Gender-related differences in myocardial inflammatory and contractile responses to major burn trauma

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Horton, Jureta W., D. Jean White, and David L. Maass. Gender-related differences in myocardial inflammatory and contractile responses to major burn trauma. Am J Physiol Heart Circ Physiol 286: H202–H213, 2004. First published September 18, 2003; 10.1152/ajpheart.00706.2003.—Gender-related differences in immune responses to hemorrhage and sepsis have been associated with hormonal fluctuation in proestrus/estrus females. In the present study, male and female (either diestrus or proestrus/estrus) Sprague-Dawley rats (250–325 g) were given a third-degree scald burn over 40% total body surface area and fluid resuscitated (4 ml/kg per %burn of lactated Ringer solution); sham burn males and diestrus as well as sham burn proestrus/estrus female rats were included to provide controls. Twenty-four hours postburn, hearts were either perfused to examine mechanical function (Langendorff, n = 8 to 9 hearts/group) or to prepare cardiomyocytes (collagenase digestion, n = 4 to 5 hearts/group). Left ventricular developed pressure and the positive and negative first derivative of left ventricular pressure responses to increases in preload were significantly lower in burned males compared with responses measured in either burned proestrus/estrus or burned diestrus females; burn trauma increased cardiomyocyte secretion of tumor necrosis factor-α, interleukin-1β, and nitric oxide to a lesser extent in proestrus/estrus females than levels secreted by either diestrus females or males. Similarly, myocytes from proestrus/estrus females accumulated significantly less sodium/calcium compared with values measured in males (P < 0.05). Our data confirm gender-related differences in myocardial function and myocardial inflammatory responses to burn injury.

MAJOR BURN INJURY PRODUCES substantial hemodynamic and cardiodynamic derangements, which contribute to the development of sepsis, multiple organ failure, and death (26, 30, 36, 48). The accumulation of isotonic fluid in both the burn tissue as well as nonburn tissue produces a fall in venous return and an initial decrease in cardiac output and stroke volume; aggressive fluid resuscitation from burn injury restores circulating volume, increasing venous return and cardiac output (11, 17). However, numerous experimental and clinical studies have shown that, despite aggressive fluid resuscitation and maintenance of pulmonary capillary wedge pressure, stroke work and ejection fraction often decrease after major burn trauma; the burn-related compromise in cardiac function is paralleled by a rise in serum troponin levels, providing evidence of burn-related myocardial injury (17, 29, 31).

Recent studies have suggested that gender is another important determinant of outcome in patients with traumatic injury and sepsis. Although age and the female gender have been associated with a worse prognosis in acute myocardial infarction (28), other studies have described a better survival rate for women with sepsis (15, 34, 38, 39, 46). The mechanisms of gender-related differences in outcome in injury and disease remain unclear, but sexual dimorphism in pro- and anti-inflammatory responses to injury have been implicated. In this regard, Schroeder and colleagues (38) suggested that the significantly improved prognoses for women with sepsis compared with men correlated with significantly lower TNF-α bioactivity and increased levels of IL-10 in women compared with levels measured in men. Similarly, Oberholzer and colleagues (34) described higher plasma IL-6 levels in severely injured males compared with levels measured in females with a similar injury severity score (ISS ≥ 25 points) during the early posttrauma period. Balteskiard and colleagues (9) described lower thromboxane B2 and TNF-α levels in young women compared with young male trauma patients, and differences in inflammatory cytokine profiles were minimized with increasing age, particularly with the onset of menopause. Collectively, these data suggest that gender-related differences in outcome after trauma and sepsis are related to hormonal status.

The role of sex hormones in determining morbidity and mortality after trauma and sepsis has received considerable attention, and several clinical studies have suggested that the rise in circulating estrogen levels and fall in testosterone levels in trauma subjects are evidence of compensatory mechanisms directed to provide protection against trauma-related morbidity/mortality (6). Testosterone depletion or blockade (flutamide) has been shown to provide considerable organ protection and to improve outcome in models of injury and sepsis. In this regard, Angele and colleagues (7) showed that testosterone receptor blockade after hemorrhage in male animals ablated immune depression and improved survival after a subsequent septic challenge, whereas Remmers and colleagues (35) described that testosterone receptor blockade with flutamide restored organ blood flow and improved tissue oxygen consumption in male rats subjected to trauma and hemorrhage. The negative effects of a rise in circulating testosterone levels have also been linked to increased thromboxane A2 receptor density in the aorta (25), inhibition of prostacyclin production by smooth muscle cells (32), and increased vasoconstriction and impaired peripheral perfusion (25, 32). The role of sex hormones in the outcome after trauma and sepsis has been further...
supported by studies that showed that either estrogen or progesterone administration improved cardiovascular function and altered macrophage cytokine release after trauma hemorrhage (3, 5, 14). Similarly, Altura and colleagues (1–3) showed that estradiol treatment of males subjected to lethal intestinal ischemia or whole body trauma prevented the reticuloendothelial system phagocytic depression that was shown to occur in untreated males subjected to these injuries; the role of estrogen in peripheral vascular function has been supported by studies from this group showing that treatment of rats with estrogen selectively enhanced vasoconstrictor responses to neurohypo-
hyalse hormones and catecholamines.

Despite a growing body of evidence suggesting gender-related differences in surgical intensive care patients, patients with myocardial infarction, and patients with trauma and sepsis, there is less information describing the effects of gender on organ function and outcome after major burn trauma. Barrow and Herndon (10) described that mortality was significantly higher in male children with severe thermal injury compared with female children with a similar percent total body surface area (TBSA) burn. In contrast, Tobiasen et al. (42) and O’Keefe and colleagues (33) described that the mortality risk was significantly increased in women 30–59 yr of age compared with men of similar age and percent TBSA burn.

Our previous experimental and clinical studies have confirmed burn-related myocardial injury and myocardial contractile dysfunction (8, 17, 37), and we have shown that postburn myocardial abnormalities correlated with myocardial inflammation (23). Therefore, the present study was designed to determine whether gender-related differences in myocardial inflammatory responses after burn injury are associated with sexual dimorphism in the myocardial contractile response to burn injury.

MATERIALS AND METHODS

Experimental model. Adult Sprague-Dawley male and female rats (300–350 g) were used in the present study. Animals obtained from Harlan Laboratories (Houston, TX) were conditioned in-house for 5–6 days after arrival with commercial rat chow and tap water available at will. All studies performed in this study were reviewed and approved by the University of Texas Southwestern Medical Center’s Institutional Review Board for the care and handling of laboratory animals and conformed to all guidelines for animal care as outlined by the American Physiological Society and the National Institutes of Health.

Identification of the estrous cycle. Vaginal smears were performed daily to profile the rat estrous cycle. Each day, between 9:00 and 10:00 AM, vaginal epithelial cells were obtained from each animal to pro-
dued the proestrus state based on the
ripped in 6 ml of enzymatic digestion solution containing a
neous leukocytes that appeared in addition to
ically swollen, corni
ed from the mark-
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10:00 AM, vaginal epithelial cells were obtained from each animal
le the rat estrous cycle. Each day, between 9:00 and
ncreased in women 30–59 yr of age com-
pared with men of similar age and percent TBSA burn.

Isolated coronary-perfused hearts. To isolate cardiomyocytes, rats
received an intraperitoneal injection of heparin (2,000 units) 20–30
min before death. The rats were decapitated, and hearts were har-
vested and placed in a petri dish containing room temperature heart
medium [113 mM NaCl, 4.7 mM KCl, 0.6 mM KH2PO4, 0.6 mM
Na2HPO4, 1.2 mM MgSO4, 12 mM NaHCO3, 10 mM KHCO3, 20
mM d-glucose, 0.5× BME amino acids (50×, GIBCO-BRL 11310-
051),10 mM HEPES, 30 mM taurine, 2.0 mM carnitine, and 2.0 mM
creatinine], which was bubbled constantly with 95% O2–5% CO2. Hearts were cannulated via the aorta and perfused with heart medium at a rate of 12 ml/min for a total of 5 min in a nonrecirculating mode. Enzymatic digestion was initiated by perfusing the heart with diges-
tion solution, which contained 34.5 ml of heart medium as described above plus 50 mg of collagenase II (Lot MOB3771, Worthington
4177), 50 mg BSA (fraction V, GIBCO-BRL 11018-025), 0.5 ml
trynpsin (2.5%, 10×, GIBCO-BRL 15090-046), 100 mM CaCl2, and
40 mM 2,3-butanediol monoxime (BDM). Enzymatic digestion was
accomplished by recirculating this solution through the heart at a flow
rate of 12 ml/min for 20 min. All solutions perfusing the heart were
maintained by 10.2±0.3°C. At the end of the
enzymatic digestion, the ventricles were removed and mechanically
dissociated in 6 ml of enzymatic digestion solution containing a
6-ml aliquot of 2× BDM-BSA solution (3 mg BSA, fraction V, to 150
ml of BDM stock, 40 mM). After mechanical dissociation with fine
forceps, the tissue homogenate was filtered through a mesh filter into

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a conical tube. The cells adhering to the filter were collected by washing with an additional 10-ml aliquot of 1× BDM-BSA solution (prepared by combining 100 ml of BDM stock, 40 mM; 100 ml of heart medium as described above; and 2 g of BSA, fraction V). Cells were then allowed to pellet in the conical tube for 10 min. The supernatant was removed, and the pellet was resuspended in 10 ml of 1× BDM-BSA. The cells were washed and pelleted further in BDM-BSA buffer with increasing increments of calcium (100, 200, and 500 μM) to a final concentration of 1,000 μM. After the final pelleting step, the supernatant was removed, and the pellet was resuspended in MEM [prepared by adding 10.8 g of 1× MEM (Sigma M-1018), 11.9 mM NaHCO₃, 10 M HEPES, and 10 ml penicillin-streptomycin (100×, GIBCO-BRL 1540-122) with 950 ml MilliQ water]; the total volume was adjusted to 1 liter. At the time of MEM preparation, the medium was bubbled with 95% O₂-5% CO₂ for 15 min and the pH was adjusted to 7.1 with 1 M NaOH. The solution was then filter sterilized and stored at 4°C until use. At the final concentration of calcium, the cardiomyocyte cell number was calculated and myocyte viability was determined (18).

Cytokine secretion by cardiomyocytes. Myocytes were pipetted into microtiter plates at 5 × 10³ cells/microtiter well (12-well cell culture cluster, Corning; Corning, NY) for 18 h (CO₂ incubator at 37°C). Supernatants were collected to measure respective-secreted TNF-α, IL-1β, IL-6, IL-10, and nitric oxide (NO) (rat ELISA, Endogen, Woburn, MA). We previously examined the contribution of contaminating cells (nonmyocytes) in our cardiomyocyte preparations using flow cytometry, cell staining (hematoxylin and eosin), and light microscopy. We confirmed that <2% of the total cell number in a myocyte preparation was noncardiomyocytes. Because our preparations are 98% cardiomyocytes, we concluded that the majority of the inflammatory cytokines measured in the cardiomyocyte supernatant was indeed cardiomyocyte derived (18).

Intracellular calcium and sodium measurements. Separate aliquots of cells were loaded with either fura-2 AM for 45 min or sodium-binding benzofurzain isophthalate (SBFI) for 1 h at room temperature in the dark. Myocytes were then suspended in 1.0 mM calcium-containing MEM and washed to remove extracellular dye; myocytes were placed on a glass slide on the stage of a Nikon inverted microscope. The microscope was interfaced with Groovey optics for epi-illumination, a triocular head, phase optics, a ×30 phase contrast objective, and a mechanical stage. The excitation illumination source (300-W compact xenon arc illuminator) was equipped with a power supply. In addition, this InCyt Im 2 Fluorescence Imaging System (Intracellular Imaging; Cincinnati, Ohio) included an imaging workstation and Intel Pentium Pro 200-MHz-based personal computer. The computer-controlled filter changer allowed alteration between the 340- and 380-nm excitation wavelengths. Images were captured by a monochrome charge-coupled device camera equipped with a television relay lens. InCyt Im2 Image software allowed measurement of intracellular calcium and sodium concentrations from the ratio of the two fluorescent signals generated from the two excitation wavelengths (340/380 nm); background was removed by the InCyt IM2 software. The calibration procedure included measurement of the fluorescence ratio with buffers containing different concentrations of either calcium or sodium. At each wavelength, the fluorescence emissions were collected for 1-min intervals, and the time between data collection was 1–2 min. Because quiescent or noncontracting myocytes were used in these studies, the calcium levels measured reflect diastolic levels.

Experimental groups. Adult male rats were randomly divided into two groups: sham burn (group 1) and burn (group 2). Sham burn males were given anesthesia, animal handling, and analgesic administration (group 1) that were identical to procedures described for the burned groups. In group 2, male rats given third-degree scald burn over 40% TBSA received fluid resuscitation and analgesics as described under Cather placement and burn procedures. Adult female rats were initially divided into proestrus/estrus and diestrus groups based on daily vaginal smears; proestrus/estrus and diestrus groups were then subdivided into sham burn (group 3, proestrus/estrus shams; and group 4, diestrus shams) and burned groups given scald burn injury, fluid resuscitation, and analgesics identical to those described under Cather Placement and Burn Procedures (group 5, proestrus/estrus burn; and group 6, diestrus burn). Twenty-four hours after burn injury (or sham burn), rats were killed and the hearts were harvested to examine left ventricular (LV) function (Langendorff, n = 7–8 rats/group) or to prepare cardiomyocytes (n = 5–7 rats/group) to examine myocyte inflammatory responses to burn injury.

Statistical analysis. All values are expressed as means ± SE. ANOVA was used to assess the overall difference among the groups for each of the variables. Levene’s test for equality of variance was used to suggest the multiple-comparison procedure to be used. If equality of variance among the groups was suggested, multiple-comparison procedures were performed (Bonferroni); if inequality of variance was suggested by Levene’s test, Tamhane multiple comparisons (which do not assume equal variance in each group) were performed. Probability values <0.05 were considered statistically significant (analysis was performed using SPSS, Windows, version 7.5.1).

RESULTS

Rats were included in the various experimental groups based on the identification of the estrous cycle on the morning of study. After burn injury, all animals moved freely about the cage and consumed food and water at will; there was no evidence of pain and no mortality over the 24-h postburn period in the present study, regardless of gender.

Table 1. Gender-related differences in hemodynamic-metabolic responses to burn injury

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Burn</th>
<th>Proestrus sham</th>
<th>Proestrus burn</th>
<th>Diestrus sham</th>
<th>Diestrus burn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>8</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>MAP mmHg</td>
<td>125 ± 4</td>
<td>94 ± 4*</td>
<td>147 ± 5†</td>
<td>139 ± 10†</td>
<td>147.6 ± 4.0†</td>
<td>107.5 ± 6.1†</td>
</tr>
<tr>
<td>HR beats/min</td>
<td>480 ± 22</td>
<td>433 ± 21</td>
<td>513 ± 10†</td>
<td>494 ± 15†</td>
<td>522 ± 8†</td>
<td>483 ± 10†</td>
</tr>
<tr>
<td>Paco₂, mmHg</td>
<td>29.2 ± 1.5</td>
<td>34.8 ± 2.2</td>
<td>29.3 ± 0.9</td>
<td>23.6 ± 1.4†</td>
<td>25.4 ± 2.1†</td>
<td>27.5 ± 1.4†</td>
</tr>
<tr>
<td>Pco₂, mmHg</td>
<td>112.9 ± 4.7</td>
<td>91.9 ± 4.0*</td>
<td>97.1 ± 4.6†</td>
<td>122.6 ± 2.8†</td>
<td>110.8 ± 3.6</td>
<td>114.8 ± 5.7†</td>
</tr>
<tr>
<td>Hct, %</td>
<td>37.4 ± 2.3</td>
<td>27.3 ± 5.1*</td>
<td>32.2 ± 2.6†</td>
<td>32.7 ± 0.7†</td>
<td>40.6 ± 1.1</td>
<td>32.5 ± 1.7†</td>
</tr>
<tr>
<td>O₂ saturation, %</td>
<td>97.9 ± 0.3</td>
<td>99.9 ± 0.1</td>
<td>99.1 ± 0.4</td>
<td>99.9 ± 0.2</td>
<td>100 ± 0.3</td>
<td>99.7 ± 0.5</td>
</tr>
<tr>
<td>Ca²⁺, mmol/l</td>
<td>1.1 ± 0.2</td>
<td>0.93 ± 0.07*</td>
<td>0.90 ± 0.04†</td>
<td>0.71 ± 0.02†</td>
<td>0.89 ± 0.05†</td>
<td>0.74 ± 0.04†</td>
</tr>
<tr>
<td>Na⁺, mmol/l</td>
<td>141.2 ± 9.6</td>
<td>137.8 ± 1.4*</td>
<td>145.8 ± 8.6</td>
<td>142.5 ± 1.1*</td>
<td>142.8 ± 0.9</td>
<td>142.0 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. MAP, mean arterial pressure; HR, heart rate; Paco₂, arterial P CO₂; Hct, hematocrit; Ca²⁺, serum ionized calcium; Na⁺, serum ionized sodium. †Statistical difference from the respective sham burn at P < 0.05 (Student’s t-test); ‡significant difference from male (sham vs. sham, burn vs. burn).
Hemodynamic-metabolic responses to burn injury. Compared with values measured in time-matched shams, mean arterial blood pressure was significantly lower 24 h after burn injury regardless of gender and despite aggressive fluid resuscitation (Table 1). Hematocrit and serum-ionized calcium levels were significantly lower 24 h after burn injury compared with values measured in burned males (P < 0.05) in both groups of burned females compared with values measured in burned females (P < 0.05) regardless of gender and despite aggressive fluid resuscitation (Table 1). Metabolic acidosis occurred after burn injury in all animals as indicated by the rise in whole blood lactate and the change in base excess (Fig. 1). Arterial lactate levels measured 24 h postburn were significantly lower (P < 0.05) in both groups of burned females compared with values measured in burned males. Serum cytokines measured 24 h after burn injury (or sham burn) are summarized in Fig. 2. These data confirm that all experimental animals responded to burn injury with a significant rise in serum TNF-α, IL-1β, IL-6 and IL-10 levels; however, these responses were significantly less in females compared with values measured in burned males (P < 0.05).

Myocardial contractile function. Hearts harvested 24 h after burn injury were perfused (Langendorff) to examine in vitro ventricular performance; Table 2 summarizes the cardiodynamic data collected after a 30-min stabilization period where hearts were perfused at a constant end-diastolic volume, constant heart rate, and constant coronary flow rate. These results were generated before implementation of incremental increases in LV volume to examine Starling relationships and before inotropic challenge (increases in perfusate Ca²⁺). There were no significant differences in cardiodynamic variables measured in the sham burn groups regardless of gender or regardless of the time of the estrous cycle. Burn trauma produced significant myocardial contractile defects as indicated by a significantly lower LV developed pressure (LVP) and positive and negative maximum first derivative of LVP (±dP/dₜ max) compared with values measured in the respective sham burn group. In addition, burn injury in males produced LVP and ±dP/dₜ max values that were significantly lower (P < 0.05) than values measured in burned females (P < 0.05) despite an identical percent body surface area burn in all animals (Table 2).

Myocardial contraction and relaxation deficits in burned males were greater as indicated by the downward and rightward shift of LV function curves (LVP and ±dP/dₜ responses to incremental increases in LV volume or preload) compared with function curves calculated for all other experimental groups (Fig. 3A). While burn injury produced cardiac contractile defects in both proestrus/estrus and diestrus females, contraction and relaxation deficits were significantly less in females compared with those measured in burned males (P < 0.05, ANOVA and Student-Neuman-Keuls test), despite an identical burn injury and an identical regimen of fluid resuscitation.

Figure 3B shows the ventricular responsiveness to increases in perfusate calcium in all experimental groups; all burned animals retained the ability to respond to incremental increases in perfusate calcium with increases in LVP and ±dP/dₜ max. However, myocardial contraction and relaxation defects were obvious in all burn groups as indicated by the lower LVP and ±dP/dₜ values in burns compared with values generated by the respective sham burn groups. In addition, burn-related myocardial contractile defects were significantly (P < 0.05) greater in male burns compared with those documented in burned proestrus/estrus or burned diestrus females despite an identical degree of burn trauma and an identical regimen of fluid resuscitation in all burn groups (ANOVA and Student-Neuman-Keuls test).

Fig. 1. Effects of gender on acid-base balance after burn injury. All values are means ± SE. *Significant difference from the respective sham burn group at P < 0.05; ± significant difference in female burns compared with the burned male group at P < 0.05.
We also considered that gender-related alterations in LV compliance may have contributed to the differences in LVP and \( \pm \frac{dP}{dt} \) responses observed in males compared with diestrus and proestrus/estrus female burns. However, as shown in Fig. 4, LV compliance was similar in sham burn rats; similarly, there were no gender-related differences in LV compliance after burn injury.

Cardiomyocyte cytokine responses to burn injury. To determine whether gender-related differences in myocardial function correlated with postburn myocardial inflammation and
cardiomyocyte secretion of cytokines, additional groups of hearts were prepared from each of the experimental groups and perfused with collagenase-containing buffer, and cardiomyocytes were isolated. As shown in Fig. 5, burn injury promoted cardiomyocyte secretion of cytokines, additional groups of cardiomyocytes prepared from the respective sham burn groups 

DISCUSSION

This present study is the first, to our knowledge, to examine gender-related differences in either postburn myocardial inflammation or myocardial contractile responses to burn. In the present study, cardiomyocytes prepared from male rats secreted significantly greater levels of cytokines than cytokine levels secreted by cardiomyocytes prepared from proestrus/estrus females; in contrast, cardiomyocyte cytokine secretion in diestrus female burns was similar to that measured in male burns. This sexual dimorphism documented in a rat model of burn injury is consistent with a report by Schroeder and colleagues (38), who described that the higher mortality in males patients with surgical sepsis (70%) correlated with the continuous increase in TNF bioactivity after the diagnosis of sepsis. In that previous study (38), the lower mortality rate (26%) in females who had ISS scores that were nearly identical to those measured in males correlated with the higher IL-10 levels. In the present study, female rats had lower IL-10 levels compared with those measured in males with identical percent body surface area burn. Although previous studies have suggested that the better outcome for females in sepsis, trauma-hemorrhage, or bacteremic infection after trauma was related to a predominantly anti-inflammatory response in the female (20, 39, 46), we attributed the lower IL-10 levels in female rats to a predominantly anti-inflammatory response to a second insult, additional in vitro studies examined TNF-α responses to lipopolysaccharide (LPS) challenge (either 0, 10, or 25 mg LPS per 5 × 10⁴ cardiomyocytes; Fig. 6). All cardiomyocytes prepared from either sham or burn animals responded to LPS challenge with a dose-dependent increase in TNF-α secretion. However, TNF-α secretion by myocytes prepared from burned animals was significantly greater than cytokine levels secreted by cardiomyocytes prepared from the respective sham groups 

male burns secreted significantly greater levels of TNF-α compared with cytokine levels secreted by myocytes prepared from proestrus/estrus females, whereas myocytes prepared from diestrus female burns had TNF-α responses to LPS challenge that closely resembled those documented in male burns.

Cardiomyocyte cation responses to burn injury. Cardiomyocytes prepared from sham burn groups had similar intracellular sodium and calcium levels, regardless of gender. Burn injury produced significant myocyte sodium and calcium accumulation compared with sodium/calcium levels measured in cardiomyocytes prepared from the respective sham burn groups 

Table 2. Cardiac stabilization data generated by perfusing hearts for 30 min at a constant LVEDP and constant HR

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Burn</th>
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<tbody>
<tr>
<td>LVP, mmHg</td>
<td>96 ± 4</td>
<td>58 ± 4*</td>
</tr>
<tr>
<td>–dP/dtmax, mmHg/s</td>
<td>2,085 ± 99</td>
<td>1,090 ± 57*</td>
</tr>
<tr>
<td>–dP/dtmax, mmHg/s</td>
<td>1,794 ± 103</td>
<td>850 ± 68*</td>
</tr>
<tr>
<td>dPmax, mmHg/s</td>
<td>1,856 ± 100</td>
<td>882 ± 54*</td>
</tr>
<tr>
<td>TFP, ms</td>
<td>87.5 ± 2.1</td>
<td>97.5 ± 4.3*</td>
</tr>
<tr>
<td>Time to 90% relaxation, ms</td>
<td>89.0 ± 4.1</td>
<td>92.4 ± 4.3</td>
</tr>
<tr>
<td>Time to max dP/dtmax, ms</td>
<td>54.8 ± 1.2</td>
<td>56.0 ± 1.0</td>
</tr>
<tr>
<td>Time to max –dP/dtmax, ms</td>
<td>58.3 ± 1.5</td>
<td>55.7 ± 1.7</td>
</tr>
<tr>
<td>CPP, mmHg</td>
<td>53.8 ± 2.3</td>
<td>51.5 ± 4.2</td>
</tr>
<tr>
<td>CVR, mmHg</td>
<td>10.8 ± 0.5</td>
<td>10.3 ± 0.9</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>261 ± 9</td>
<td>264 ± 8</td>
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</table>

Values are means ± SE; n, no. of rats. LVEDP, left ventricular (LV) end-diastolic pressure; LVP, LV pressure; –dP/dtmax, maximum first derivative of LVP; dPmax, developed pressure at 40 mmHg; TFP, time to peak pressure; CPP, coronary perfusion pressure; CVR, coronary vascular resistance. *Significant difference in burn compared with the respective sham groups (sham male vs. burn male, etc.) at P < 0.05; †significant difference from male at P < 0.05.

Our finding that diestrus female burns had myocardial cytokine profiles resembling those documented in male burns is consistent with a previous report showing that the improved outcome in female trauma and septic patients appeared to be age related, and this improved outcome was lost with the onset of menopause. In this regard, McLauchlan and colleagues (27) showed that mortality was higher in female patients with abdominal sepsis and a mean age of 66 yr compared with males.
patients of a comparable age range and comparable sepsis. Similarly, Watanakunakorn (44) described that the female gender was associated with a higher mortality rate in a group of patients age 60 yr or older who were diagnosed with *Staphylococcus aureus* endocarditis. Our finding that myocardial cytokine responses to burn injury were significantly different in proestrus/estrus males and diestrus females is also consistent with a report by Krzych et al. (21), who described altered immune responses in different phases of the estrous cycle, and a report by Cannon and Dinarello (13), who described that plasma IL-1 activity was increased in women after ovulation. We also considered that the greater myocyte inflammatory cytokine secretion in male rats was related to endotoxin contamination of the collagenase used to digest hearts and to prepare myocytes; alternatively, we considered that myocytes from diestrus females and male rats are more sensitive to

Fig. 3. A: burn trauma impaired ventricular performance in all groups regardless of gender or regardless of the estrous phase. Contractile dysfunction was evident from the downward shift of the left ventricular (LV) function curves from function curves calculated for the respective sham burn group. Function curves for males burns were shifted downward and to the right of function curves calculated for either proestrus/estrus burns or diestrus burns, indicating greater postburn myocardial abnormalities in the male rats. LVP. LV developed pressure. B: effects of increasing perfusate calcium on ventricular performance. Ventricular function was examined as perfusate calcium concentration was incrementally increased from 0.5 to 2.5 in all experimental groups. LVP and positive and negative maximum first derivatives of LVP were significantly lower in burn males as perfusate calcium was increased compared with values measured in either proestrus/estrus burns or diestrus burns ($P < 0.05$). All values are means ± SE. *Significant difference from the respective sham burn group at $P < 0.05$. 

LVP, LV developed pressure. LVP and positive and negative maximum first derivatives of LVP were significantly lower in burn males as perfusate calcium was increased compared with values measured in either proestrus/estrus burns or diestrus burns ($P < 0.05$). All values are means ± SE. *Significant difference from the respective sham burn group at $P < 0.05$. 

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minuscule amounts of LPS, which may have contaminated our collagenase preparation. In the present study, the collagenase used to digest cardiomyocytes was tested and shown to be endotoxin free. However, our data showed that a second challenge of cardiomyocytes (prepared from all experimental groups) with LPS confirmed a greater sensitivity to LPS challenge in cardiomyocytes prepared from either diestrus females or males as indicated by the higher TNF-α levels secreted by cardiomyocytes from these groups and compared with TNF-α levels secreted by myocytes prepared from proestrus/estrous females challenged with an identical dose of LPS (25 μg LPS for 18 h). In contrast, lower dose LPS challenge (10 μg for 18 h) produced nearly identical levels of TNF-α secretion by myocytes regardless of gender or hormonal status.

Our finding that proestrus/estrous females had less myocardial contractile dysfunction compared with function measured in diestrus females is consistent with previous reports showing that hormonal status plays a significant role in injury and disease (4, 6, 7, 24, 34, 35, 46, 47). That estrogen modulates numerous injury- and disease-related responses has been supported by the finding that administration of low levels of estrogen in males improved peripheral vasoconstrictor responses to catecholamines (1, 2) and improved immune function and outcome in models of trauma-hemorrhage or polymicrobial sepsis. Similarly, administration of testosterone receptor blockade or castration have been associated with improved organ blood flow, improved tissue oxygen consumption, and improved cardiac and hepatic function in males subjected to trauma-hemorrhage (35).

In the present study, burn-related myocardial inflammation, indicated by the increased cardiomyocyte secretion of TNF-α, IL-1β, IL-6, and NO, is consistent with numerous reports describing cardiomyocyte secretion of inflammatory cytokines in response to a variety of insults including burn trauma, ischemia-reperfusion, and sepsis (18, 19, 23, 45). The role of inflammation in myocardial dysfunction has been further supported by the finding that patients with heart failure have increased circulating levels of IL-1 and TNF-α, and the extent to which concentrations of these inflammatory mediators were elevated correlated with greater myocardial dysfunction (22, 43). An additional observation linking myocardial inflammation with myocardial contractile abnormalities is the finding that acute infection and inflammatory disease precipitate transient heart failure (40), as well as the finding that the addition of TNF-α to LV muscle preparations or to cardiomyocyte cultures produce contractile defects as well as cardiac myocyte apoptosis (19). In addition, transgenic mice overexpressing TNF-α exclusively in the myocardium have myocardial TNF-α levels sufficient to cause dilated cardiomyopathy and severe congestive heart failure (12). Finally, anti-TNF strategies have been shown to provide significant cardioprotection in injury models such as burn and sepsis (16, 19). However, in the present study, it was surprising that ventricular function in diestrus burned females was significantly better than function measured in males despite the fact that proinflammatory cytokine profiles in diestrus female burns closely resembled those of male burns. The disparity between cardiomyocyte secretion of inflammatory cytokines and ventricular dysfunction in diestrus female burns suggests that burn-related myocardial abnormalities are not related solely to myocardial proinflammatory cytokine responses to burn injury, and other mediators play a role. In this regard, we considered that gender-related differences in cardiomyocyte sodium/calcium homeostasis may have played a role in cardiac performance, and measures of cardiomyocyte sodium/calcium accumulation provide another index of cardiomyocyte integrity and cellular function.

**Fig. 4.** There were no gender-related differences in LV compliance in hearts from sham burn rats (A); similarly, LV compliance was similar in all burned rats regardless of gender (B). All values are means ± SE.
However, burn injury promoted significantly greater myocyte calcium/sodium accumulation in males compared with values measured in female burns, and there were no differences in sodium/calcium levels in diestrus compared with proestrus females.

In the present study, myocardial inflammation and function were examined 24 h after burn injury; this time was selected based on our previous time-course studies confirming that myocardial inflammatory cytokine responses are maximal and myocardial dysfunction reaches a nadir at this time (23).

Fig. 5. Effects of gender on myocardial proinflammatory cytokine responses to burn trauma. Burn trauma promoted a significant increase in cardiomyocyte TNF-α (A), IL-1β (B), and nitric oxide (C) compared with the respective sham burn groups. However, male burns secreted significantly more inflammatory mediators than levels secreted by cardiomyocytes prepared from proestrus/estrus females ($P < 0.05$). In contrast, diestrus female burns had cardiomyocyte cytokine levels similar to those measured in male burns. Cardiomyocyte anti-inflammatory cytokine profiles paralleled the proinflammatory responses [IL-6 (D) and IL-10 (E)]. All values are means ± SE. *Significant difference from the respective sham burn group at $P < 0.05$; +gender-related differences among burns (males vs. females).
Although we did not examine mortality in this present study, we have shown that mortality over 8 days after burn injury over 40% TBSA in male rats was <10% if fluid resuscitation was based on clinical formulas (4 ml/kg per %burn).

In summary, male rats as well as diestrus and proestrus/estrus females were given a third-degree scald burn over 40% TBSA and fluid resuscitated by a standard clinical protocol. LVP and ±dP/dt responses to increases in preload were significantly lower in burn males compared with responses measured in burn proestrus/estrus females. Burn diestrus females had measures of contraction and relaxation that were significantly better than those measured in burn males but were less than those measured in burn proestrus/estrus females. Similarly, myocytes from proestrus/estrus females secreted significantly more TNF-α than myocytes from time-matched shams. All values are means ± SE.

*Significant difference from the respective sham burn group at P < 0.05; +gender-related differences among burns.

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Fig. 6. TNF-α responses to an in vitro lipopolysaccharide (LPS) challenge in cardiomyocytes prepared from males (top), diestrus females (middle), and proestrus females (bottom). Myocytes from each experimental group (both sham and burn) were plated (5 × 10^6 cells/microtiter well) and incubated for 18 h in the presence or absence of LPS; TNF-α was measured in the supernatant by ELISA. Myocytes prepared from burns secreted more TNF-α than myocytes from time-matched shams. All values are means ± SE.

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Fig. 7. Effects of burn injury and gender on cardiomyocyte calcium (top) and sodium (bottom) accumulation measured in all experimental groups. [Ca^{2+}], intracellular Ca^{2+} concentration; [Na^{+}], intracellular Na^{+} concentration. All values are means ± SE. *Significant difference from the respective sham burn group at P < 0.05; +gender-related differences among burns.
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REFERENCES


