ventricle, from its equator between the apex and base. Specimens were dehydrated through an ethanol series and embedded in paraffin. Following microtome sectioning, the 4-μm tissue specimens underwent Verhoeff-Van-Gieson (VVG) staining for assessment of collagen and elastin fibers. Sections were examined by light microscopy (25× objective) using Zeiss AxioCam digital camera and AxioVision software (Zeiss; München-Hallbergmoos, Germany) by an observer who had no knowledge of treatment groups and previous results. Multiple digital images were obtained and percent perivascular collagen was calculated as the ratio of total perivascular collagen area – vessel luminal area/total perivascular collagen area × 100 using the ImageJ program from National Institutes of Health.^{26,27} For each left ventricle, an average of 5 non-epicardial coronary arteries were found suitable (e.g., cross-sectional cut) for morphometric analysis. One average value for the perivascular collagen area ratio was used for each specimen.

Cardiac Biopterins

Cardiac tissue BH₄ levels were measured by HPLC with fluorescence detection as previously documented.^{20,21} Total biopterins [BH₄, dihydrobiopterin (BH₂), and oxidized biopterin (B)] were measured after acid oxidation of the deproteinized cardiac supernatant. BH₂ plus B was determined by alkali oxidation, followed by iodine reduction and acidification. The resulting samples were centrifuged and small aliquots injected into a 250-mm long, 4.6-mm inner diameter Spherisorb ODS-1 column (5-μm particle size; Alltech Associates, Inc., Deerfield, IL, USA). Fluorescence (350 nm excitation, 450 nm emission) was detected by a fluorescence detector (RF10AXL; Shimadzu Co., Columbia, MD, USA). BH₄ concentration was calculated by subtracting BH₂ and B from total biopterins.

Cardiac Nitrite

Nitrite levels were determined using a chemiluminescence-based nitric oxide analyzer (Sievers, Inc., GE Analytical Instruments, Boulder, CO, USA) as previously described.^{20,21} Standard curves were obtained and used for quantitative measurements.

Statistical analysis

All values are expressed as mean $\pm$ SEM. All statistical analyses were performed using GraphPad Prism version 5 (GraphPad, San Diego, CA, USA). All endpoints were statistically evaluated by one-way analysis of variance with *Tukey's* multiple comparisons test for the post-*hoc* determination of significant differences. Differences were considered significant at $p < 0.05$.

We performed a linear trajectory analysis to estimate the predicted systolic blood pressure over time for each group. We utilized this model to examine the difference in slopes between groups before and after the 11-week intervention. (For further details regarding this model, please see Figure, Supplemental Digital Content 1, <http://links.lww.com/MENO/A49>.) The findings were consistent with the observations in this study as discussed in the results.

Results

The OVX-mRen2.Lewis rat is a well established animal model of female sex-specific diastolic dysfunction that emulates the cardiovascular phenotype of the postmenopausal woman,^{19–23} specifically systolic hypertension, left ventricular hypertrophy, impaired relaxation and elevated filling pressures. As expected, animals in the OVX group had significantly greater body weights than those in the two E2-treated groups or the sham-operated group (OVX: 269 ± 20 grams vs. Sham: 239 ± 7 grams, E2-early: 198 ± 22 grams, E2-late: 241 ± 14 grams, $p < 0.05$). Body weights in the OVX group receiving E2 supplementation beginning at the time of surgery, E2-early, were lower than sham and E2-late therapy groups ($p < 0.05$). The efficacy of surgical bilateral oophorectomy was also confirmed by the significant reduction in serum 17β -estradiol levels in OVX-rats compared to their cycling, sham-operated littermates, 27 pg/ml vs. undetectable level (< 5 pg/ml), respectively. Four (E2-late) and ten (E2-early) weeks of subcutaneous 17β -estradiol supplementation to OVX-rats increased plasma estradiol levels up to 37 and 131 pg/ml, respectively, which were within the physiological range of cycling rodents.^{23,28,29}

Consistent with our previous studies,^{20,21} substantial increases in systolic blood pressure were observed by 8 weeks of age in OVX-rats when compared to intact littermates, with a subsequent plateau by 11 weeks of age or at a systolic blood pressure of about 165 mmHg (Figure 1). In the “early” E2 therapy group, systolic blood pressure peaked by 8 weeks of age, or at 125 mmHg, and this pressure was maintained until the completion of the protocol. Interestingly, the blood pressure profile of the “early” group was lower than that observed among sham-operated, estrogen-intact rats. The blood pressure rise and plateau profile of the “late” estrogen treatment group was not different from that of the OVX-vehicle treated rats.

The early surgical loss of ovarian estrogens (at 5 weeks of age) led to significant increases in heart weight by 15 weeks of age (Table 1). The whole heart weight increased by 13% in the OVX- compared with sham-operated rats ($p < 0.05$). Since the body weight in OVX rats was also increased, the heart weight/body weight ratios were not different among groups. Even so, the wall thicknesses and LV mass, as determined by M-mode echocardiography, were significantly increased in OVX rats compared with their sham-operated littermates. While early E2 therapy limited the effects of ovarian estrogen loss on anterior wall thickness and LV mass ($p < 0.05$), its effects on heart weight and relative wall thickness were only modest.

Moreover, late E2 treatment attenuated OVX-related increases in LV mass, and tended to attenuate increases in anterior, posterior, and relative wall thicknesses ($p>0.05$) (Table 1).

The diastolic functional potential of early and late estrogen administration in OVX-mRen2.Lewis rats was evaluated in this study using both conventional and tissue Doppler echocardiography. While the conventional Doppler indices of diastolic function were not different among treatment groups (Table 2), the tissue Doppler-derived index of left ventricular filling pressure, or E/e' , was adversely affected by the loss of ovarian estrogens when compared to sham-operated and E2-treated rats (Figure 2). Myocardial relaxation, or e' , was reduced in OVX vs. intact and estrogen-treated rats but statistical significance was only apparent between OVX and late E2 therapy. Interestingly, there were no differences in e' or E/e' between the early and late E2 treated OVX-rats nor were there differences between E2-treated rats and age-matched cycling littermates. Systolic function, as determined by fractional shortening (FS), was not affected by the loss of estrogens or the administration of estradiol (Table 1).

Consistent with our previous findings, the perivascular collagen deposition was significantly enhanced in LV sections from OVX-mRen2.Lewis rats when compared with sham-operated littermates (OVX: $0.49 \pm 0.10\%$ vs. Sham: $0.36 \pm 0.09\%$, $p<0.05$). This increase in perivascular fibrosis was attenuated by both early and late estradiol supplementation ($p<0.0001$). There were no differences in collagen deposition between early and late E2-treated rats (Figure 3). Our previous data show enhanced ROS generation and reduced NO production in hearts from OVX mRen2.Lewis rats,²⁰ presumably due to a relative deficiency in tetrahydrobiopterin. Consistent with our previous findings,^{20,21} cardiac BH2 levels were increased while BH4 concentrations were decreased in hearts from OVX-rats compared to sham-operated rats (Figure 5). The OVX-related changes in BH2 were significantly inhibited by 4 weeks (late) and 10 weeks (early) of E2 supplementation. Notably, the levels of this biopterin component were similar to that of cycling, sham-operated littermates. Cardiac BH4 levels were modestly increased by early and late E2 therapy compared to hearts from OVX rats, although levels did not achieve significance. Nitrite has been shown to be a good indicator of NOS activity.³⁰ As we reported previously,²⁰ the cardiac indicator of NO production, nitrite, was significantly reduced in OVX mRen2.Lewis rats when compared to sham-operated rats. The OVX-related reduction in cardiac NO production was significantly inhibited by late E2 therapy and there was a tendency for cardiac NO to also increase in the E2-early group ($p = 0.13$). Moreover, cardiac nitrite levels in OVX-treated rats were similar to that of cycling animals (Figure 4).

Discussion

Although results of preclinical studies in animal models documented a cardioprotective effect of estrogen therapy,^{23,31–33} large clinical trials did not show protective cardiovascular benefits in women who received conjugated equine estrogen after the onset of menopause.^{11,13,14,34} Estrogen administration in the human studies usually occurred many years postmenopause. In contrast, previous studies using animal models often administered estrogen immediately after OVX. The present study compared the cardiac effects of early and late E2 administration, and resulted in several important findings. First, estrogen therapy immediately or initiated 6 weeks after OVX had an equally similar impact on decreasing LV filling pressures, or E/e' , and improving cardiac remodeling. Second, these cardioprotective effects of E2 were independent of systolic blood pressure since the pressures in the early-E2 rats were actually lower than that measured in sham-operated, estrogen-intact rats, while the pressures in the late-E2 rats were comparable to that assessed in untreated-OVX littermates. Third, the effects of E2, particularly when initiated after the blood pressure reached a

hypertensive plateau, appears to be dependent on decreases in cardiac BH2 in the presence of augmentations in BH4 and nitrite.

In the present study, estrogen therapy was started either early (immediately after OVX), or late (six weeks after OVX), a time when exacerbated increases in blood pressure reach a hypertensive plateau in this model.^{20,21} In the early E2 group, blood pressure decreased significantly compared to the control group, starting four weeks after pellet implantation. The change in blood pressure post-OVX and the effect of early E2 treatment is consistent with results of previous studies of ovariectomized mRen2.Lewis rats,²³ spontaneously hypertensive rats,³⁵ stroke-prone SHR,³⁶ deoxycorticosterone acetate salt-induced hypertensive rats,³⁷ and Dahl salt-sensitive rats.³⁸ However, once blood pressure reached a higher hypertensive plateau, late E2 therapy did not reduce the pressure, even though serum estradiol levels should have been similar four weeks after pellet implantation in the early and late groups, and not significantly different from that in intact rats. The irreversibility of increased blood pressure in the late E2 rats may have been a consequence of the adverse structural remodeling of resistant vessels brought about by the changed neurohormonal mechanisms associated with the evolution of hypertension.^{39,40} Another explanation for the absence of an overt estrogenic response on blood pressure in the late E2 group might be due to estradiol dosing and/or its potency to the novel receptor estrogen receptor GPR30 on vascular smooth muscle. While Lindsey and colleagues show that GPR30 contributes to the estrogen-dependent regulation of blood pressure in mRen2.Lewis rats,⁴¹ the 400 µg/kg/day dosing of the GPR30-specific agonist, G1, used in their studies was based on a higher dose of estradiol (1.0 mg/pellet)²³ than that of the current study (0.36 µg/pellet). Indeed, the activation of GPR30 by different doses of G1 or E2 could have different effects on blood pressure. We recently reported that chronic G1 administration (50 µg/kg/day) to mRen2.Lewis rats did not alter exacerbated elevations in blood pressure six weeks after OVX.²²

Despite the significant differences between early and late E2 treatments in final blood pressure and blood estradiol concentration, the two groups did not differ in diastolic function or measured indices of cardiac remodeling. These data suggest that ventricular relaxation processes are uncoupled from the load that the increased vascular resistance imposes on cardiac dynamics. Although our original idea was that early E2 treatment might offer additional benefit over late E2 treatment in attenuating the adverse effects of OVX on diastolic function and cardiac structure, the current findings did not support this hypothesis. Although the reasons for this are not clear, our results indicate that the protective effects of E2 may have been due to inhibition of OVX-induced increases in renin angiotensin system activity.²³ Previous studies also showed that estradiol directly attenuated angiotensin II, phenylephrine, or endothelin-1-induced hypertrophy in cardiomyocytes from isolated neonatal rats, through the cGMP-dependent protein kinase, FAK, or ERK and PKC pathways.^{40–44} Furthermore, while there were no significant differences between early and late E2 supplementation on measured outcomes, late E2 more profoundly limited the adverse effects of ovarian estrogen loss on early mitral annular velocity (e') and cardiac NO production. Whether the relative reduction in blood pressure in the long-term, or early E2 supplementation group led to compensatory increases in sympathetic activation, offsetting any direct E2 benefits, remains speculative. As reported by Pechenino et al., delayed administration of estrogen is associated with elevations in inflammatory genes such as inducible nitric oxide synthase and TNF- α as opposed to early E2,⁴⁵ which prevents these increases. Therefore, the timing of estrogen loss may potentially lead to mechanistic differences between early and late E2 initiation that reinforce the cardiac phenotype.

Previous studies concluded that one of the mechanisms by which endogenous estrogens maintain cardiac structure and diastolic function in the adult female mRen2.Lewis rat may

be through improved availability of BH₄, an essential cofactor that allows nNOS to produce nitric oxide rather than reactive oxygen species.²⁰ Results of the present study confirmed previous findings that estrogen deprivation by OVX reduced cardiac nitrite and BH₄, and enhanced BH₂. These effects may increase production of superoxide in the heart, as opposed to nitric oxide, and also increase filling pressures and deposition of cardiac collagen in comparison to intact littermates. Although the exact mechanisms by which estrogen regulates the bioavailability of cardiac BH₄ are not yet clear, the direct effects of estrogen on the expression of GTP cyclohydrolase I (GTPCH), a key enzyme in the synthesis of BH₄, may be involved.⁴⁶ Perivascular fibrosis is an important cause of diastolic dysfunction in hypertensive and aging hearts,^{27,47–49} affecting coronary hemodynamics independently of blood pressure.⁴⁷ Coronary vascular resistance and flow reserve have been shown to affect diastolic function in both humans and animal models.^{27,49} A number of studies have demonstrated that E₂ inhibits cardiac fibroblast proliferation and collagen synthesis.^{50,51} In the present study, bilateral oophorectomy increased perivascular fibrosis, and this effect was prevented by E₂ treatment. Despite the differences in serum estradiol and blood pressure, the early and late treatment groups exhibited no differences in inhibition of cardiac fibrosis, which is consistent with the lack of differences in cardiac function and structure. In contrast, estradiol treatment for 8 weeks prevented cardiac perivascular fibrosis in SHR ovariectomized at 10 weeks, but not at 25 weeks.³⁵ Inconsistencies between studies may be related to the differences in age at which time treatment commenced. Structural changes in the wall of coronary arteries may have been more pronounced in the older SHR rats used in the study, and could not be restored by estrogen treatment.

While one primary goal of hormone therapy in peri- and early postmenopausal women is the relief of vasomotor and/or urogenital symptoms, several small studies provide clinical evidence that exogenous estrogens might reverse or delay menopause-associated diastolic dysfunction. Fak et al. showed that a single oral dose of conjugated equine estrogen (0.625 mg) significantly improved isovolumic relaxation time and nearly normalized the early-to-late (E/A) transmitral filling ratio in hypertensive postmenopausal women whereas the same dose given to normotensive, postmenopausal women had no effect.⁵² Alecrin et al. showed that 12 weeks of oral estradiol (1.0 mg/day) administration significantly improved isovolumic relaxation and early deceleration times, and increased the E/A ratio in hypertensive postmenopausal women when compared to placebo-treated women with similar characteristics.⁵³ Using both conventional and tissue Doppler derived indices of diastolic function, Duzenli et al. demonstrated that 6 months of hormone therapy (oral 0.625 conjugated estrogen + 2.5 mg medroxyprogesterone acetate/day) positively affected diastolic function and improved exercise capacity in normotensive, women who were within 3 years of menopause.⁵⁴ Whether hormone therapy can improve diastolic function of older hypertensive women with more than 10 years of estrogen deficiency is not known.

When extrapolating to the clinical scenario, there are a few practical limitations that should be considered. First, the OVX rodent model leads to abrupt menopause and does not simulate the hormonal cycling of perimenopausal women. Additionally, unlike OVX rats, 90% of menopausal women still have their ovaries. The postmenopausal ovary remains hormonally active, secreting significant amounts of androgens and estrogens,⁵⁵ which could influence cardiovascular tissue and function. Future studies using 4-vinylcyclohexene diepoxide (VCD) in the mRen2.Lewis could help delineate differences in diastolic function between transitional and surgical menopause in women.^{56,57} Second, we did not monitor the physical activity of the rats, which could have positive effects on diastolic function. Clinical and experimental studies show that aerobic training limits age-related changes in diastolic function and arterial stiffness^{58–60} and might also add to the cardiovascular benefits of hormone therapy.⁶¹ Even so, the rats in this study were pair-housed, and were not exposed to freewheel running.

Conclusion

In summary, results of the present study demonstrated that early and late E2 treatments attenuated estrogen deprivation-induced diastolic dysfunction and cardiac remodeling to similar extents in female mRen2.Lewis rats, independent of final blood estradiol concentration and blood pressure. Previous studies using animal models found that a protracted delay between estrogen deprivation and replacement led to significant changes in cardiac gene expression, so that the status of cardiac tissue was markedly different when estrogen production ceased and replacement started.⁴⁵ It is likely that gene expression and the status of cardiac tissue were different when the early and late treatments were initiated in the present study, but further studies are necessary to determine the specific differences and their effects on diastolic function and LV remodeling.

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Figure 1. Tail-cuff systolic blood pressure in conscious, sham-operated and oophorectomized female mRen2.Lewis rats untreated or treated with estradiol immediately following OVX (early group) or 6 weeks post-OVX (late group). Values are means $\pm$ SEM; OVX, oophorectomized; E2, estradiol. $n = 7-13$ /group.

Figure 2.

Early mitral annular velocity (e'), and the ratio of transmitral early filling-to-early mitral annular descent (E/e') in sham-operated and oophorectomized female mRen2.Lewis rats untreated or treated with early or late estradiol. Values are mean $\pm$ SEM; * $P < 0.05$ vs. sham, # $P < 0.05$ vs. OVX. $n = 7-13$ /group.

Figure 3. Quantification (lower panel) and representative collagen staining (upper panel) of perivascular collagen in the hearts of sham-operated and oophorectomized female mRen2.Lewis rats untreated or treated with early or late estradiol. Data are expressed as mean $\pm$ SEM; *** $P < 0.0001$ vs. other groups. $n = 7-13$ /group.

Figure 4.

Cardiac concentrations of nitrite, biopterin, dihydrobiopterin, and tetrahydrobiopterin in sham-operated and oophorectomized female mRen2.Lewis rats untreated or treated with early or late estradiol. Values are mean $\pm$ SEM; * $P < 0.05$ vs. sham, # $P < 0.05$ vs. OVX. $n = 7-13$ /group.

Table 1

Body and Heart Weight Values, M-mode-derived Measures of LV Wall Thickness and Systolic Function

Variables	Sham	OVX	E2-early	E2-late
Body weight (g)	239 ± 7	269 ± 20 [*]	198 ± 22 [†]	241 ± 14 ^{†,‡}
Heart weight (g)	0.83 ± 0.06	0.94 ± 0.10 [*]	0.80 ± 0.09	0.89 ± 0.05
Heart index (mg/kg)	3.47 ± 0.25	3.50 ± 0.36	4.04 ± 0.27	3.69 ± 0.19
RWT	0.37 ± 0.10	0.71 ± 0.15 [*]	0.60 ± 0.18	0.60 ± 0.09
PWT	0.18 ± 0.03	0.23 ± 0.03 [*]	0.19 ± 0.05	0.20 ± 0.03
AWT	0.17 ± 0.02	0.20 ± 0.01 [*]	0.17 ± 0.01 [†]	0.18 ± 0.01
LV mass	0.41 ± 0.04	0.71 ± 0.07 [*]	0.38 ± 0.07 [†]	0.51 ± 0.08 [†]
LVESD (cm)	0.35 ± 0.05	0.37 ± 0.04	0.32 ± 0.06	0.37 ± 0.06
LVEDD (cm)	0.67 ± 0.06	0.68 ± 0.04	0.63 ± 0.04	0.67 ± 0.04
FS (%)	47.4 ± 4.8	46.3 ± 3.6	50.2 ± 7.3	44.1 ± 8.1

Data are expressed as mean ± SEM. OVX=oophorectomized; E2-early=17β-estradiol-early; E2-late=17 β-estradiol-late; RWT=relative wall thickness; PWT=posterior wall thickness; AWT=anterior wall thickness; LV=left ventricular; LVESD=left ventricular end-systolic dimensions; LVEDD=left ventricular end-diastolic dimensions; FS=fractional shortening

^{*}
p<0.05 vs. sham;

[†]
p<0.05 vs. OVX;

[‡]
p<0.05 vs. E2-early. n=17–13/group

Table 2

Conventional Doppler Indices of Diastolic Function

Variables	Sham	OVX	E2-early	E2-late
HR (beats/min)	251 ± 28	245 ± 33	234 ± 24	255 ± 24
Edec time (sec)	0.057 ± 0.010	0.062 ± 0.010	0.050 ± 0.009	0.058 ± 0.007
Edec slope (cm/sec ²)	11.5 ± 1.2	10.7 ± 2.8	11.1 ± 2.0	11.9 ± 2.4
Amax (cm/sec)	42.0 ± 8.3	39.8 ± 7.7	42.5 ± 12.6	44.2 ± 8.6
E/A	1.54 ± 0.26	1.64 ± 0.38	1.51 ± 0.39	1.46 ± 0.33

Data are expressed as mean ± SEM. OVX=oophorectomized; E2-early=17 β -estradiol-early; E2-late=17 β -estradiol-late