

Estrogen and testosterone have opposing effects on chronic cardiac remodeling and function in mice with myocardial infarction

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Cavasin, Maria A., Steadman S. Sankey, Ai-Li Yu, Shreevidya Menon, and Xiao-Ping Yang. Estrogen and testosterone have opposing effects on chronic cardiac remodeling and function in mice with myocardial infarction. *Am J Physiol Heart Circ Physiol* 284: H1560–H1569, 2003. First published January 30, 2003; 10.1152/ajpheart.01087.2002.—Premenopausal women are much less prone to develop cardiovascular disease than men of similar age, but this advantage no longer applies after menopause. We previously found that male mice have a significantly higher rate of cardiac rupture than females during the acute phase of myocardial infarction (MI); however, the effects of sexual hormones on chronic remodeling are unknown. We hypothesized that estrogen (E) may protect the heart from chronic remodeling and deterioration of function post-MI, whereas testosterone (T) may have adverse effects. Mice (4 wk old) of both genders were divided into four groups: female groups consisted of 1) sham ovariectomy (S-Ovx) + placebo (P) (S-Ovx + P), 2) S-Ovx + T, 3) Ovx + P, and 4) Ovx + T; and male groups consisted of 1) sham castration (S-Cas) + P (S-Cas + P), 2) S-Cas + 17 β -estradiol (E), 3) Cas + P, and 4) Cas + E. MI was induced 6 wk later. Echocardiography was performed to assess cardiac function and left ventricular dimensions (LVD). Myocyte cross-sectional area (MCSA) was measured at the end of the study. In females, both testosterone and ovariectomy decreased ejection fraction (EF) and increased LVD, and when combined they aggravated cardiac function and remodeling further. Testosterone significantly increased MCSA. In males, castration or estrogen increased EF and reduced LVD, whereas castration significantly reduced MCSA. Our data suggest that estrogen prevents deterioration of cardiac function and remodeling after MI, but testosterone worsens cardiac dysfunction and remodeling and has a pronounced effect when estrogen levels are reduced.

sexual hormones; cardiac dysfunction

PREMENOPAUSAL WOMEN are much less prone to develop cardiovascular disease than men of similar age, but this advantage no longer applies after women reach menopause, suggesting that estrogen has a cardioprotective effect (15, 28). However, little is known about the long-term outcome or prognosis after myocardial infarction (MI) in premenopausal women or how it differs from men, because these women rarely have heart attacks. Many observational studies have shown

that postmenopausal women who receive estrogen replacement therapy (ERT) have a lower rate of coronary heart disease (CHD) and cardiac death than those not receiving ERT (7, 18, 42, 48). These protective effects have been associated with a reduced ratio of cholesterol and high-density lipoproteins (HDL) (10) and promotion of endothelium-dependent relaxation via increased nitric oxide (NO) bioavailability (37).

In general, most investigations have been concerned with the possible cardioprotective effects of estrogen, indirectly revealed after the onset of menopause, whereas the hypothesis that androgens may contribute to cardiovascular risk has received less consideration. In humans and experimental animals, testosterone has been associated with an increased risk of coronary artery disease by adversely affecting the plasma lipid and lipoprotein profile, thrombosis, and cardiac hypertrophy (1, 16, 36, 49). Perhaps the observed gender differences and the increased incidence of cardiac events in women after menopause are not entirely due to depletion of estrogen, but are also related to significant amounts of circulating testosterone, because postmenopausal ovaries reportedly produce significant amounts of androgens in the form of testosterone and androstenedione (47). A positive correlation has been found between age and testosterone levels in the ovarian vein (3), and increased testosterone levels in menopausal women are associated with hypertension, decreased HDL, impaired vascular reactivity, and coronary artery disease (21, 35, 40, 41).

Previously, we (11) found that in mice with surgically induced MI, mortality and cardiac rupture during the first week were significantly higher in males than females. However, the differential effects of sexual hormones on prognosis and structural remodeling of the heart post-MI have not been studied closely. We hypothesized that estrogen may protect the heart from chronic remodeling and deterioration of function post-MI, whereas testosterone may have adverse effects. Using male and female mice subjected to gonadectomy and/or hormone replacement, we studied whether 1) cardiac dysfunction and remodeling post-MI are less severe in females than males, 2) ovariectomy and/or

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testosterone supplementation worsen and accelerate cardiac dysfunction and remodeling, and 3) castration and/or estrogen supplementation can ameliorate cardiac dysfunction and remodeling.

METHODS

Animals. Male and female C57BL/6J mice (4 wk old) were obtained from Jackson Laboratories (Bar Harbor, ME). They were housed in an air-conditioned room with a 12:12-h dark-light cycle and given standard chow with free access to tap water. All surgical procedures were conducted with the mice under pentobarbital anesthesia (50 mg/kg ip). The study was approved by the Institutional Animal Care and Use Committee of Henry Ford Health System.

Gonadectomy. Females were randomly subjected to bilateral ovariectomy (Ovx) or sham ovariectomy (S-Ovx), for which they were placed prone and surgery performed via a pair of flank incisions. They were then divided into the following groups: 1) S-Ovx + placebo (P), 2) S-Ovx + testosterone (T), 3) Ovx + P, and 4) Ovx + T. Males were randomly subjected to castration (Cas) or sham castration (S-Cas), for which they were placed supine and the testes removed or left intact via a low-middle abdominal incision. They were then divided into the following groups: 1) S-Cas + P, 2) S-Cas + 17 β -estradiol (E), 3) Cas + P, and 4) Cas + E. Some mice of both genders were left intact to be used as controls. Hormones and placebo were administered by subcutaneous pellets inserted on the day of surgery, which lasted 60 days and were replaced as needed (Innovative Research of America; Sarasota, FL). Dosage was 23 μ g/day 17 β -estradiol (1.7 mg in 60 days) and 208 μ g/day testosterone (12.5 mg in 60 days).

Induction of myocardial infarction. Six weeks after gonadectomy and hormone manipulation, MI was induced by coronary artery ligation as described previously (11, 53). Mice were anesthetized, intubated, and ventilated with room air using a positive-pressure respirator (680 Harvard; South Natick, MA). A left thoracotomy was performed via the fourth intercostal space, the lungs were retracted to expose the heart, and the pericardium was opened. The left anterior descending coronary artery was ligated with an 8-0 silk suture near its origin between the pulmonary outflow tract and the edge of the left atrium. Acute myocardial ischemia was considered successful when the anterior wall of the left ventricle (LV) turned pale and obvious ST segment elevation was observed on the electrocardiogram. The lungs were inflated by increasing positive end-expiratory pressure, and the thoracotomy site was closed. Animals were kept on a heating pad until they awakened. Sham MI surgery was performed on mice that had sham gonadectomy.

Cardiac function and blood pressure assessment. Trans-thoracic echocardiography was performed in conscious mice before induction of MI and every 4 wk until the end of the study (53). LV ejection fraction (EF), systolic and diastolic dimensions (LVDs and LVDD), respectively, and heart rate (HR) were calculated from the echocardiograms. EF, a measurement of LV systolic function, was calculated as follows

$$EF (\%) = [(LVAd - LVAs)]/LVAd \times 100$$

where LVAd and LVAs are LV diastolic and systolic areas, respectively, measured from the LV cross-sectional area (two-dimensional short-axis view). LVDs and LVDD were measured from the M-mode view. Systolic blood pressure (SBP) was measured in awake mice at the end of the study using a noninvasive computerized tail-cuff system (Visitech BP-2000; Apex, NC) (4).

Determination of 17 β -estradiol and testosterone in plasma. Twelve weeks after MI, mice were anesthetized and blood was collected from the vena cava; plasma was separated out and kept at -70°C until assay. Plasma concentrations of 17 β -estradiol and testosterone were determined by enzyme immunoassay using a commercially available kit (Cayman Chemicals; Ann Arbor, MI) to ensure drug delivery.

Histopathological study. After blood was collected, the chest was opened and the heart was stopped at diastole by intraventricular injection of 15% KCl. After the heart was weighed, the LV was sectioned transversely into three slices, rapidly frozen in isopentane, and stored at -70°C for determination of MI size (31), cardiomyocyte cross-sectional area (MCSA), and interstitial collagen fraction (ICF) (32).

Statistical analysis. Data are expressed as means \pm SE. The primary method for group comparison was analysis of variance with repeated measures or Student's *t*-test when comparing only two groups. Most groups did not exhibit significant deviations from normality. When nonnormality was observed, a nonparametric test (Wilcoxon's rank sum) was used. $P < 0.05$ was considered statistically significant. Mortality and cardiac rupture rates were compared using chi-square tests or Fisher's exact test for sparse data. To test gender effect, MI + S-Ovx + P was compared with MI + S-Cas + P. Comparisons among females were: 1) sham MI vs. MI + S-Ovx + P (to test effects solely due to MI); 2) MI + S-Ovx + P vs. MI + S-Ovx + T and MI + Ovx + P vs. MI + Ovx + T (to test the effects of testosterone in the presence or absence of significant levels of estrogen); and 3) MI + S-Ovx + P vs. MI + Ovx + P (to test the effects of reduction of endogenous estrogen); comparisons among males were: 1) sham MI vs. MI + S-Cas + P (to test effects solely due to MI); 2) MI + S-Cas + P vs. MI + S-Cas + E and MI + Cas + P vs. MI + Cas + E (to test the effects of estrogen in the presence or absence of significant levels of testosterone); and 3) MI + S-Cas + P vs. MI + Cas + P (to test the effects of reduction of endogenous testosterone).

RESULTS

Mortality and cardiac rupture after MI. For mortality and cardiac rupture, mice from a pilot study were combined with the present study to reach a sufficient number of animals for statistical analysis. Absolute numbers of mice are given in Table 1. Mortality during the first week post-MI was significantly higher in

Table 1. Absolute number of total mice and cumulative deaths and ruptures

	MI+S-Ovx+P	MI+S-Ovx+T	MI+Ovx+P	MI+Ovx+T
Females				
Total mice	31	46	32	45
Total deaths	3	20	0	14
Death due to rupture	2	17	0	11
	MI+S-Cas+P	MI+S-Cas+E	MI+Cas+P	MI+Cas+E
Males				
Total mice	36	29	27	29
Total deaths	11	5	1	1
Death due to rupture	9	3	0	1

MI, myocardial infarction; S, sham; Ovx, ovariectomy; T, testosterone; P, placebo; Cas, castration; E, estrogen.

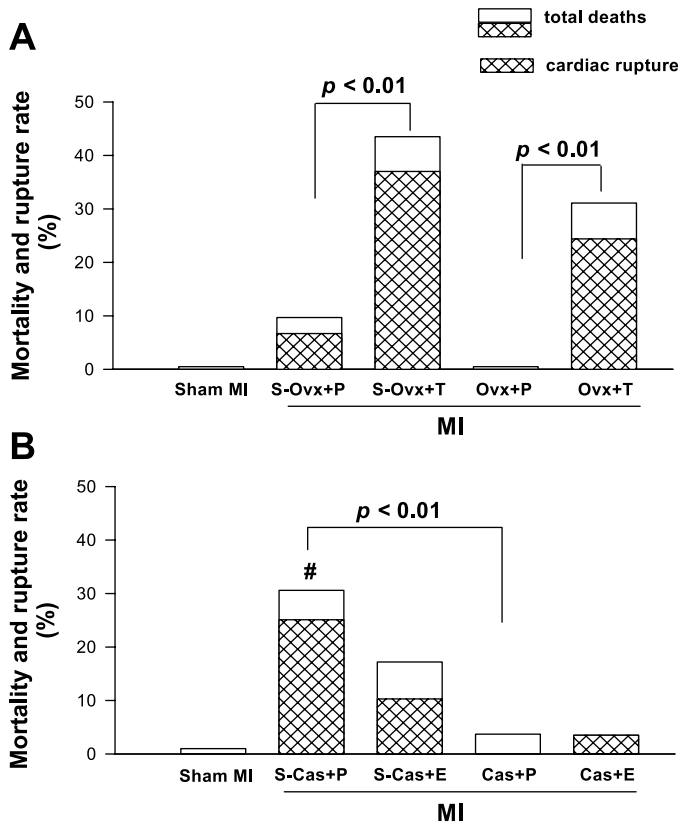


Fig. 1. Mortality rate and cardiac rupture during the first week of myocardial infarction (MI). *A*: females; *B*: males. Entire bar (white plus crosshatch) represents mortality; crosshatched portion represents death from cardiac rupture. S-Ovx, sham ovariectomy; S-Cas, sham castration; P, placebo; T, testosterone; E, 17 β -estradiol. Gender effect: #*P* < 0.01 vs. S-Ovx + P (females).

S-Cas + P (“normal males”) than in S-Ovx + P (“normal females”). The major cause of death was cardiac rupture, which generally occurred 3–5 days after MI. In females, supplemental testosterone increased mortality and rupture significantly (Fig. 1*A*), whereas ovariectomy did not. In males, castration significantly reduced both mortality and rupture. Supplemental estrogen reduced rupture in noncastrated males, though

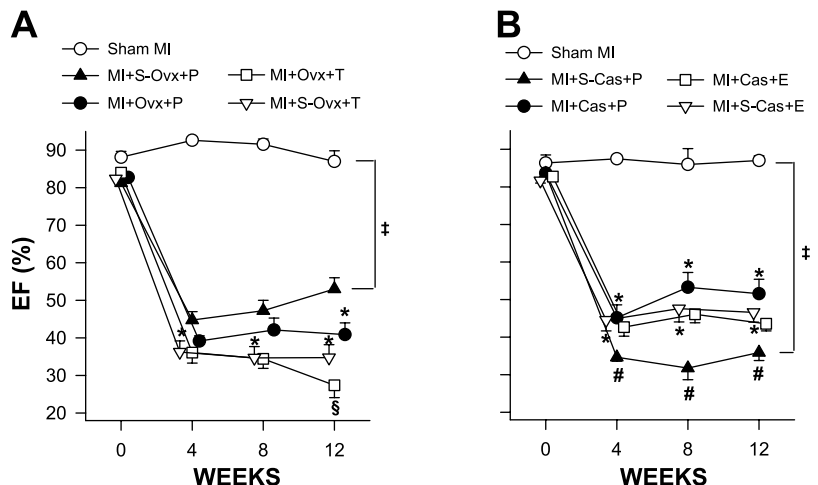
the difference was not significant (Fig. 1*B*). Late mortality (weeks 2–12) was somewhat low and was similar in all groups. No mice with sham MI died during the study.

Effects of gonadectomy and hormone manipulation on LV performance and dilatation after MI. Figure 2 shows the time course of EF. MI significantly degraded cardiac performance, as evidenced by decreased EF (sham MI vs. MI + S-Ovx + P in females and MI + S-Cas + P in males); however, cardiac dysfunction was more severe in normal males than in normal females. Among females, testosterone or ovariectomy significantly reduced EF compared with MI + S-Ovx + P; testosterone reduced EF even more in ovariectomized females, becoming significant at 12 wk (Fig. 2*A*). Among males, either castration or estrogen significantly increased EF compared with MI + S-Cas + P (Fig. 2*B*); however, there were no differences between MI + Cas + P and MI + Cas + E.

Figure 3 shows the time course of LVDD and LVDs. MI induced significant LV dilatation, which was more pronounced in normal males than in normal females. Among females, testosterone or ovariectomy increased LVD compared with MI + S-Ovx + P (Fig. 3, *A* and *C*), although these effects only reached significance at 8–12 and 8 wk, respectively; testosterone increased LVD further in ovariectomized females, becoming significant at 12 wk. Among males, either castration or estrogen significantly reduced LVD compared with MI + S-Cas + P (Fig. 3, *B* and *D*); however, there were no differences between MI + Cas + P and MI + Cas + E.

Effects of gonadectomy and hormone manipulation on systolic blood pressure and heart rate after MI. MI alone significantly decreased SBP in females but not in males (Fig. 4, *A* and *B*). Testosterone increased SBP in nonovariectomized females, but the difference was only marginally significant. Ovariectomy also raised SBP, but the difference was not significant. Among males, castration significantly lowered SBP; estrogen also tended to reduce SBP, particularly in castrated males,

Fig. 2. Effect of gonadectomy and/or hormone manipulation on left ventricular ejection fraction (EF) in female (*A*) and male mice (*B*) with MI. In females: ‡*P* < 0.01 vs. sham MI; **P* < 0.05 vs. MI + S-Ovx + P; §*P* < 0.05 vs. MI + Ovx + P. In males: ‡*P* < 0.001 vs. sham MI; **P* < 0.05 vs. MI + S-Cas + P. Gender effect: #*P* < 0.01 vs. S-Ovx + P (females).



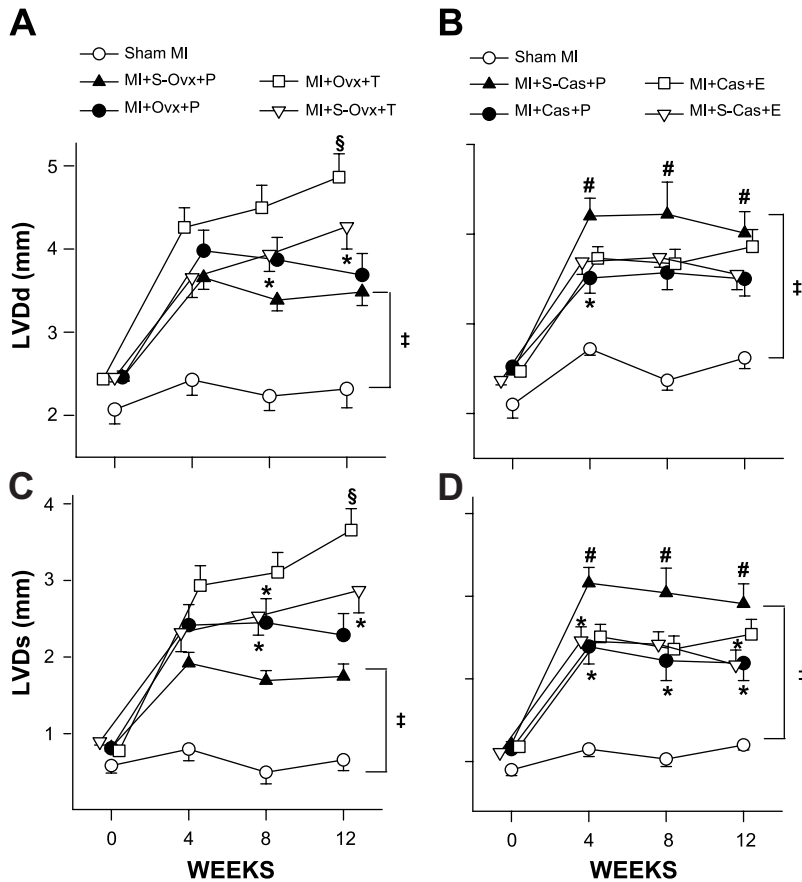


Fig. 3. Effect of gonadectomy and/or hormone manipulation on left ventricular diastolic dimension (LVDD) and systolic dimension (LVDs) in female (A and C) and male mice (B and D) with MI. In females: ‡ P < 0.001 vs. sham MI; * P < 0.05 vs. MI + S-Ovx + P; § P < 0.05 vs. MI + Ovx + P. In males: ‡ P < 0.001 vs. sham MI; * P < 0.05 vs. MI + S-Cas + P. Gender effect: # P < 0.01 vs. S-Ovx + P (females).

but the differences were not significant. Within gender, HR did not differ among groups (Fig. 4, C and D).

Effects of gonadectomy and hormone manipulation on myocyte hypertrophy and collagen deposition after MI. Figure 5 shows MCSA and ICF. MI alone significantly increased myocyte size and interstitial collagen deposition in both males and females. Myocyte hypertrophy and collagen deposition were more pronounced in normal males, but only MSCA reached significance. Testosterone further increased MCSA post-MI in females with or without ovariectomy (Fig. 5A), whereas ovariectomy alone did not enhance myocyte hypertrophy. In males, castration reduced MCSA, whereas estrogen had no significant effect (Fig. 5B). Within gender, ICF was similar among groups with MI (Fig. 5, C and D). Figure 6 shows representative histological sections of cardiac tissue from the following groups: female sham MI (A), MI + S-Ovx + P (B), MI + S-Ovx + T (C), male sham MI (D), MI + S-Cas + P (E), and MI + Cas + P (F).

Effects of gonadectomy and hormone manipulation on cardiac morphology, infarct size, and hormone levels. MI significantly increased LV and total heart weight compared with sham MI in males and females. In females, testosterone increased LV and total heart weight further post-MI, with or without ovariectomy, although LV-to-BW and HW-to-BW ratios were only significant in ovariectomized females (Table 2). Ovariectomy alone had no significant influence on LV or

total heart weight. In males, castration alone significantly decreased LV and total heart weight, and this effect was ameliorated by estrogen supplementation (Table 3). Estrogen decreased LV and total heart weight in sham-castrated mice, but this effect was not significant. There were no significant differences in body weight or MI size among groups, except that MI size was greater in MI + Cas + E than in the MI + Cas + P group. As expected, ovariectomy significantly decreased plasma estrogen and castration decreased plasma testosterone. Supplemental testosterone increased plasma testosterone in females but had no effect on estradiol levels. Supplemental estrogen increased plasma estrogen in males, but also increased testosterone in castrated males to about the same level as in noncastrated mice.

DISCUSSION

We found that in mice with MI there was an obvious gender difference in cardiac remodeling and function, as evidenced by the fact that males given sham castration plus placebo ("normal males") had a higher incidence of cardiac rupture, decreased EF, and increased LVD and MSCA compared with females with sham ovariectomy plus placebo ("normal females"). In females, we found that 1) supplemental testosterone increased cardiac rupture in the acute phase of MI, whereas reduction of estrogen levels by ovariectomy

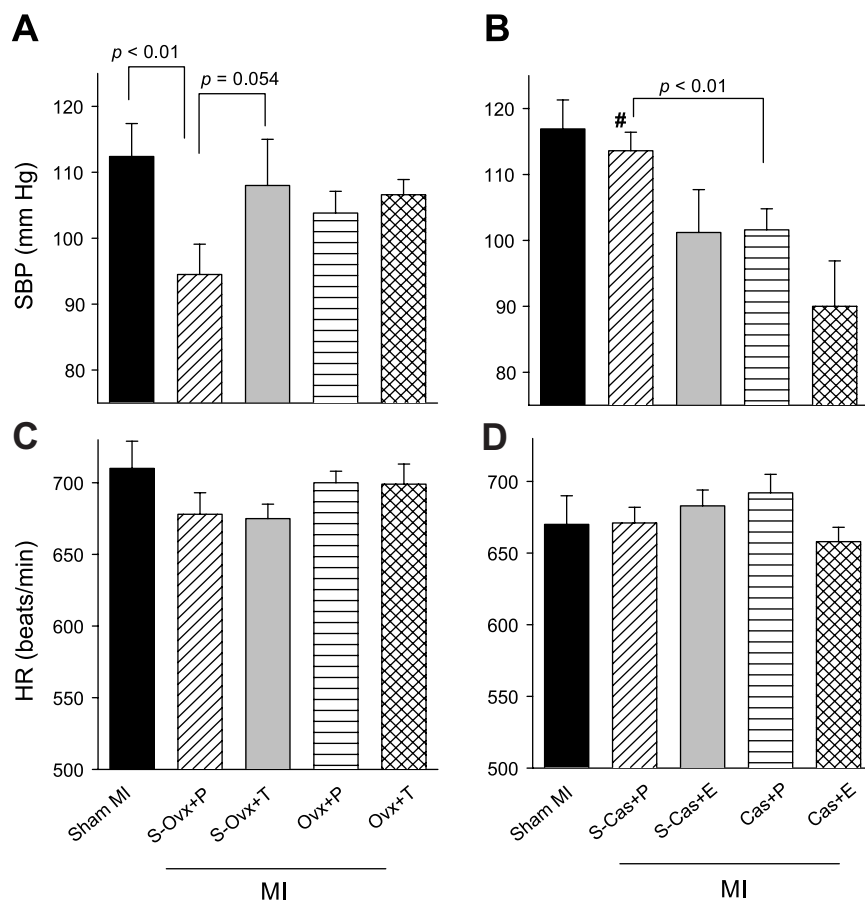


Fig. 4. Effect of gonadectomy and/or hormone manipulation on systolic blood pressure (SBP) and heart rate (HR) in female (A and C) and male mice (B and D) with MI. Gender effect: # $P < 0.05$ vs. MI + S-Ovx + P (females).

had no effect; 2) during the chronic phase of MI, testosterone or ovariectomy worsened LV dysfunction and dilatation in females, as indicated by decreased EF and increased LVD, and the adverse effects of testosterone were more pronounced when estrogen levels were reduced due to ovariectomy; and 3) testosterone further increased myocyte size regardless of ovariectomy, while ovariectomy and testosterone increased LV hypertrophy. In males, we found that 1) castration significantly reduced cardiac rupture in the acute phase of MI, whereas supplemental estrogen had no significant effect; 2) during the chronic phase of MI, castration or supplemental estrogen improved LV function and prevented dilatation, as indicated by increased EF and decreased LVD; 3) castration significantly decreased myocyte size and LV hypertrophy, while estrogen tended to decrease LV mass; and 4) no additive protective effects were observed with combined castration and estrogen. Taken together, our data suggest that testosterone has a detrimental effect on myocardial healing and aggravates cardiac dysfunction and chronic remodeling, whereas estrogen seems to have a cardioprotective effect only in the chronic phase post-MI in both genders.

Effects of estrogen and testosterone on cardiac rupture post-MI. The myocardium undergoes a dynamic repair process after MI, which is regulated by mechanical, hormonal, and genetic factors (8, 17) and characterized by removal of necrotic tissue, scar formation,

myocyte hypertrophy, and chamber dilatation, all of which ultimately lead to changes in LV size, shape, and function (so-called "cardiac remodeling") (8, 25, 33). In the acute phase post-MI, weakening of the myocardium and increased wall stress may result in rapid LV dilatation and infarct expansion, contributing to cardiac rupture, but we do not know how sexual hormones affect the healing process, nor the mechanism(s) involved. It has been reported that estrogen prompts healing of endometrial and cutaneous wounds (5, 24), but it is still unclear whether this is true for myocardial tissue as well. We previously found a significant difference in early mortality and cardiac rupture between male and female mice (11), which was confirmed in the present study. Rupture rate was not altered by ovariectomy in females, while supplemental estrogen tended to reduce it in males although not to a significant degree; thus these observations challenge the assumption that estrogen is cardioprotective, at least in the acute phase post-MI. The important finding of the present study is that supplemental testosterone dramatically increased cardiac rupture and mortality in females with or without ovariectomy, while castration significantly decreased both events in males. These findings suggest that rather than estrogen being protective, testosterone may adversely affect myocardial healing and early remodeling during the acute phase of MI, causing the observed "gender difference." We are currently investigating how sexual hormones influence

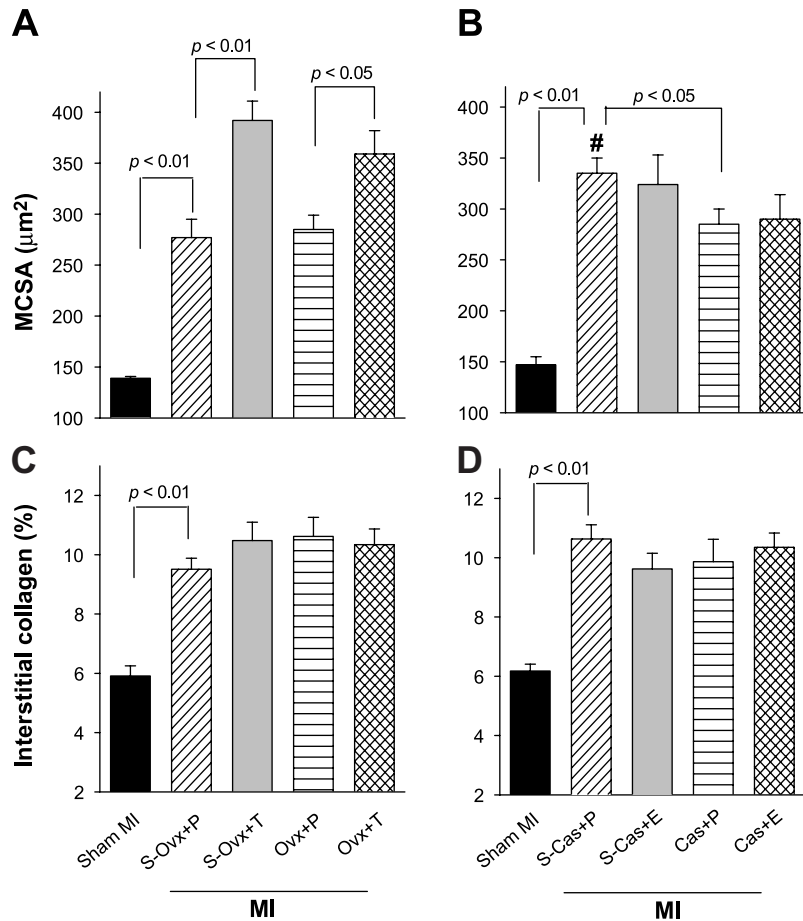


Fig. 5. Effect of gonadectomy and/or hormone manipulation on myocyte cross-sectional area (MCSA) and interstitial collagen fraction (ICF) in female (A and C) and male (B and D) mice with MI. Gender effect: # $P < 0.05$ vs. MI + S-Ovx + P (females).

myocardial repair, inflammation, and early remodeling post-MI.

Effects of estrogen on cardiac function and late remodeling after MI. We found that 1) cardiac dysfunction and chronic remodeling post-MI were less severe in normal females compared with normal males, 2) ovariectomy significantly reduced circulating levels of estrogen and worsened LV performance and remodeling in females, and 3) estrogen supplementation significantly improved cardiac function and ameliorated remodeling in noncastrated males. These observations support the hypothesis that estrogen, either endogenous or supplemental, is cardioprotective in mice with MI. In humans and animals, administration of estrogen causes rapid dilatation of coronary arteries, an effect mediated largely by the generation of NO (20, 26, 51). It has been suggested that the cardioprotective effects of estrogen involve lowering low-density lipoprotein and cholesterol, improving endothelial function, inhibiting inflammatory cell activation, and/or acting as an antioxidant (10, 37, 39). However, the recently published Heart and Estrogen/Progestin Replacement Studies (HERS) I and II (22, 23) and Women's Health Initiative (WHI) report (52) failed to demonstrate that conjugated estrogen plus progestin or medroxyprogesterone reduces the overall incidence of cardiac events in patients with established CHD or the risk of cardiovascular disease in healthy postmeno-

pausal women. Still, as the authors pointed out, these trials could not distinguish the effects of estrogen from those of progestin because progestin may be important in breast cancer and atherosclerotic disease, including CHD and stroke (52). Therefore, whether estrogen alone is cardioprotective in humans needs to be clarified. The WHI investigators have been conducting a separate trial to test whether estrogen alone prevents CHD in women with hysterectomy, but these results will not likely be available until 2005. Another important factor that needs to be considered is methylation of the estrogen receptor α gene ($ER\alpha$), which causes downregulation of gene expression. Methylation of $ER\alpha$ has been found to be associated with aging and atherosclerosis (43), which could be another explanation for the lack of benefits of ERT in older women.

Effects of testosterone on cardiac function and late remodeling after MI. Testosterone adversely affects function and remodeling of the heart after MI. We found that 1) normal males had more severe deterioration of LV performance, enhanced dilatation, and increased MCSA compared with normal females; 2) in females, supplemental testosterone significantly increased cardiac rupture, worsened cardiac performance, enhanced LV dilatation and hypertrophy, and increased myocyte size; and 3) in males, castration significantly reduced cardiac rupture, prevented deterioration of cardiac function and LV dilatation, de-

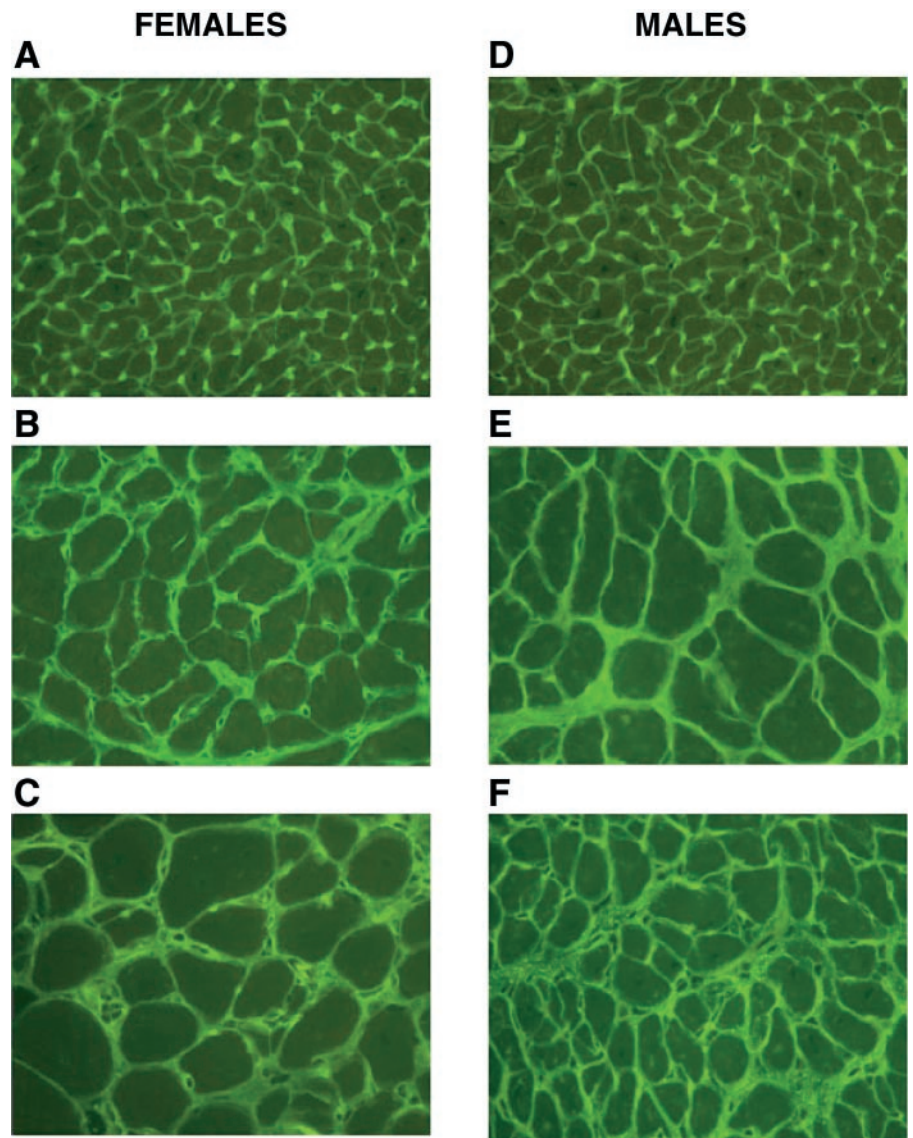


Fig. 6. Representative histological sections of cardiac tissue stained with fluorescein-labeled peanut agglutinin for interstitial collagen and myocyte cross-sectional area determination. In females: sham MI (A), MI + S-Ovx + P (B), MI + S-Ovx + T (C); in males: sham MI (D), MI + S-Cas + P (E), and MI + Cas + P (F). All photos have magnification of 100.

creased myocyte size, and tended to decrease LV weight. The detrimental effects of testosterone might be due to 1) impairment of coronary artery relaxation (12, 50), thereby lessening myocardial irrigation and causing dysfunction; 2) enhancement of myocyte hy-

peretrophy via androgen receptors, which are present in both genders (34); and/or 3) an increase in the number of dead myocytes because it has been reported that anabolic-androgen steroids induce dose-dependent myocyte apoptosis (34, 55), which is gender dependent

Table 2. *Body weight, cardiac morphology, plasma hormone levels, and infarct size*

Females	Sham MI	MI+S-Ovx+P	MI+S-Ovx+T	MI+Ovx+P	MI+Ovx+T
<i>n</i>	6	13	10	10	9
BW, g	21.6 ± 0.7	22.9 ± 0.2	23.8 ± 0.6	24.6 ± 0.5	24.7 ± 0.3
LV, mg	65.2 ± 2.3	94.8 ± 2.8 ^a	114.7 ± 7.8 ^c	98.2 ± 4.6	122.0 ± 5.7 ^e
Total heart weight, mg	91.5 ± 3.0	130.1 ± 3.64 ^a	156.2 ± 10.6 ^c	137.3 ± 7.0	166.7 ± 6.9 ^e
LV/BW, mg/10 g	30.31 ± 1.54	41.56 ± 1.37 ^a	48.01 ± 3.01	40.06 ± 2.04	49.48 ± 2.32 ^e
HW/BW, mg/10 g	42.47 ± 1.94	58.69 ± 1.73 ^a	65.31 ± 3.96	59.31 ± 3.93	65.95 ± 3.10 ^f
Estradiol, pg/ml	164 ± 32	147 ± 14	197 ± 25	66 ± 6 ^b	66 ± 7
Testosterone, pg/ml	244 ± 31	142 ± 18 ^a	4,324 ± 519 ^b	99 ± 12	3,710 ± 387 ^d
MI size, %	NA	31 ± 2	28 ± 3	38 ± 3	34 ± 4

Values are means ± SE; *n*, number of mice. MI, myocardial infarction; BW, body weight; RV, right ventricle; LV, left ventricle; HW, heart weight; NA, not applicable. ^a*P* < 0.001 vs. Sham MI; ^b*P* < 0.001 vs. S-Ovx + P; ^c*P* < 0.05 vs. S-Ovx + P; ^d*P* < 0.001 vs. Ovx + P; ^e*P* < 0.01 vs. Ovx + P; ^f*P* < 0.05 vs. Ovx + P.

Table 3. *Body weight, cardiac morphology, plasma hormone levels, and infarct size*

Males	Sham MI	MI+S-Cas+P	MI+S-Cas+E	MI+Cas+P	MI+Cas+E
<i>n</i>	7	10	11	11	14
BW, g	31.9 ± 1.1	28.5 ± 0.5	26.1 ± 0.3	24.4 ± 0.3	26.2 ± 0.4
LV, mg	87.0 ± 4.2	111.6 ± 5.8 ^a	105.6 ± 5.0	88.0 ± 2.4 ^c	104.9 ± 4.0 ^e
Total heart weight, mg	120.8 ± 5.6	154.7 ± 7.7 ^a	145.3 ± 6.7	125.0 ± 4.2 ^c	144.0 ± 6.4 ^f
LV/BW, mg/10 g	27.24 ± 0.74	39.16 ± 1.94 ^a	40.38 ± 0.96	36.22 ± 0.94	40.09 ± 1.51
HW/BW, mg/10 g	37.81 ± 0.86	54.32 ± 2.74 ^a	55.76 ± 1.51	51.46 ± 1.56	54.95 ± 2.29
Estradiol, pg/ml	112 ± 16	75 ± 3 ^a	915 ± 84 ^b	60 ± 5	1,009 ± 97 ^d
Testosterone, pg/ml	515 ± 68	401 ± 33	425 ± 62	56 ± 8 ^b	359 ± 45 ^d
MI size, %	NA	32 ± 3	30 ± 3	28 ± 3	36 ± 2 ^f

^a*P* < 0.001 vs. sham MI; ^b*P* < 0.001 vs. S-Cas + P; ^c*P* 0.01 vs. S-Cas + P; ^d*P* < 0.001 vs. Cas + P; ^e*P* < 0.01 vs. Cas + P; ^f*P* < 0.05 vs. Cas + P.

in failing hearts (19). We should point out that testosterone has more pronounced adverse effects in females when circulating estrogen levels are reduced due to ovariectomy, which may indicate that endogenous estrogen somehow opposes the detrimental effects of testosterone.

Effects of estrogen and testosterone on SBP. The present study also showed that 1) MI significantly decreased SBP in normal females but not in normal males, 2) testosterone tended to increase SBP in females with MI; and 3) reduction of testosterone levels by castration significantly reduced SBP in males, while supplemental estrogen reduced it further. As discussed previously, estrogen may exert part of its cardioprotective effect by causing vasorelaxation (via the release of NO), lowering BP, and reducing cardiac work after MI. Conversely, testosterone may have vasoconstrictor properties, because testosterone impaired porcine coronary artery relaxation *in vitro* (50); male spontaneously hypertensive rats had higher BP than females of the same age, and castration decreased BP in males, whereas testosterone increased BP in females (44). Even though cardiac output was not measured at the same time as BP, we cannot disregard the possibility that the differential effects of sexual hormones on cardiac function seen in our study were due to decreased peripheral resistance. We measured SBP only once during the chronic stage after MI (12 wk), and only females exhibited a significant decrease in SBP after MI. To the best of our knowledge, this is the first study comparing SBP after MI in female and male mice; thus we do not have any precedent. However, there is some evidence that acute ischemia induced by coronary artery occlusion decreases arterial blood pressure more significantly in females than in males; this was probably due to the effects of circulating estrogen on autonomic function, because vagotomy abolished the decrease in blood pressure (2, 14).

Other possible mechanisms. There are other mechanisms that may help explain the observed estrogen-induced cardioprotection and the detrimental effects of testosterone. For example, sexual hormones interact with other neurohormones such as angiotensins and endothelin and indirectly influence myocardial adaptation and function after MI. Estrogen has been shown to decrease angiotensin II and increase angiotensin (1–7)

in plasma of hypertensive rats (9), downregulate expression of the angiotensin II type 1 receptor (AT₁) (38), and inhibit endothelin-1 synthesis (13, 27, 54), whereas testosterone stimulates AT₁ expression (29) and increases circulating levels of angiotensin-converting enzyme (30).

Unexpected findings. First, MI per se seemed to reduce both estrogen and testosterone levels after MI in both genders; however, only testosterone in females and estrogen in males reached statistical significance. It could be that 1) as reported in humans with severe heart failure (46), plasma volume increases with progression of disease, and as plasma volume expands blood components are diluted; because mice have such a small volume of blood, a small change in plasma volume could make a big difference in terms of concentration of its components; and/or 2) decreased cardiac performance due to heart failure may lead to decreased organ perfusion and impaired organ function, including the glands that release sexual hormones, so that levels of sexual hormones decrease. However, we do not have any evidence to support these speculations in mice with MI. Second, it is known that estradiol can be synthesized from testosterone by aromatase, although we did not observe a great increase in estrogen levels with testosterone supplementation in the present study. On the contrary, we found that estrogen supplementation significantly increased testosterone levels in castrated males; however, we found no reported mechanisms by which estradiol could be converted to testosterone, or any document showing testosterone levels during estrogen supplementation in castrated animals. Therefore, we have no precedent to support our observation regarding this point. Third, we did not observe further improvement of LV function and remodeling and reduction of hypertrophy in castrated mice that received estrogen compared with placebo as we expected. The possible explanations could be: 1) although infarct size was similar within gender in most groups, it was significantly larger in MI + Cas + E compared with MI + Cas + P, and a larger infarct leaves less myocardium to recover and compensate after injury; 2) the infarct was too large (30–40% of the LV) and the injury too severe, so that the compensatory capacity of the residual noninfarcted myocardium in response to castration and/or estrogen supplementation

had reached its limit and no further functional and/or histopathological difference could be detected; and 3) plasma levels of testosterone in castrated mice that received estrogen were similar to those in noncastrated mice, so that testosterone may have opposed the beneficial effect of estrogen.

Limitations of the present study. First, although physiological hormone levels of these mice were comparable to those found in rats (45), plasma estrogen and testosterone in males and females that received hormone supplementation were much higher than physiological levels; thus one should be cautious when interpreting these results, because they may largely reflect pharmacological significance of sexual hormone manipulation. Testosterone levels found in supplemented mice resembled those found in cases of androgen abuse, where they can increase six to eightfold (6). Second, infarct size was significantly larger in MI + Cas + E compared with MI + Cas + P, and this difference may explain why estrogen did not show further cardioprotection in castrated mice as we expected.

Conclusions. Estrogen (either endogenous or supplemental) prevents maladaptive chronic remodeling and further deterioration of cardiac performance, whereas testosterone (either endogenous or supplemental) adversely affects myocardial healing (as indicated by increased cardiac rupture), degrades cardiac dysfunction and remodeling, and exerts pronounced effects when estrogen levels are reduced. We believe this is the first study to show that estrogen and testosterone play different and opposing roles in the development of heart failure and long-term remodeling after MI in mice.

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