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REVIEW

Estrogenic Impact on Cardiac Ischemic/Reperfusion Injury

Sivaporn Sivasinprasasn [1,2,3,5] · Krekwit Shinlapawittayatorn [1,2,5] · Siriporn C. Chattipakorn [1,4,5] · Nipon Chattipakorn [1,2,5]

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Abstract The increase in cardiovascular disease and metabolic syndrome incidence following the onset of menopause has highlighted the role of estrogen as a cardiometabolic protective agent. Specifically regarding the heart, estrogen induced an improvement in cardiac function, preserved calcium homeostasis, and inhibited the mitochondrial apoptotic pathway. The beneficial effects of estrogen in relation to cardiac ischemia/reperfusion (I/R) injury, such as reduced infarction and ameliorated post-ischemic recovery, have also been shown. Nevertheless, controversial findings exist and estrogen therapy is reported to be related to a higher rate of thromboembolic events and atrial fibrillation in post-menopausal women. Therefore, greater clarification is needed to evaluate the exact potential of estrogen use in cases of cardiac I/R injury. This article reviews the effects of estrogen, in both acute and chronic treatment, and collates the studies with regard to their in vivo, in vitro, or clinical trial settings in cases of cardiac I/R injury and myocardial infarction.

Keyword Estrogen · Menopause · Heart · Ischemia · Reperfusion injury

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✉ Nipon Chattipakorn
nchattip@gmail.com

[1] Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

[2] Cardiac Electrophysiology unit, Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

[3] School of Medicine, Mae Fah Luang University, Chiang Rai, Thailand

[4] Department of Oral Biology and Diagnostic Science, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand

[5] Center of Excellence in Cardiac Electrophysiology Research, Chiang Mai University, Chiang Mai, Thailand

Abbreviations

eNOS	Endothelial nitric oxide synthase
ER	Estrogen receptor
I/R	Ischemic and reperfusion
GPR30	G protein-coupled receptor
ICAM-1	Intercellular adhesion molecule-1
I/R	Ischemic/reperfusion
LDL	Low-density lipoprotein
LVDP	Left ventricular developed pressure
LVP	Left ventricular pressure
mPTP	Mitochondria permeability transition pore
OVX	Ovariectomy
ROS	Reactive oxygen species
RPP	Rate pressure product
TNF	Tumor necrosis factor
VCAM-1	Vascular cell adhesion protein-1
VEGF	Vascular endothelial growth factor

Introduction

Cardiovascular disease (CVD) has been a major cause of death worldwide for the past several decades and is expected to remain so until 2030 [1]. Women have a lower incidence of CVD than men at a similar age, but the incidence shows an increase after the onset of menopause [2]. A bilateral ovariectomy (OVX) in females causes an increase in mortality from CVD suggesting related beneficial effects of female sex hormones [3]. It has been shown that systolic and diastolic blood pressure is higher in women following menopause than in age-matched men, suggesting the role of estrogen deprivation in the development of hypertension [4]. Vascular expansion and the upregulation of endothelial nitric oxide synthase (eNOS) level, in response to high-blood flow, were found to be absent in the aorta of OVX rats, indicating the impact of estrogen deprivation on vascular dysfunction which may further contribute to atherosclerosis and myocardial ischemia [5].

In addition, data from a study of 12,134 post-menopausal women indicates that earlier deprivation of endogenous estrogen, or younger menopausal age, is associated with an increasing rate of mortality from ischemic heart disease [6], suggesting that the impact of estrogen deprivation is not only on the cardiovascular status, but also on the lifespan of women.

Menopausal women not only exhibit an increased cardiovascular risk but also have an increased prevalence of metabolic syndrome including dyslipidemia, insulin resistance, visceral fat deposition, and central obesity [7]. Female sex hormone withdrawal during menopausal transition causes decreasing body energy expenditure and a buildup of excessive visceral fat [8], which augments the production of reactive oxygen species (ROS) and inflammatory adipokine release resulting in oxidative stress and non-infectious systemic inflammation [9, 10]. Chronic systemic inflammation is known to correlate with the generation of endothelial dysfunction and atherosclerosis [11]. Moreover, the other menopause-associated features, such as increased total cholesterol, LDL cholesterol, triglycerides, and hypertension, participate in the development of coronary artery disease and myocardial ischemia, which are indicated as leading causes of mortality in women in the Western world and many developing countries [12]. The recent report from our team involving estrogen-deprived female rats demonstrated that chronic consumption of a high-fat diet aggravates metabolic disorders, accelerates the impairment of cardiac autonomic regulation and mitochondrial function, and subsequently results in reduced cardiac performance [13]. These findings suggest an increased influence of obesity, on both metabolic and cardiac performance, in estrogen-deprived states.

Treatment with estrogen supplements has been reported to improve cardiac performance in cases of both non-ischemic conditions and ischemic/reperfusion (I/R) injury. In cases of I/R injury, estrogen reduced oxidative stress, protected cardiac mitochondria, and lowered myocardium infarction [14, 15]. However, there are few studies that report the functional roles of estrogen on the heart in the conditions of I/R combined with metabolic problems [16]. The influence of female sex hormones on the metabolic status and cardiac I/R must be investigated, to clarify the underlying mechanisms and to elucidate its cardiometabolic effects.

Estrogen and the Heart

It is well known that estrogen exerts its effects by binding to estrogen receptors. There are two types of classic estrogen receptors (ER) including ER alpha (ERα) and ER beta (ERβ). ER are expressed in cardiac myocytes, fibroblasts, and cardiac mitochondria and also in the endothelium, smooth muscle cells, adventitial cells, and macrophages of blood vessels [17]. The receptors of estrogen are located in the plasma membrane, cytosol, and nucleus of the cells and work as ligand-activated transcription factors which translocate into the nucleus and activate gene transcription after estrogen has bound with them [17]. Previous studies indicate cardiac expression of ERα in the nucleus, cytosol, intercalated disc, and membrane of T-tubule and plasmalemma and involvement in the estrogenic non-genomic signaling pathway [18, 19]. It is known that the expression of ERα is influenced by estrogen levels, as it has already been reported that exposure to estrogen for approximately 4 h results in downregulation of overall ERα expression in heart tissue [19]. Interestingly, expressions of ERα messenger RNA (mRNA) and protein have been shown to be elevated in patients suffering end-stage heart failure with dilated cardiomyopathy. It is also upregulated in the myocardium of patients with aortic stenosis [20, 21], suggesting that its expression is also manipulated by pressure overload on the heart. Depletion of ERα resulted in higher heart weight [22] and inhibition of estrogenic protective effects on vascular injury [23]. Activation of ERα has been shown to play a pivotal role in cardiac metabolism, as it upregulates cardiac glucose utilization [24]. In the heart, acute treatment with an ERα agonist showed potential effects in post-I/R recovery of cardiac function and reduced infarction [25, 26]. However, chronic ERα activation by PPT had no significant improvement in post-I/R arrhythmias [27], whereas chronic activation of ERα by ERA-45 resulted in increased free radicals and necrosis reduction [28].

ERβ is an estrogen receptor which exhibits its localization in the mitochondria of neurons and cardiocytes, suggesting that estrogen could mediate its direct effect on mitochondrial function [29]. Treatment with estrogen (50 μg/kg) or an ERβ agonist (diarylpropionitrile (DPN); 5 μg/kg) following trauma–hemorrhage showed an increase in mitochondrial ERβ DNA-binding activity and resulted in elevated mitochondrial respiratory complex activity as well as inhibition of apoptotic signaling pathways in cardiac mitochondria [30]. Activation of ERβ by DPN also restored cardiac function which had been suppressed in untreated rats after trauma–hemorrhage [30]. ERβ could therefore possibly directly exert its function through activating transcription factors. Moreover, ERβ can also function in a counter-balanced manner with ERα, as the binding of ERβ can result in either activation or inhibition of ERα-induced gene transcription. At sub-saturating concentrations of estrogen, increasing ERβ expression vectors could suppress the transcriptional activity and decrease the sensitivity to estrogen of ERα [31]. These suppressing responses of ERβ, which are controlled by the concentration of estrogen hormone or ERβ agonists, suggest its potential in the modulation of cell function [31]. Depletion of ERβ caused a reduction of mitochondrial electron transport enzyme activities in platelets, increased the number of microvesicles, and raised thrombin-generating capacity in the plasma [32]. These results of ERβ deficiency and altered platelet mitochondrial energy

production suggest a mechanism that may be responsible for the development of thromboembolism and consequent development of myocardial infarction.

Another type of estrogen receptor is G protein-coupled (GPR30, GPER) which is located in the plasma membrane and endoplasmic reticulum. Activation of GPR30 reduced the expression of inflammatory cytokines, ICAM-1 and VCAM-1, which were induced by the tumor necrosis factor (TNF) in endothelial cells, suggesting that the antiinflammatory effects of GPR30 stimulation occur via a non-genomic pathway [33]. According to its location, the binding of estrogen to GPR30 caused intracellular calcium mobilization and synthesis of phosphatidylinositol 3,4,5-trisphosphate in the nucleus, thought to be involved in the mediation of the physiological effects of estrogen on cells [34]. Using isolated Langendorff-perfused hearts, GPR30 activation by G-1 administration enhanced heart rate (HR), decreased left ventricular pressure (LVP), and increased rate pressure product (RPP), initially through ERK and subsequently via eNOS phosphorylation pathways [35]. Mice with GPR30 deficiency showed enlargement of the left ventricle and impairment of cardiac function. In addition, myocardial contraction and relaxation in these mice were diminished and left ventricular end-diastolic pressure (LVEDP) was increased [36].

Estrogen mitigated cardiac fibroblasts prevented cardiac fibrosis, hypertrophy, and cardiomyocyte apoptosis, suggesting benefits in the treatment of myocardial ischemia and heart failure progression [37, 38]. Loss of estrogen signaling in females resulted in cardiac function impairment and accelerated responses of the heart to pathological insults [39]. After estrogen deprivation, left ventricular (LV) hypertrophy and dilatation were more extensive due to chronic volume overload, which suggested more severe LV myocardial dysfunction and extensive cardiac remodeling [40]. A patch clamp study showed that estrogen receptor knockout (ERKO) mice had an elevated number of cardiac L-type Ca^{2+} channels in the cardiomyocyte membrane, leading to increased abnormalities in cardiac excitability as well as increased risk of arrhythmia and CVD [41].

However, there is controversy regarding the exact effects of estrogen on the heart. Previous studies using a dog model showed that estrogen treatment could reduce infarct size and prevent post-ischemic cardiac arrhythmia [42, 43]. In contrast, experimental evidence exists that estrogen has arrhythmogenic effects in female rat hearts by increasing the sarcoplasmic reticulum calcium leak via ERβ signaling [44]. A study using subcutaneous- implanted estradiol pellets reported that estradiol helps improve lung congestion but fails to improve cardiac cellular redox balance and right ventricular function in OVX rats with pulmonary arterial hypertension [45]. Clinical studies also reported undesirable effects of estrogen supplements on the cardiac electro-activity in post-meno pausal women such as increased QT interval, QT dispersion, and augmented atrial fibrillation incidence [46, 47]. Moreover, estrogen therapy in combination with progesterone may be associated with an increased rate of breast cancer [48]. Further study into the effects of estrogen on the heart is still needed to demonstrate its precise effects and to clarify the underlying mechanisms involved.

Estrogen and Cardiac I/R Injury

Existence of gender dependence response to cardiac ischemic/reperfusion (I/R) revealed higher cardiac stress tolerance, better cardiac functional recovery, and greater survival rate in females than in males [49–53]. Estrogen deprivation resulted in removal of these cardioprotective effects [25, 52, 54–58], thus indicating the beneficial role of this female sex hormone in cases of I/R injury.

Acute Treatment Using Estrogen in I/R

Acute estrogen treatment exerted protective effects in both preischemic and post-ischemic administration. Administration of estrogen before the ischemic period in an in vivo setting resulted in the protection of cardiac mitochondria [14], a decrease in infarct size [14, 59], and reduction of cardiac ischemic and inflammatory markers [60]. Intravenous injection of 17β-estradiol either before ischemic induction or prior to the reperfusion period similarly resulted in a lower myocardial superoxide anion level, a reduced expression of connexin43 protein phosphorylation, and decreased myocardial infarction in an in vivo study using a dog model [15]. Treatment with the synthetic17β-aminoestrogen, (17β-(3-hydroxy-1-propylamino)-1,3,5(10)-estratrien-3-ol), which is synthesized from estrone, then given to the rats before reperfusion also, improved cardiac function via increased HR, LVESP, and ±dP/dt during reperfusion and protected the heart from myocardial I/R injury [61].

In isolated rat hearts, cardiac preconditioning using 5-min cycles of perfusion with estrogen prior to ischemic introduction resulted in an increase in intracellular reactive oxygen species (ROS) and reduced myocardial infarction after the transient hormonal treatments [62]. A single dose of 17β-estradiol (100 nM) administered to isolated rat hearts before the ischemic period improved the post-I/R recovery, decreased infarct size, and decreased norepinephrine levels in coronary effluence [63, 64]. Estrogen infusion during reperfusion was also able to revive myocardial function, preserve coronary flow, and lower lactate dehydrogenase (LDH) release [65]. The acute effects of estrogen, in either

Table 1 In vivo studies on the acute effects of estrogen administration on cardiac I/R

Model	I/R (min)	Treatment/dose	Major findings	Interpretation	Ref.
Rats	30/90	- E2, 12.5 μg/kg, or - Prolame (a synthetic 17β-aminoestrogen) 75 μg/kg; via an intravenous bolus through the tail veins at 5 min before reperfusion	• E2 and prolame similarly increased HR, LVESP and ±dP/dt, and decreased LVEDP during reperfusion • Both E2 and prolame diminished infarct area and maintained myocardial NO level, but prolame showed the superior effect on these results.	E2 and the synthetic 17β-aminoestrogen prolame protected cardiac function against I/R injury	[61]
Rabbits	30/240	- E2, 20 μg/rabbit; IV injection at 30 min before I/R	• Decreased infarct size as a % of area at risk • Pretreatment with inhibitors of COX2 and PGI2 receptor diminished this effect of E2.	E2 reduces infarction from I/R through COX2 activation pathway.	[59]
Rabbits	30/240	- 30 min before I/R, IV injection with either, (1) E2, 20 μg/rabbit (2) ERβ agonist (DPN), 3 μg/kg (3) ERα agonist (PPT), 3 μg/kg	• E2 and ERα agonist decreased infarct size, reduced cardiac troponin-I, reduced the tissue deposition of the membrane attack complex and C-reactive protein.	E2 and ERα activation protects the myocardium from I/R injury.	[60]
Dogs	60/120	E2, 10 μg/kg; by (1) IV injection prior to 60-min occlusion or (2) IV injection 3 min just prior to reperfusion after the 57-min occlusion.	• E2 treatments either prior to ischemia or prior to reperfusion lowered concentration of superoxide anion, Cx43 expression, p-Cx43, and infarct size.	E2 has cardioprotective and antioxidant effects in I/R due to reduced levels of phosphorylated connexin43 and myocardial infarction.	[15]
Dogs	5/20	Raloxifene (a selective E2 receptor modulator) 5 μg/kg/min; infused in to LAD via bypass tube for 20 min during perfusion.	• Raloxifene increased coronary blood flow and fractional shortening, improved myocardial anaerobic metabolism, and increased both Akt activity and the NO level.	Activation of E2 receptor upregulates PI3K/Akt activity and NO production, resulted in improved cardiac recovery from I/R.	[101]
Rabbits	45/180	GST, 1.0 mg/kg; IV injected 5 min before ischemia	• GST reduced infarction, inhibited DNA ladder pattern in myocardium, prevented apoptosis in cardiomyocytes, lowered Fas and Bax protein expression, and increased Bcl-2/Bax ratio.	Phytoestrogen prevented cardiocyte apoptosis and reduced myocardial infarction from I/R.	[89]
Rabbits	30/240	17β-estradiol, 20 μg or 17α-estradiol, 1 mg; IV injection 30 min before occlusion of LAD coronary artery	• 17β-estradiol-treated group showed intact mitochondria and smaller infarct size compared with 17α-estradiol or vehicle groups.	E2 protected mitochondria and myocardial tissue from I/R injury.	[14]
Dogs	90/360	Raloxifene (selective E2 receptor modulator), 5 μg/kg/min; infusion into LAD coronary artery at 10 min before coronary occlusion and continued until 1 h after reperfusion started	• Raloxifene reduced myocardial infarct size and the incidence of ventricular fibrillation that may be modulated via NO and the opening of K_{Ca} channel-dependent mechanisms	Activation of E2 receptor caused suppression of p38 MAPK and myeloperoxidase activities and resulted in reduced cardiac I/R injury.	[88]

Cx43 connexin43, *DPN* the ERβ-selective agonists 2,3-bis(4-hydroxyphenyl)-propionitrile, *±dP/dt* rate of LV pressure rising during systole and diastole, *E2* 17β-estradiol, *ER* estrogen receptor, *GST* genistein (phytoestrogen), *IV* intravenous, *I/R* ischemic/reperfusion, *K_{Ca} channel* calcium-activated potassium channel, *LAD* left anterior descending, *MAPK* mitogen-activated protein kinase, *NO* nitric oxide, *PGI2* prostaglandins I2, *PPT* the ERα-selective agonists 4,4′,4″-[4-propyl-(1H)-pyrazole-1,3,5-triyl]tris-phenol

in vivo or in vitro classifications, are summarized in Tables 1 and 2, respectively.

Chronic Treatment of Estrogen in I/R

Chronic treatment of estrogen exhibited prominent advantages to ischemic hearts in both in vivo and in vitro (or ex vivo) studies (categorized in Tables 3 and 4, respectively). Subjects who had chronic subcutaneous estrogen injections for 10 days prior to I/R showed a higher level of cardioprotection than from a 10-min direct estrogen perfusion before ischemia, as evidenced by the greater decrease in ventricular fibrillation duration and increasing of coronary flow during reperfusion [66].

Estrogen treatment for 1 week completely reversed I/R-induced cardiac dysfunction, suppressed norepinephrine overflow, and preserved the nitric oxide level [49]. Twenty-one-day estrogen treatment via a subcutaneous-implanted pellet increased cardiac stromal cell-derived factor-1 (SDF-1) production, which is involved in cardioprotective effects, reduced myocardial necrosis, and lowered free radical production by the heart [28, 51]. Treatment with a 60-day release of

Table 2 In vitro or ex vivo studies on the acute effects of estrogen administration on cardiac I/R

Model	I/R (min)	Treatment/dose	Major finding	Interpretation	Ref.
H9C2 cells	20/120	E2, 1 nM, or G-1, 10 nM; administered 5 min before ischemia	• Both E2 and G-1 reduced Bax and TNF-α and increased Bcl-2, SOD, and ATPase in post-I/R cardiomyocyte	E2 and G-1 protected cardiomyocyte from I/R injury by inhibiting apoptosis and enhancing antioxidant activity and ATP production.	[87]
Isolated mice heart	25/40	E2, 100 nM was treated to mouse cardiac stem cells (CSC) for 24 h; then, this E2-treated CSC was infused into isolated mouse hearts through the aortic root within 1 min immediately before ischemia or during the initiation of reperfusion	• E2 increased VEGF and SDF-1 production in CSC. • E2-treated CSC increased cell viability of cardiocyte subjected to hypoxia. • E2-treated CSC reduced LDH and caspase-3, increased myocardial STAT3, and improved LVDP and ±dP/dt in I/R heart.	E2 increased protective factors from CSC, and the treatment with E2-treated CSC provided cardioprotective effects against I/R injury by decreased apoptosis and improved cardiac function.	[109]
Isolated mice heart	20/40	G-1, 1 μM, and G1 (1 μM) together with Erk inhibitor PD-98059 (5 μM); via perfusion	• G1-treated hearts had increased functional recovery, decreased infarct size, and inhibited mPTP opening • Protective effect of G-1 was abolished by PD-98059.	Stimulation of GPR30 caused inhibiting opening of mPTP, decreasing infarction, and improving myocardial recovery via Erk pathway.	[81]
Isolated rat heart	40/30	E2, 100 nM; perfused 15 min before the start of ischemia to 5 min after the onset of reperfusion.	• Greater recovery of LVDP, LVEDP, and +dP/dt • Suppressed excessive norepinephrine release in coronary effluent from the post-ischemic heart	E2 prevented I/R-induced cardiac dysfunction that may involve with suppressing of NE overflow and NO production.	[63]
Isolated rat heart	47/60	ERβ agonist diarylpropionitrile (DPN), 5 μg/kg; 45 min prior to I/R	• No effect on functional recovery following I/R injury	Acute activation of ERβ did not affect the cardiac function after I/R induction.	[80]
Isolated rat heart	30/120	E2, 100 nM; perfused prior to ischemia	• Decreased infarct size and improved post-I/R recovery • Treatment with the GPER agonist G1 prior to ischemia and reperfusion significantly reduced infarct size.	Treatment with E2 and stimulation of GPER, administrated before ischemia, demonstrated the beneficial effects on the heart under I/R stress.	[64]
Isolated rat heart	25/40	G-1, 10 nM or 100 nM; via perfusion	• G-1 administration improved LVDP, +dP/dt, −dP/dt, and lower TNF-α, IL-1β, and IL-6 levels in myocardium.	Activation of GPR30 improved myocardial function recovery and decreased cardiac tissue inflammation following I/R.	[83]
Isolated rat heart	20/120	G-1, 110 nM; perfused before ischemia induction	• G-1 induced less post-ischemic contractile dysfunction, reduced infarct sizes, and instigated activation of both Akt and ERK1/2. • These cardioprotective effects were abolished by PI3K inhibitor.	GPR30 activation enhanced cardiac functional recovery and decreased myocardial infarction via PI3K-dependent manner.	[82]
Isolated rat heart	25/40	PPT (selective ERα agonist) or DPN (selective ERβ agonist) 1, 10, or 100 nM: perfused throughout reperfusion	• Both PPT and DPN improved post-ischemia cardiac recovery, although had no effect on TNF-alpha or IL-1beta productions.	Both activations of ERα and ERβ are involved in mediating E2-induced rapid cardioprotection after I/R injury.	[26]
Isolated rat heart	47/60	PPT (selective ERα agonist), 5 μg/kg body; SC 45 min before euthanasia	• PPT decreased infarct size and increased RACK2 mRNA levels (for the PKCε anchoring protein) in both adult and aged hearts after I/R. • PPT restored particulate and mitochondrial ERα expressions and increased nuclear and mitochondrial PKCε levels in aged hearts.	Activation of ERα decreased cardiac ischemic injury in the aged, estrogen-deficient female via non-genomic mechanism associated with redistribution of ERα and PKCε activation.	[25]
Isolated rat heat	25/40	Normal mesenchymal stem cell (MSC) or E2-treated male MSC, 1 million MSCs; via transcoronary delivery immediately prior to ischemia	• E2-treated MSCs provoked significant more VEGF production, greater post-ischemic recovery of LVDP and LVEDP at the end of reperfusion.	Infusion of MSC prior to ischemia helped protecting post-ischemic myocardial contractile function and viability.	[90]

Table 2 (continued)

Model	I/R (min)	Treatment/dose	Major finding	Interpretation	Ref.
Isolated rat heart	25/40	E2, 1, 10, 25, or 50 nM; post-ischemic perfused throughout reperfusion	• 1 and 10 nm E2 improved post-ischemic LVDP, LVEDP recovery, preserved coronary flow and decreased lactate dehydrogenase level. • E2 at all concentrations increased ERK phosphorylation.	Infusion of E2 during reperfusion enhanced post-ischemic myocardial functional recovery and viability.	[65]
Isolated rat heart	30/120	Water-soluble cyclodextrin-encapsulated E2, 10 μM three times; preconditioning with three 5-min perfusion cycles before ischemia	• Reduced infarct size • Elevated intracellular ROS which is similar to the effect of classic preconditioning • Induced phosphorylation of PKB, PKCα, and PKCε and membrane translocation of PKCε and PKCδ.	Preconditioning with E2 induced cardioprotective effects through the signaling pathways similar to the ischemic preconditioning.	[62]
Isolated mouse heart	20/120	- αERKO, βERKO female mice - Isoproterenol, 10 nM: perfused for 1 min just prior to ischemia (to induce hypercontractile conditions)	• βERKO female hearts showed significantly lower ischemic pH, less post-ischemic function and phosphocreatine (PCr) recovery, and decrease in ATP citrate lyase, fatty acid synthase, and stearoyl CoA desaturase which may lead to enhanced ischemic injury.	ERβ plays an important role in the protection observed in the female heart.	[50]
Isolated rat heart	20/30	- E2, 40 μg/kg; IP injection - 6 h after E2 treatment, rats were placed in a water bath at 43 °C (HT) to induce heat-shock protein expression - 24 h later, I/R was induced in hearts	• Male has more increased expression of HSP72 in response to hyperthermia and better LV function recovery than female. • E2 reduced HT-induced cardiac HSP72 overexpression and abolished better LV functional recovery.	E2 had inhibitory effects on HSP72 expression which exterminated the functional recovery of LV.	[110]
Isolated rat heart	40/40	E2, 1 nM; included in the 20-min perfusion before I and during R.	• Limited the changes in $pH_{(i)}$, $Na^{+}_{(i)}$, and $Ca^{2+}_{(i)}$ during ischemia • Improved recovery of LVDP and diminished release of LDH during reperfusion.	Acute treatment with E2 limits myocardial Na^{+} and, therefore, Ca^{2+} accumulation, which inhibited I/R injury.	[84]
Isolated rat heart	30/40	1. E2, 0.7 ng/mL of coronary flow; via 15-min perfusion before ischemia 2. E2, 250 μg/kg; via injection at 3 h before ischemia 3. Long-term E2, 0.5 mg pellet; via SC implantation at 6 weeks before ischemia All groups were duplicated to combined with ischemic preconditioning (IPC; two cycles of 5 min ischemia and 10-min reperfusion before 30-min ischemia induction)	• Short-term E2 treatment improved post-ischemic recovery of myocardial contractility and coronary function and correlated with reduced cardiac TNF-α level. • When combined with preconditioning, E2 did not provided additional benefit when compared with preconditioning alone or E2 treatment alone.	Short-term E2 treatment regulated cardiac TNF-α level and protected the heart from I/R injury.	[100]

ATPase adenosine triphosphatase, *DPN* the ERβ-selective agonists 2,3-bis(4-hydroxyphenyl)-propionitrile, *±dP/dt* rate of LV pressure rising during systole and diastole, *E2* 17β-estradiol, *ER* estrogen receptor, *Erk* extracellular signal-regulated kinase, *G-1* GPR30 agonist, *HT* high temperature, *I/R* ischemic/reperfusion, *LV* left ventricle, *LVEDP* left ventricular end-diastolic pressure, *LVDP* left ventricular diastolic pressure, *mPTP* mitochondrial permeability transition pore, *MSC* mesenchymal stem cell, *NE* norepinephrine, *NO* nitric oxide, *PCr* phosphocreatine, *PKCε* protein kinase C-ε, *PLB* phospholamban, *PPT* the ERα-selective agonists 4,4′,4″-[4-propyl-(1H)-pyrazole-1,3,5-triyl]tris-phenol, *RACK2* receptor for activated C kinase2, *SC* subcutaneous, *SDF-1* stromal cell-derived factor-1, *SOD* superoxide dismutase, *VEGF* vascular endothelial growth factor

estrogen, via subcutaneous-implanted pellets, inhibited cardiac expression of tumor necrosis factor alpha (TNF-α) [67]. Subcutaneous injections of estrogen for 2 weeks before I/R helped inhibit reperfusion arrhythmia and myocyte apoptosis indicating its antiarrhythmic, antiapoptotic features [27]. Chronic estrogen treatment not only prevented endothelial and myocardial dysfunction but also promoted estrogenic antioxidant activity by the reduction of superoxide anion concentration in the arterial segment [68]. However, a study using isolated rat hearts

Table 3 In vivo studies on the effects of chronic estrogen administration on cardiac I/R

Model	I/R (min)	Treatment/dose	Major finding	Interpretation	Ref.
Mice	30/60	E2, 0.1 mg; via 60-day release pellets, SC implanted 2 weeks before the induction of I/R	• Prevented coronary endothelial dysfunction • Decreased ROS generation and limited infarct size in wild-type mice but not in mice deficient in endothelial ERα	E2 limited cardiac tissue infarction and ROS production and also preserved coronary endothelial function and structure after I/R via ERα.	[75]
K_{ATP}-KO mice	30/90	E2, 0.1 μg/g/day; via 21-day release pellet for 18–20 days before I/R	• E2 decreased infarct size in wild-type rats, but not KATP-KO rats. • E2 increased SUR2 protein, which is the regulatory subunit for the cardiac KATP channels.	E2 regulated SUR2 expression which enhances KATP channel density and diminished cardiac I/R injury.	[111]
Rats	20/30	- SC injected for 2 weeks before I/R with either: 1. E2, 100 μg/kg/day 2. ERβ agonist (DPN), 5 μg/kg/day 3. ERα agonist (PPT), 5 μg/kg/day	• Prevented reperfusion-induced arrhythmias, VPB, VT • Decreased duration of VT, VF, Cx43 de-phosphorylation, and myocyte apoptosis • ERβ agonist reduced reperfusion-induced VPB and VT incidence, while ERα agonist had no significant influence	E2 protected against reperfusion-induced cardiac arrhythmia, evidently through ERβ activation.	[27]
Rats	45/120	- E2, 2 μg/day; released for 21 days from SC-implanted pellets - Treated randomly by ERA-45 (ERα agonist, 75 μg/kg; twice daily by gavage), ERB-88 (ERβ-antagonist, 75 μg/kg; SC) or vehicle for 5 days prior to I/R	• Reduced the size of necrosis within the AAR • Reduced the amount of MPO activity and produced fewer free radicals from the AAR • Treatment by ERα agonist also reduced necrotic tissue and decreased free radicals.	Treatment with E2 resulted in reduction of cardiomyocyte death, neutrophil infiltration, and oxygen-free radical availability, which are primarily mediated via ERα activation.	[28]
Rats	7/7	Tamoxifen (selective E2 modulator), 1 or 10 mg/kg; SC daily for 14 days	• Tamoxifen (both 1, 10 mg/kg) reduced the incidence of VT and VF, prevented fall in GSH and GSH-Px, and prevented increase in MDA and CK levels. • Tamoxifen treatment (10 mg/kg) reduced MDA levels.	Stimulation of E2 receptor by tamoxifen had antioxidant activity and beneficial cardioprotective effects on I/R-induced myocardial injury and arrhythmia.	[112]
Rats	60/120	E2, 30 μg/kg/day; daily SC injection for 8 weeks	• Restored UCN-induced cardioprotection as reduced infarction, serum CK, serum LDH, and apoptosis and increased CRHR2 expression level	E2 maintained myocardial CRHR2 expression and enhanced cardioprotection induced by UCN against I/R injury	[113]
Dogs	15/120	E2, 100 μg/kg/day; daily SC injection for 2 weeks	• Prevented reperfusion arrhythmia • Maintained systolic shortening • Preserved vasodilatory responses to Ach, increased serum nitrite/nitrate concentration, and lowered concentration of superoxide anion in arterial segments.	E2 prevented endothelial and myocardial dysfunction resulting from I/R which may be related to its antioxidant effect.	[68]

AAR area at risk, *CK* creatine kinase, *CRHR2* corticotropin-releasing hormone receptor 2, *Cx43* connexin43, *DPN* the ERβ-selective agonists 2,3-bis(4-hydroxyphenyl)-propionitrile, *E2* 17β-estradiol, *ER* estrogen receptor, *GSH* glutathione, *GSH-Px* glutathione peroxidase, *HT* high temperature, *I/R* ischemic/reperfusion, K_{ATP} ATP-sensitive potassium channels, *KO* knockout, *LDH* lactate dehydrogenase, *MDA* malondialdehyde, *MPO* myeloperoxidase, *PPT* the ERα-selective agonists 4,4′,4″-[4-propyl-(1H)-pyrazole-1,3,5-triyl]tris-phenol, *ROS* reactive oxygen species, *SC* subcutaneous, *VF* ventricular fibrillation, *VPB* ventricular premature beats, *VT* ventricular tachycardia, *UCN* urocortin

reported that estrogen treatment, via subcutaneous injection for 5 weeks before myocardial ischemia, stimulated cardiac fatty acid oxidation that resulted in diminished post-ischemic recovery of cardiac function [69].

Table 4 In vitro and ex vivo studies into the effects of chronic estrogen administration on cardiac I/R

Model	I/R (min)	Treatment/dose	Major finding	Interpretation	Ref.
Isolated rat hearts	40/30	E2, treated daily 20 μg/kg/day; SC, for 7 days	• Inhibited OVX-elicited aggravation of cardiac dysfunction • Suppressed NE overflow • Recovered NOx level after I/R	Estrogen protected the heart from I/R injury by suppressing NE overflow through elevating the level of NO production.	[49]
Isolated rat or mouse hearts	25/40	E2, 75 mg; SC-implanted 21-day release pellets	• Restored SDF-1 expression in ovariectomized females and entire males. • Ablation of ERα markedly decreased SDF-1 production	After I/R, E2 induced increasing of SDF-1 expression which may involve cardioprotection of E2 via ERα.	[51]
Isolated rat heart	45/120	Estrogen, 1.5 mg; SC-implanted 60-day release pellets	• E2 regulated [Ca2+]i transients in ventricular myocytes and reduced apoptosis and infarct size • E2 also restored LDH, CaMKIIδ, and phosphorylated CaMKII to the normal level.	E2 regulated [Ca2+]i transients and CaMKIIδ and phospho-CaMKII levels and referred greater cardioprotection in I/R.	[56]
Isolated rat heart	30/30	E2, 40 μg/kg; SC, daily injections of for 4 weeks	• Decreased cardiomyocyte contraction and the expression of β_1-AR • Increased expression of β_2-AR • Lowered LDH release.	E2 altered the expressions of β-adrenoceptors and is associated with reduced cardiomyocyte contraction after I/R.	[58]
Isolated mouse heart	20/40	DPN, 0.8 and 0.1 mg/kg/day of E2; SC, delivered for 2 weeks by implanted micro-pump	• DPN induced better functional recovery, upregulation of protective genes, growth arrest and DNA damage 45β, and cyclooxygenase 2.	Activation of ERβ improved post-ischemic functional recovery and induced protective effects against cardiac cell death.	[57]
Isolated rat heart	40/40	E2 (1 μM) and testosterone (testosterone propionate, 1 μM); SC injected for 10 days prior to the experiment or direct-treated via perfusion (10 min) before ischemia	• E2 pretreatment for 10 days was more efficient in cardioprotection including increased coronary flow, decreased LDH release rates, and decreased duration of ventricular fibrillation.	10-day pretreatment with E2 was more effective in protecting the heart from I/R injury and limiting cardiac arrhythmias.	[66]
Isolated rat heart and in situ	45/120	Estrogen (3 mg), MPA (3 mg), E + MPA; via SC-implanted pellets	• E2 alone reduced necrotic zone, but MPA and E in combination resulted in a significantly larger necrotic zone and necrotic area.	Combination of E2 and progesterone may cause adverse effects on the heart unlike the use of E2 alone.	[91]
Cultured rat cardiomyocytes	18 h/1 h	Estrogen, 10 nM; treated overnight before being placed into an anaerobic chamber to induce hypoxia	• Blocked ROS generation and p38α activation during H/R • Inhibited apoptosis of cardiomyocytes.	E2 suppressed ROS and apoptotic pathways after hypoxia.	[85]
Isolated rat hearts	25/40	- Estrogen, 1.5 mg/pellet; 60-day release - Etanercept (inactivates TNF-α), 30 μg/ml; via perfusion 10 min before the onset of ischemia and remained throughout reperfusion period	• Decreased TNF-α in coronary artery effluent and LV • Increased ventricular TNFR1 • Reduced TUNEL-positive myocytes • Decreased caspase-3 cleavage product expression	E2 downregulated apoptotic signaling proteins and improved cardiac ischemia tolerance.	[67]
Isolated rat heart and isolated myocytes	30/120	E2, 1.5 mg; implanted with subcutaneous 60-day release pellets	• Restored the infarct size to a level comparable with the female counterparts • Decreased cAMP level • Suppressed LDH, mRNA, and protein levels of β1-AR.	E2 limited infarction, suppressed LDH and B1-AR expression in the hearts which underwent I/R.	[112]
Isolated mice heart	40/60	- E2, 0.1 mg ; implanted with subcutaneous 60-day release pellets - Preconditioned with two cycles of 2-min global ischemia and 5-min reperfusion, or perfused for 14 min before I/R	• Estrogen deprivation showed reduced LVDP and increased infarct size compared with normal females, and preconditioning did not protect the heart of OVX rats. • These alterations were not reversed by E2 substitution.	Endogenous E2 is essential for preserving cardiac function, limiting post-ischemic infarction and cardioprotection by ischemic pre-conditioning.	[92]
Isolated rat hearts	U/K	E2, 0.1 mg/kg/day; SC, for 5 weeks	• Induced lower post-ischemic cardiac function, lower recovered after ischemia, greater fatty acid oxidation	E2 lowered cardiac recovery and increased oxidation of fat following ischemia.	[69]

Table 4 (continued)

Model	I/R (min)	Treatment/dose	Major finding	Interpretation	Ref.
Isolated rat heart	30/30	Estradiol propionate, 450 μg/kg; by weekly IM injections for 10 weeks before heart isolation	• During reperfusion, ±dP/dt, LVEDP, and LVDP were restored by E2. • E2 also improved cardiomyocyte Ca^{2+}i transient recovery through decreasing PLB and Ser16-phosphorylated PLB levels.	E2 maintained cardiac function and enhanced cardiomyocyte Ca^{2+} cycling by regulating PLB expression and activity.	[114]
Isolated rat heart	30/150	E2; via subdermal implantation of adhesive tubes filled with E2 powder (0.3–0.4 cm)	• E2 reduced infarct size in female rat heart but increased infarct size in male rat heart. • E2 reduced mTOR activity and caspase-9 and -3 processing and increased XIAP and Bcl-xL in female rat heart.	E2 regulated autophagy and reduced apoptosis of cardiomyocyte resulting in decreased myocardial infarction after I/R.	[115]
Isolated rat heart	15/30	E2, 0.5 μg/kg/day; via injection for 4 days	• E2 prolonged QT interval, reduced heart rate, and averaged arrhythmia severity index in young female but not middle-aged female rats.	E2 inhibited I/R arrhythmia in young female rats.	[116]

Bcl-xL B cell lymphoma-extra large, *β1-AR* β1-adrenergic receptor, *β2-AR* β2-adrenergic receptor, *CaMKII* calcium/calmodulin-dependent protein kinase II, *cAMP* cyclic adenosine monophosphate, *DPN* the ERβ-selective agonists 2,3-bis(4-hydroxyphenyl)-propionitrile, *E2* 17β-estradiol, *ER* estrogen receptor, *I/R* ischemic/reperfusion, *LDH* lactate dehydrogenase, *LV* left ventricle, *LVEDP* left ventricular end-diastolic pressure, *LVDP* left ventricular developed pressure, *MPA* medroxyprogesterone acetate, *NE* norepinephrine, *NO* nitric oxide, *NOx* NO2/NO3, *OVX* ovariectomy, *SDF-1* stromal cell-derived factor 1, *SC* subcutaneous injection, *TNF-α* tumor necrosis factor alpha, *TNFR1* tumor necrosis factor receptor 1, *XIAP* X-linked inhibitor of apoptosis protein

Effects of Estrogen on Myocardial Ischemia and Infarction Conditions in Clinical Studies

Up to present, verified data regarding the effects of estrogen on myocardial ischemia and reperfusion in clinical trials is limited. Some clinical studies reporting the effects of estrogen supplements in patients, with a history of CVD, are summarized in Table 5. Acute administration of estrogen in nine patients just before coronary angiography intervention resulted in a decrease of myocardial ischemia, evidenced by lower levels of chest pain, ST-segment shift, and myocardial lactate depletion. Endothelin-1 levels, from the great cardiac vein, were suppressed by estrogen treatment, which correlated with attenuated coronary vasoconstriction. Mechanistically, estrogen limited these ischemic indications and vascular dysfunction through K_{ATP} channel activation [70].

The evidence concerning the incidence rate of post-MI cardiovascular events in patients with estrogen therapy is still conflicting. A nationwide study in Denmark, involving women who were admitted with myocardial infarction from 1997 to 2009, reported the positive role of estrogen, suggesting that post-menopausal women who received estrogen hormone therapy exhibited a lower incidence of atrial fibrillation (AF) after myocardial infarction, when compared to those without estrogen supplement therapy [71]. Estrogen replacement did not affect the rate of reinfarction, cardiovascular mortality, or all-cause mortality, when comparing estrogen therapy data in post-menopausal women with a history of myocardial infarction [72]. Although estrogen replacement therapy helped lower the LDL and raised the HDL levels in post-menopausal women with established coronary disease, it also augmented coronary heart disease (CHD) events, venous thromboembolic events, and gallbladder disease [73]. Moreover, estrogen treatment in patients with prostate cancer, who had a history of CVD, provoked cardiovascular events during treatment [74]. Therefore, estrogen hormone therapy should be prescribed with caution to patients with cardiovascular disorders, due to its unclear effects on coexisting disease status.

Roles of ER in I/R Injury

Several studies reported beneficial effects of ERα activation. After I/R, myocardial infarct size was reduced by protein kinase C epsilon (PKCε) stimulation through ERα activation [25]. Depletion of ERα decreased recovery of cardiac function, related to endothelial dysfunction following I/R [75, 76]. Treatment with ERα agonists reduced cardiac troponin-I, C-reactive protein, and post-ischemic infarction in rabbit hearts [60]. Ovariectomized female rats and ERα-knockout mice showed more impaired mitochondria with abnormal shape, loss of matrix, disruption of inner and outer mitochondrial membranes, and also lower mitochondrial enzyme activity [77, 78].

Table 5 Clinical studies into estrogenic effects on myocardial ischemia and infarction

Model (Number)	Treatment/dose	Major finding	Interpretation	Ref.
Patients with coronary angioplasty (41)	Estrogen (Premarin), 5 mg; administered intracoronary via a guiding catheter, prior or after coronary angiography	• Limited myocardial ischemia by activation of $K_{(ATP)}$ channels • Suppressed endothelin-1 release which is related to changes in coronary vasomotion	E2 limited myocardial ischemia through $K_{(ATP)}$ activation and attenuated vasoconstriction via suppression of endothelin-1 release.	[70]
Post-menopausal women with a history of myocardial infarction (32,925)	Estrogen hormone replacement therapy in all types	• Decreased incidence rates of AF during the first year after MI in female patients with use of HRT	Hormone therapy correlated with a decreased risk of AF in post-menopausal women with myocardial infarction.	[71]
Post-menopausal women with a history of myocardial infarction (3322)	Usage of prescribed hormone replacement therapy at the time of myocardial infarction	• No difference in the risks of re-infarction, cardiovascular mortality, or all-cause mortality between use vs. disuse of HRT.	Neither a modest benefit nor a worrisome increase in risks of adverse cardiovascular events with continuing or discontinuing hormone replacement therapy after myocardial infarction.	[72]
Post-menopausal women with coronary disease (2763)	Usage of hormone replacement therapy or placebo; for 1–3 years with follow-up averaged 4.1 years	• E2-treated group had lower LDL and higher HDL levels but experienced more coronary heart disease (CHD) events, venous thromboembolic events, and gallbladder disease.	E2 supplement did not reduce the overall rate of CHD events in post-menopausal women with established coronary disease but did increase the rate of thromboembolic events and gallbladder disease.	[73]
Patients with prostate cancer and history of CVD (915)	Parenteral poly-estradiol phosphate (PEP); 240 mg, i.m., twice a month for 2 months and thereafter monthly	• Increased cardiovascular events during treatment with PEP	Patients with previous cardiovascular disease are at considerable risk of cardiovascular events during treatment with high-dose PEP.	[74]

AF atrial fibrillation, *CHD* coronary heart disease, *CVD* cardiovascular disease, *HDL* high-density lipoprotein, *HRT* hormone replacement therapy, *LDL* low-density lipoprotein, *LV* left ventricle, *MI* myocardial infarction, *PEP* parenteral poly-estradiol phosphate

However, the abnormalities of mitochondrial structure and physiological function were restored to normal by treatment with estrogen, implying an important role of estrogen and ER, especially ERα, in the regulation of mitochondrial structure and function after I/R.

Acute stimulation of ERβ increased myocardial function, upregulated numbers of cardioprotective genes, and decreased the incidence of reperfusion-induced arrhythmias, such as ventricular tachycardia (VT) and ventricular premature beats (VPB) [26, 27, 57]. Knockout of ERβ decreased left ventricular developed pressure (LVDP), lessened functional recovery, increased TNF production, and reduced PI3K and Akt activation which correlated with the increased caspase-3 and caspase-8 activity in the apoptotic pathway in cardiac muscle [50, 53, 79]. However, there is controversial data from a study using isolated rat hearts, demonstrating that acute activation of ERβ prior to ischemia did not influence the ischemic tolerance or cardiac functional recovery following I/R, thus indicating that ERβ might not be responsible for cardioprotection during I/R injury [80].

G protein-coupled estrogen receptor 1 (GPER, GPR30) activation exhibited beneficial effects in cardiac protection after I/R. Hypoxia caused an increase of GPER mRNA in cardiac tissue which became more augmented during reperfusion [64]. Administration of a GPER agonist (G-1) improved functional recovery of the myocardium, reduced infarction, and increased the threshold for mitochondria permeability transition pore (mPTP) opening [81, 82]. G-1 perfusion prior to ischemia enhanced LVDP and ±dP/dt and lowered myocardial inflammatory agents after I/R [83].

Pathways Involved in Cardioprotection Against I/R Injury

Estrogenic cardioprotective effects against I/R injury are mediated by several mechanisms. Apoptotic cell death was found to be reduced via suppression of CaMKIIδ, P38α, and calpain activity and the activation of the JNK, PKCε, and PI3K/Akt pathways [25, 53, 54, 56, 81]. Estrogen and its receptor agonists helped regulate cardiac ROS production and decreased LDH levels [62, 75, 84, 85]. Estrogen prevented intracellular Na^+ and Ca^+ accumulation and thereby reduced cardiomyocyte injury

Table 6 Summary of good, neutral, and bad effects and the underlying mechanisms of estrogen and cardiac ER activation in I/R condition

Good effect	Underlying mechanism
Reduce cardiomyocyte apoptosis and myocardial infarction	• Reduce Fas and Bax protein expression [87, 89]
	• Increase Bcl-2 expression [87, 89]
	• Activate COX2 pathway [59]
	• Induce phosphorylation of PKB, PKCα, and PKCε and membrane translocation of PKCε and PKCδ [62]
	• Reduce myocardial connexin43 activity [15]
	• Reduce troponin-I [60]
	• Decreased LDH level [65]
	• Lower superoxide anion [15]
	• Reduce myocardial ROS production [75]
	• Enhance antioxidant activity by increase in SOD level [87]
	• Maintain GSH and GSH-Px levels [117]
	• Suppress p38 MAPK [88]
	• Suppress MPO activities [28, 88]
	• Reduce lipid peroxidation by inhibiting MDA increase [117]
	• Decrease caspase-3 cleavage product expression [67]
	• Reduced neutrophil infiltration, oxidant stress, and necrosis [28]
	• Promote ATP production [87]
	• Inhibit HSP72 overexpression [110]
	• Increase SUR2 expression which enhances K_{ATP} channel density [111]
	• Suppressing CaMKII [56]
	• Inhibit DNA ladder pattern [89]
Enhance cardiac function and post-I/R recovery	• Suppress NE overflow in coronary effluent [63]
	• Maintain myocardial NO production [61, 101]
	• Limit myocardial Na^+ and inhibit Ca^{2+} accumulation [84]
	• Decrease cardiomyocyte shortening after I/R [58]
	• Decrease expression of β1-AR and increase expression of β2-AR [58]
Reduce inflammation	• Reduce myocardial C-reactive protein [60]
	• Reduce TNF-α [67, 83, 87, 100]
	• Lower IL-1β and IL-6 levels [83]
	• Increase ERK phosphorylation [65]
Increased coronary blood flow	• Upregulate Akt-PI3K pathway and increase NO level [101]
	• Preserve vasodilatory responses to Ach [68]
	• Increase serum nitrite/nitrate concentration [68]
	• Lower arterial superoxide anion concentration [68]
	• Suppress endothelin-1 release [70]
Protect cardiac mitochondria	• Increase amount of intact mitochondria [14]
	• Inhibit mPTP opening [81]
Inhibit cardiac arrhythmia	• Regulate the opening of K_{Ca} channels [88]
	• Decrease duration of VT and VF [27]
Neutral and bad effect	**Underlying mechanism**
No effect on the reduction of reinfarction risk and mortality	• N/A [72]
Decrease post/I/R cardiac function	• E2 supplement increased myocardial fatty acid oxidation [69].
Increase cardiovascular events	• High-dose E2 therapy increased cardiovascular events in patients with previous CVD; however, the mechanism is still unclear [74].

β1-AR β1-adrenergic receptor, *β2-AR* β2-adrenergic receptor, *CaMKII* Ca^{2+}/calmodulin-dependent protein kinase II, *CHD* coronary heart disease, *CVD* cardiovascular disease, *E2* 17β-estradiol, *ER* estrogen receptor, *GSH* glutathione, *GSH-Px* glutathione peroxidase, *HSP72* heat shock protein-72, *IL* interleukin, K_{ATP} ATP-sensitive potassium channels, K_{Ca} calcium-activated potassium channels, *LDH* lactate dehydrogenase, *MDA* malondialdehyde, *MPA* medroxyprogesterone acetate, *MPO* myeloperoxidase, *NE* norepinephrine, *NO* nitric oxide, *PI3K* phosphoinositide 3-kinase, *SOD* superoxide dismutase, *SUR2* sulfonylurea receptor, *TNF-α* tumor necrosis factor alpha, *VF* ventricular fibrillation, *VT* ventricular tachycardia

and contractile dysfunction from I/R injury [84]. Estrogen helped protect the cardiac mitochondria from I/R injury by inhibiting the opening of mPTP which may lead to mitochondrial swelling and rupture, thereby preventing cardiac cell death [81, 86]. Administration of estrogen treatment resulted in decreased β1 adrenergic receptor expression and increased β2 adrenergic receptor expression in hearts subjected to I/R, which correlated to the reduction of cardiomyocyte shortening after I/R [58]. This alteration in adrenergic receptor expression might help by regulating the basal ratio of the β1/β2 adrenergic receptors, which could be changed due to I/R [58]. The antioxidant effect of estrogen was also shown to have a role in cardioprotection following I/R as it reduced ROS production and increased SOD level in the myocardium [75, 87]. Furthermore, estrogen treatment reduced myeloperoxidase enzyme activity by decreasing the amount of free radicals released from the area at risk of ischemic heart and a lowered superoxide anion concentration generated in arterial segments [28, 68].

Under the I/R setting, activation of ERα triggered portioning of ERα to the particulate, suggesting that estrogen had an effect on the redistribution of ERα and increased non-genomic ER effect which confirms the rapid action of estrogen [25]. Estrogen also mediated its non-genomic action via a NO-dependent pathway and opening of K_{Ca} channels, to protect the heart from I/R injury by limiting the size of the infarction [88]. Moreover, norepinephrine overflow, which correlated to post-ischemic cardiac dysfunction, was also inhibited via the estrogenic-stimulated increase of nitric oxide levels [49, 63].

Administration of Estrogen Treatment in I/R

Utilization of phytoestrogen demonstrated the potential to reduce myocardial apoptosis and infarction in rabbit hearts which had been subjected to I/R [89]. Infusion with estrogen-treated mesenchymal stem cells (MSCs), before induction of ischemia, showed an increase in vascular endothelial growth factor (VEGF) production, improved LV functional recovery, and myocardial viability after I/R [90]. Contrary to the effects seen from estrogen alone, treatment with a combination of estrogen and progesterone caused a reduction of the antiinflammatory effects of estrogen and aggravated the accumulation of inflammatory cells, which resulted in greater tissue injury and more necrosis [91]. However, there is a controversial report that estrogen substitution could not reverse I/R-induced cardiac injury in ovariectomized animals, showing fatty acid oxidation and impaired post-ischemic cardiac functional recovery in rat hearts, indicating a possible risk and poor outcome of estrogen administration in I/R management [69, 92].

Estrogen in Obesity, Insulin Resistance, and I/R Injury

In addition to CVD, estrogen deprivation had been reported in clinical studies to be associated with an increased prevalence of obesity, insulin resistance, and metabolic syndrome [93–95]. These findings are consistent with the results from animal studies that showed that estrogen deprivation reduced

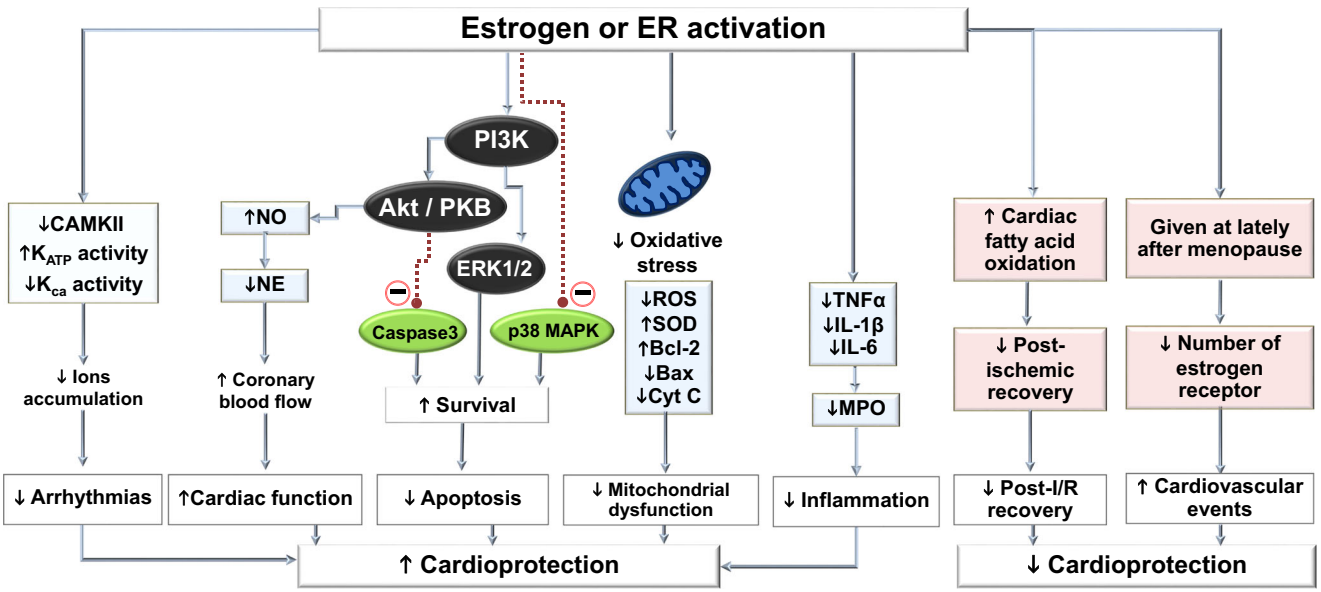

Fig. 1 The schematic illustrating estrogen effects under I/R condition. *Bax* Bcl2-associated X protein, *Bcl-2* B cell lymphoma 2, *CaMKII* Ca^{2+}/calmodulin-dependent protein kinase II, *Cyt C* cytochrome C, *ER* estrogen receptor, *IL* interleukin, *I/R* ischemic/reperfusion, K_{ATP} ATP-sensitive potassium channels, K_{Ca} calcium-activated potassium channels, *MPO* myeloperoxidase, *NE* norepinephrine, *NO* nitric oxide, *PI3K* phosphoinositide 3-kinase, *ROS* reactive oxygen species, *SOD* superoxide dismutase, *TNF-α*, tumor necrosis factor alpha, *VF* ventricular fibrillation, *VT* ventricular tachycardia

insulin sensitivity and induced metabolic impairment [16, 96, 97], leading to cardiovascular disorders. After OVX, female rats had an increased serum insulin level within 5 weeks [16]. Ovariectomized female rats became obese within 10 weeks and had a greater decrease in cardiac contractile function and reduced cardiac recovery after I/R [16]. Treatment with exogenous estrogen improved insulin sensitivity, enhanced post-ischemic cardiac contractility, and deceased myocardial tissue infarction suggesting the positive effects of this hormone on I/R [16]. A previous study showed that post-reperfusion cardiac arrhythmia incidence (e.g., ventricular fibrillation) occurred at a significant level in male rats fed with high-sucrose water, but was absent in females, and could be prevented by insulin infusion. Moreover, females also showed a lower level of plasma lipid oxidation than males after consumption of 30 % sucrose water for 8 months [98]. This study, using high-caloric-diet-induced obese rats, showed that male rats had increased body weight, visceral fat, plasma triglyceride, and glucose levels and also developed insulin resistance. After I/R induction, male obese rats also experienced more severe I/R injury, evidenced by higher myocardial infarct size, than female obese rats, suggesting beneficial effects of estrogen on the metabolic and cardiovascular systems [99]. However, the mechanisms that could explain the estrogenic protection in females have not been well established and the negative effects of estrogen on metabolism, cardiac electrophysiology, and cardiac function after I/R have been well documented. In summary, the exact, positive or negative, effects of estrogen are still controversial and have not yet been fully explored. The effects of estrogen on cardiac function and injury from I/R in female rats with metabolic disorders are still unclear and need to be investigated further.

Good and Bad Effects of Estrogen Treatment in Cardiac I/R Condition

The findings from several studies in various experimental settings, i.e., in vitro, in vivo, and clinical settings, indicated possible beneficial effects of estrogen in cardiac I/R condition. Treatment with estrogen provided its protective effects against I/R injury by reducing inflammation, protecting cardiac mitochondria, and, therefore, reducing cardiomyocyte apoptosis and myocardial infarct area [14, 67, 81, 83, 87, 100]. Moreover, estrogen treatment enhanced blood flow to the heart and improved post-I/R cardiac function and recovery as well as preventing arrhythmias [27, 58, 61, 63, 68, 70, 84, 88, 101]. Estrogen therapy, either immediately or after estrogen deprivation, was also reported to be effective in the preservation of diastolic function and cardiac structure [102]. Therefore, exogenous estrogen might be a useful regimen for the treatment of myocardial ischemia after endogenous estrogen deprivation. However, the results from clinical studies have demonstrated that estrogen treatment might increase cardiovascular events in women who already had established CVD [73, 74]. Timing of estrogen therapy is an important factor to be considered. It appears that hormone therapy, initiated early following menopause, may reduce CHD risk. However, it also appears that hormone therapy in older women, 10 years or more post-menopause, results in an increased CHD risk [103]. Previous reports have indicated that estrogen therapy reduced CHD and overall morality when prescribed for women who are less than 10 years post-menopausal and/or less than 60 years of age [104]. The adverse effects of estrogen given long after menopause, or at an older age, might be related to the diminished ER expression and the impaired inflammatory response of macrophages and vascular smooth muscle cells [105]. Estrogen treatment has also been reported to increase the level of myocardial lipid peroxidation resulting in decreased post-I/R cardiac function in isolated rat heart [69]. This particular finding might limit the use of E2 in impaired glucose tolerance, obese insulin resistance, and diabetes mellitus conditions which previously exhibited higher levels of myocardial lipid peroxidation [106–108]. The good, neutral, and bad effects of estrogen treatment and ER activation under I/R conditions as well as the underlying mechanisms are summarized in Table 6 and Fig. 1. In summary, estrogen exhibits both advantages and disadvantages on the cardiovascular system. However, since it has effects on various tissues, not only the myocardium, further studies to clarify the estrogenic effects, as well as its safety, on cardiovascular function under I/R conditions are still need to be investigated.

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Compliance with Ethical Standard

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