Estrogen Replacement Therapy Attenuates Post-infarction Left Ventricular Remodeling in Rats

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**KEY WORDS:** gender, heart failure, hormones, infarction, remodeling

**ABSTRACT**

**Objective:** Estrogens are known to have multiple effects following myocardial infarction and may attenuate left ventricular remodeling. Using a coronary artery ligation model in the rat we examine the effects of 17β-estradiol on cellular hypertrophy in remodeling myocardium remote to the infarct zone.

**Methods:** Sprague-Dawley rats underwent ovariectomy at 8 weeks of age, and then at 9 weeks of age were randomized to either coronary artery ligation or sham procedure, and to receive either placebo or 17β-estradiol 0.5 mg subcutaneous pellet. After 6 weeks an invasive hemodynamic study and histologic analysis were performed.

**Results:** At follow up there was no difference in systemic pressures between the estrogen- and placebo-treated rats. Mean infarct size was 22.3 ± 2.4% circumference in the placebo-treated rats and 21.7 ± 3.2% circumference in the estrogen-treated rats ($P=0.84$). In placebo-treated rats myocardial infarction resulted in an increase in left ventricular volume, $192 ± 15 \mu L$ to $257 ± 26 \mu L$ ($P<0.05$). Following myocardial infarction placebo-treated rats had a significantly greater increase in left ventricular mass than estrogen-treated rats (from $546 ± 19 \text{ mg}$ to $617 ± 14 \text{ mg}$ vs $561 ± 25 \text{ mg}$ to $547 ± 12 \text{ mg}$ [$P<0.05$]). Estrogen therapy abolished the increase in cardiac myocyte cross-sectional that was present in the remodeled myocardium of the placebo-treated rats ($306 ± 12 \mu m^2$ to $361 ± 22 \mu m^2$ vs $315 ± 16 \mu m^2$ to $305 ± 11 \mu m^2$ [$P<0.05$]).

**Conclusions:** We conclude that estrogen replacement attenuates post-infarction left ventricular remodeling in the rat, and that this occurs in the absence of a demonstrable hemodynamic effect.

**INTRODUCTION**

In epidemiologic studies, postmenopausal estrogen replacement therapy has been associated with a decrease in cardiovascular morbidity and mortality.\(^1\)\(^2\) However the Heart and Estrogen/progestin Replacement Study (HERS)
found no overall cardiovascular benefit with an early increase risk of coronary heart disease events. The pattern of early harm and later benefit in this large prospective study of conjugated equine estrogens and medroxyprogesterone highlights the complex nature of the clinical actions of female sex hormones. Estrogen therapy appears to have an early prothrombotic effect that is adverse, but these actions may be offset with time by a number of beneficial effects. It has been suggested that estrogen may have favorable effects on vascular function, components of the lipid profile, ischemia-reperfusion injury, and cardiac remodeling.

Left ventricular remodeling is the major determinant of late survival following myocardial infarction. At a macroscopic level the remodeling process is characterized by left ventricular hypertrophy and dilatation. Left ventricular mass increases and both end-systolic and end-diastolic volumes also increase. The increase in left ventricular mass is the result of hypertrophy of the cardiac myocytes in non-infarcted myocardium remote to the infarct zone. These cells are central to the remodeling process. Following myocardial infarction both cell length and width increase, but it is the predominant increase in length that is the major determinant of the increase in left ventricular volume. The cardiac myocytes are known to express estrogen receptors and estrogen may influence the remodeling process.

The rat coronary artery ligation model is a well-characterized model of post-infarction remodeling. In the weeks following the infarction there is hypertrophy and dilatation of the left ventricle. This can be favorably influenced by angiotensin-converting enzyme (ACE) inhibition. Additionally estrogen therapy has been shown to normalize wall tension and inhibit left ventricular dilatation post infarction. In this study, we use the rat coronary artery ligation model to assess the effects of estrogen on indices of left ventricular remodeling, including cardiac myocyte size.

**METHODS**

**Experimental Protocol**

Approval for the study was obtained from the Christchurch School of Medicine Animal Ethics Committee. At 8 weeks of age female Sprague-Dawley rats underwent ovariectomy. One week following ovariectomy rats were randomized to undergo either coronary artery ligation or sham procedure, and to receive either estrogen replacement or placebo. Six weeks following myocardial infarction, an invasive hemodynamic study was performed, and the rats were killed and the tissue harvested.

**Surgical Procedures**

All surgical procedures were performed under anesthesia with 50 mg/kg intraperitoneal pentobarbital. Ovariectomy was carried out using a posterior approach through bilateral paramedian incisions. During coronary artery ligation rats were ventilated using a 7025-10 rodent ventilator (Hugo Sachs, March-Hugstetten, Germany). A ligation was placed on the left coronary artery through a left lateral thoracotomy. Successful ligation was confirmed through the observation of pallor of the left ventricular wall distal to the ligation site. In the sham procedure the suture was not tightened around the coronary artery. The rat was extubated when spontaneous respirations had returned. Estrogen pellet or placebo was then inserted subcutaneous through a small interscapular dorsal midline incision. Sixty-day release preparations containing 0.5 mg of 17β-estradiol were used (Innovative Research of America, Sarasota, FL). On recovery from anesthesia the rats were returned to the animal holding facility where they received
A 20-gauge intravenous cannula was inserted across the aortic valve into the left ventricle and secured with a suture around the atrio-ventricular groove. The right ventricle was incised to prevent right ventricular compression from influencing left ventricular compliance. The left ventricle was evacuated, and sequential 0.025-mL normal saline increments were injected using a Hamilton syringe with a repeating microdispenser (Hamilton model PB600-1, Hamilton Company, Reno, NV). Pressure was measured following each volume increment and pressure-volume curves were formulated for each animal. From this, left ventricular volume at a common distending pressure of 10 mmHg was derived. The heart was then excised and the atria and great vessels trimmed away. The right ventricular free wall was separated from the left ventricle with septum intact. The ventricles were individually weighed and fixed in paraformaldehyde.

**Histological analysis**

Serial 3-µm transverse sections of the left ventricle were obtained. These were stained with Sirius red F3B (0.1% solution in saturated aqueous picric acid) to allow a clear discrimination between cardiomyocytes and collagen matrix. A

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<th>Placebo</th>
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<th>Estrogen</th>
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<td></td>
<td>Sham</td>
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<td>Sham</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>401 ± 11</td>
<td>416 ± 7</td>
<td>381 ± 36</td>
<td>425 ± 14</td>
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<td>SBP (mmHg)</td>
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<td>135 ± 4</td>
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<td>138 ± 4</td>
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<tr>
<td>DBP (mmHg)</td>
<td>111 ± 5</td>
<td>111 ± 3</td>
<td>108 ± 5</td>
<td>111 ± 3</td>
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<td>LVEDP (mmHg)</td>
<td>2.0 ± 0.9</td>
<td>1.7 ± 1.4</td>
<td>1.4 ± 0.7</td>
<td>2.0 ± 1.5</td>
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<tr>
<td>+dP/dt&lt;sub&gt;max&lt;/sub&gt; (mmHg/s)</td>
<td>12,506 ± 1,233</td>
<td>9,288 ± 730</td>
<td>11,753 ± 552</td>
<td>10,631 ± 1,134</td>
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<tr>
<td>-dP/dt&lt;sub&gt;max&lt;/sub&gt; (mmHg/s)</td>
<td>11,465 ± 1,140</td>
<td>8,535 ± 516</td>
<td>12,215 ± 606</td>
<td>8,926 ± 899*</td>
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*P<0.01 vs sham
bpm=beats per minute
SBP=systolic blood pressure
DBP=diastolic blood pressure
LVEDP=left ventricular end diastolic pressure

**Hemodynamic Study**

The anterior cervical region of the rat was shaved and a right paramedian incision made. A venotomy was performed on the external jugular vein with the placement of PET 50 tubing for the later administration of potassium chloride. The right carotid artery was mobilized, ligated distally, and a hemostasis suture placed proximally. An arteriotomy was performed and a 2-F Miller catheter advanced into the ascending aorta. Heart rate, systolic blood pressure, and diastolic blood pressure were derived from the online digitized pressure recording. The Miller catheter was then advanced in retrograde fashion across the aortic valve in order for left ventricular end diastolic pressure, +dP/dt<sub>max</sub> and -dP/dt<sub>max</sub> to be obtained. Next a laparotomy was performed and 2 mL of blood was obtained through venipuncture of the inferior vena cava. The rat was then killed with a central venous injection of 2 mEq/mL solution of potassium chloride arresting the heart in diastole.

Next a median sternotomy was performed. Left ventricular passive pressure-volume curves were generated from sequential injections of normal saline. A 20-gauge intravenous cannula was inserted across the aortic valve into the left ventricle and secured with a suture around the atrio-ventricular groove. The right ventricle was incised to prevent right ventricular compression from influencing left ventricular compliance. The left ventricle was evacuated, and sequential 0.025-mL normal saline increments were injected using a Hamilton syringe with a repeating microdispenser (Hamilton model PB600-1, Hamilton Company, Reno, NV). Pressure was measured following each volume increment and pressure-volume curves were formulated for each animal. From this, left ventricular volume at a common distending pressure of 10 mmHg was derived. The heart was then excised and the atria and great vessels trimmed away. The right ventricular free wall was separated from the left ventricle with septum intact. The ventricles were individually weighed and fixed in paraformaldehyde.
basal section of each ventricle was digitized using an Olympus OP10 (Olympus America Inc., Center Valley, PA) digital camera. Infarct sizing was performed on apical Sirius red sections. An enlarged photograph of each section was traced around on a digitizing palette (GTCO CalComp Inc., Columbia, MD). Infarct size was expressed as (infarct circumference x 2/endocardial + pericardial circumference) x 100%. Myocyte cross-sectional area was measured from images captured from reticulin-stained 3-μm sections. The outline of 40 subepicardial cells from a basal section remote to the infarct zone was traced around using ScionImage software (Scion Corporation, Frederick, MD). Measurements were made in duplicate and averaged. Calibration was from a graticule.

All data is expressed as mean ± SEM. The results were analyzed by two-way analysis of variance (ANOVA) with procedure and treatment as factors.

RESULTS
Fifty three ovariectomized rats underwent thoracotomy. Coronary artery ligation was performed in 30 and sham procedure in 23. Of the infarcted rats, 14 were randomized to estrogen and 16 to placebo. Four estrogen- and 8 placebo-treated rats died within 24 hours of myocardial infarction (Fisher’s Exact test, P=NS). Ten sham animals were randomized to estrogen and 13 to placebo, with 1 acute death in the estrogen group and 3 in the placebo group. There were no further deaths in the 6-week study period.

At follow-up in the estrogen-treated rats, serum estrogen was 28.0 ± 1.9 pg/mL in the sham-operated rats and 30.1 ± 2.4 pg/mL in infarcted rats (P=0.51). Serum estrogen was not detectable in any of the placebo-treated animals. Mean infarct size was 22.3 ± 2.4% circumference in the placebo infarct rats and 21.7 ± 3.2% circumference in the estrogen infarct rats (P=0.84).

Hemodynamic Results
Myocardial infarction resulted in a considerable decrease in both +dP/dt\textsubscript{max} and -dP/dt\textsubscript{max}, (P<0.05) (Table 1). There was a trend for infarcted rats to have higher
heart rates than sham-treated ones, 420 ± 8 bpm vs 391 ± 18 bpm, respectively ($P=0.17$). Estrogen therapy was not associated with a significant hemodynamic effect, with heart rate and systemic pressures being no different between estrogen- and placebo-treated animals (Table 1).

**Morphometric and Histologic Results**
In the placebo-treated rats, myocardial infarction resulted in a marked increase in LV mass as compared to placebo-treated rats who underwent the sham procedure; 617 ± 14 mg vs 546 ± 19 mg, respectively ($P=0.01$). This effect was abolished with estrogen, $P=0.027$ (Figure 1). Right ventricular mass was not increased in the infarcted animals and was not influenced by estrogen therapy (Table 2). Left ventricular volume was significantly increased with infarction in the placebo-treated rats but not in the estrogen-treated animals (Table 2). Cardiac myocyte size increased significantly from baseline in the placebo group. This response was significantly different from that observed in the estrogen group.

**DISCUSSION**
This study adds to existing data that estrogen can attenuate post-infarction left ventricular remodeling. In placebo-treated rats myocardial infarction resulted in an increase in left ventricular mass and volume. Cardiac myocyte size in the non-infarcted left ventricular myocardium was also increased following myocardial infarction. Estrogen treatment abolished the increase in both cardiac myocyte size and left ventricular mass.

Estrogen has previously been implicated as a potential mediator of left ventricular remodeling by Douglas et al. They examined the effects of gender on ventricular remodeling in rats with intact gonadal function by studying the response to pressure overload in a weanling aortic banding model. After 20 weeks male but not female rats showed evidence of pathologic remodeling and a transition to heart failure. Female rats appeared to be protected from cavity dilatation, elevated wall stress, and diastolic dysfunction. Their study design did not however allow for the effects of estrogen to be separated from other gender-related factors such as body size and other gonadal hormones. More recently in a rat coronary artery ligation model similar to ours, 17β-estradiol has been shown to normalize wall tension and inhibit left ventricular dilatation and hypertrophy. However cardiac myocyte size was not measured. Our study has shown that estrogen can abolish both the increases in LV mass and cardiac myocyte size following moderate-sized myocardial infarction.

In rats, left ventricular mass may be
lower than control for up to 19 days post-infarction, owing to thinning and scar formation in the infarct zone. After that time, progressive left ventricular hypertrophy produces an increase in mass. Placebo rats in the current study developed this hypertrophy. Increased right ventricular mass following myocardial infarction was not seen. Right ventricular hypertrophy is reported to develop only in rodents with large infarcts. Our surviving rats had not had large myocardial infarctions, their mean infarct size of 21% is consistent with medium-sized infarcts.

In this study, rats receiving estrogen replacement therapy had physiologic estrogen levels. Mean level at 6 weeks was 29.1 ± 1.5 pg/mL. Levels ranging from 7 to 88 pg/mL have been reported during the normal estrus cycle in the Sprague-Dawley rat.

While we noted both +dP/dt\textsubscript{max} and -dP/dt\textsubscript{max} to fall with myocardial infarction, there was no difference in systemic hemodynamics between the estrogen and placebo rats. The absence of a demonstrable hemodynamic effect indicates that alternative mechanisms of action may be important. A direct receptor-mediated effect is possible as both cardiac myocytes and fibroblasts contain functional estrogen receptors. In the myocytes the estrogen receptor may function as a transcriptional regulator for hypertrophy-related genes such as myosin heavy chain and structural matrix proteins.

It is also possible that estrogen may have attenuated remodeling through interactions with activated neurohormonal systems. A number of studies have found that estrogen can attenuate activity within the renin angiotensin system. Estrogen has been shown to decrease tissue angiotensinogen gene expression in ovariectomised Sprague-Dawley rats and to reduce ACE mRNA concentration and tissue ACE activi-

ty. A similar attenuation of ACE activity occurred when post-menopausal cynomolgus monkeys were treated with chronic conjugated equine estrogens. In addition there is the potential for estrogen to decrease the end organ effects of angiotensin through the down-regulation of angiotensin\textsubscript{1} receptor expression.

The results of this study are not inconsistent with the observation that the prognosis for women who have suffered a myocardial infarction is poorer than it is for men. In premenopausal women factors such as small size, less aggressive treatment, variability in presentation, and any prothrombotic effect of estrogen may offset a beneficial effect on ventricular remodeling. In post-menopausal women estrogen levels are similar to those of men. Selective estrogen receptor modulators can exert estrogen-like effects on the vasculature in both male and female rats. While the specific cardiac effects of the selective estrogen receptor agonists have not been examined, it is possible that these agents could attenuate remodeling and become an important therapeutic modality.

**Limitations**

It is a limitation of our study that estrogen levels and hemodynamic measurements were only recorded at a single time point. We can not exclude the possibility that estrogen may have had an important hemodynamic effect at an earlier time point in the study. However the hemodynamics were recorded with a high fidelity transducer at a time when we demostated physiologic estrogen levels. Rats surviving thoracotomy in this study had infarctions of moderate size. The myocardial infarction size was not sufficiently large to induce right ventricular hypertrophy, but there was significant left ventricular remodeling in the placebo-treated animals. This remod-
eling was evidenced by increased left ventricular volume and mass, and occurred without hemodynamic changes. Systemic hypotension is a very late manifestation of cardiac failure and would not have been expected to be present as a result of the myocardial infarction.

CONCLUSION
We have shown that estrogen replacement therapy can attenuate aspects of post-infarction left ventricular remodeling in the rat. This occurred in the absence of a demonstrable hemodynamic effect. Alternative mechanisms such as a direct receptor mediated cardioprotective effect and neurohormonal modulation may be responsible.

Acknowledgments
The authors gratefully acknowledge the assistance with the histologic specimens of Harold Neal PhD, Christchurch Hospital.

REFERENCES


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**ERRATUM**

Due to a printer’s error in *The Journal of Applied Research*, Volume 6, Number 2, some of the figures in Endothelial Nitric Oxide Synthase Deficiency Enhanced Carotid Artery Ligation-Induced Remodeling by Promoting Vascular Inflammation by Zhang et al were missing important data. All of the corrected figures are printed here. To view the complete article with the corrected figures please go to www.JARCET.com and click on “Published Articles.”

*The Journal of Applied Research* apologizes for this error.

![Figure 1](image_url)  
**Figure 1.** Representative hematoxylin and eosin-stained (H&E) sections of the control nonligated (0-week), 1-week, and 4-week ligated carotid arteries from wildtype (WT) and endothelial nitric oxide synthase knockout (eNOS-KO) mice. IEL, internal elastic lamina; EEL, external elastic lamina. Original magnifications X 200.

*Continued*
Figure 2. Representative hematoxylin and eosin (H&E), Mac-3, and α-actin stained sections of the 1-week ligated carotid artery from wildtype (WT) and the 1-week and 4-week ligated carotid arteries from endothelial nitric oxide synthase knockout (eNOS-KO) mice. Original magnifications X 400.

Figure 3. Quantitative analysis of vascular remodeling in response to carotid artery ligation in wildtype (WT), endothelial nitric oxide synthase (eNOS) deficient, and NG-nitro-l-arginine methyl ester (L-NAME)–treated WT mice. Con = control; Lig = ligated
Figure 4. Morphometric changes of each individual component of the carotid arteries from wildtype (WT), endothelial nitric oxide synthase (eNOS) deficient, and N⁵-nitro-L-arginine methyl ester (L-NAME)–treated WT mice in response to carotid artery ligation.

Figure 5. Representative hematoxylin and eosin-stained (H&E) sections for inflammatory response scoring. IEL, internal elastic lamina; EEL, external elastic lamina. Arrows indicate inflammatory cells. Original magnification X 400.
Figure 6. Time course of inflammatory scoring and quantitative analysis of macrophage infiltration in vascular wall and small muscle cell proliferation in the neointima after carotid artery ligation. WT, wildtype, eNOS-KO, endothelial nitric oxide synthase knockout.

Continued
Figure 7. Ex vivo vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1) secretion by the nonligated (Time 0) and ligated carotid arteries isolated from wildtype (WT) and endothelial NO synthase knockout (eNOS-KO) mice.

# P<0.05 vs. WT, * P<0.05 vs. 0 week