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The intrinsic resistance of female hearts to an ischemic insult is abrogated in primary cardiac hypertrophy

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Bell JR, Porrello ER, Huggins CE, Harrap SB, Delbridge LM. The intrinsic resistance of female hearts to an ischemic insult is abrogated in primary cardiac hypertrophy. Am J Physiol Heart Circ Physiol 294: H1514–H1522, 2008. First published February 1, 2008; doi:10.1152/ajpheart.01283.2007.—Important sex differences in cardiovascular disease outcomes exist, including conditions of hypertrophic cardiomyopathy and cardiac ischemia. Studies of sex differences in the extent to which load-independent (primary) hypertrophy modulates the response to ischemia-reperfusion (I/R) damage have not been characterized. We have previously described a model of primary genetic cardiac hypertrophy, the hypertrophic heart rat (HHR). In this study the sex differences in HHR cardiac function and responses to I/R [compared to control normal heart rat (NHR)] were investigated ex vivo. The ventricular weight index was markedly increased in HHR (29.0 ± 6.3%), a resistance to postischemic dysfunction not evident in NHR hearts (decreased functional recovery, and increased lactate dehydrogenase efflux). Female NHR hearts exhibited a significantly greater recovery (dP/dmax) post-I/R relative to male NHR (95.0 ± 12.2% vs. 60.5 ± 9.4%, a resistance to postischemic dysfunction not evident in female HHR (29.0 ± 5.6% vs. 25.9 ± 6.3%). Ventricular fibrillation was suppressed, and expression levels of Akt and ERK1/2 were selectively elevated in female NHR hearts. Thus the occurrence of load-independent primary cardiac hypertrophy undermines the intrinsic resistance of female hearts to I/R insult, with the observed abrogation of endogenous cardioprotective signaling pathways consistent with a potential mechanistic role in this loss of protection. Sex differences; intracellular signaling; cardiac function; ischemia-reperfusion

IMPORTANT DIFFERENCES EXIST between men and women with respect to cardiovascular disease, and much of this differential is cardiac specific. There is a growing awareness of the extent to which sex differences and sex hormones can influence both cardiac function and the outcomes of cardiovascular diseases, including hypertrophic cardiomyopathy and cardiac ischemia (24). Epidemiological evidence has supported the view that in women, estrogen affords a level of cardiovascular protection. However, clinical trials relating to the use of estrogen in hormone replacement therapy indicate that it may actually have a deleterious effect (37). Androgenic influence is generally considered to be a cardiovascular liability in men, although recent reports of the use of testosterone supplementation as a treatment to improve function in heart failure (26, 36) suggest that androgens can be of cardiac benefit. A greater understanding of the role of sex differences in cardiac pathophysiology is required.

Cardiac hypertrophy is a major independent risk factor predictive of cardiovascular disease and death (25). Sex differences in the characteristics of left ventricular hypertrophic modeling and in ischemia-related arrhythmogenesis are evident (17, 31, 45). In men, ischemic heart disease incidence is high compared with that in premenopausal women, with this difference diminishing at postmenopause. In younger women, the postinfarction mortality is greater than in men of similar age, yet paradoxically, the salvage of ischemic myocardium is enhanced after reperfusion compared with men (30, 40). The differential predisposition to functional cardiac impairment between men and women when hypertrophy and ischemia coincide is not yet understood.

Experimental investigations of sex differences in ischemia-reperfusion (I/R) injury have produced conflicting results. In Langendorff-perfused hearts, a number of studies have shown females to have improved postischemic functional recovery compared with males (2, 28, 39, 42, 43), with a loss of this protection in ovariectomized animals (32, 46), which is reversed with exogenous estradiol administration (46). In contrast, other reports show no sex differences in the extent of I/R injury (6, 10, 11, 13, 21), although some investigations using transgenic models have shown female protection can be revealed in hypercontractile states (10, 11, 16, 21, 33). Studies of animal models of load-induced cardiac hypertrophy have shown that the hypertrophied myocardium is more susceptible to I/R injury (1, 3). The manner in which load-independent (primary) hypertrophy modulates the response to I/R has not been characterized.

Studies of sex differences in the extent to which hypertrophy influences the vulnerability to I/R damage have been limited.
and equivocal, possibly reflecting the confounding contributions of loading in the models investigated (6, 13, 35, 38). The signaling pathways that mediate the cardiac hypertrophic response to pathological stimuli are complex, involving the activation of mitogen-activated protein kinases (MAPKs), including p38, c-Jun NH2-terminal kinases (JNKs), and extracellular signal-regulated kinases (ERKs) (20). Physiological growth of the heart has been linked to activation of the phosphatidylinositol 3-kinase (PI3K)-Akt/PKB signaling cascade (29). Differences in the I/R response have also been noted among these signaling cascades. Whereas ERK activation and PI3K/Akt signaling improve cardiac functional recovery after I/R (15, 23, 27), the role of JNK activation following I/R injury is context specific and can be associated with either myocardial dysfunction or cardioprotection (22). There is suggestive evidence that cardioprotection in the female is mediated via modulation of these signaling pathways, including MAPKs and the PI3K pathway (2, 43).

We have previously described a model of primary genetic cardiac hypertrophy, the hypertrophic heart rat (HHR). Relative to control strain normal heart rats (NHR), male HHRs exhibit marked cardiac and cardiomyocyte hypertrophy in the absence of pressure loading (19). In this study, using Langendorff ex vivo recording methods, we evaluated the sex differences in HHR basal cardiac function. The sex-specific responses to ischemic insult and induction of signaling pathways involved in protection from I/R injury were contrasted in this model of primary cardiac hypertrophy.

**MATERIALS AND METHODS**

**Experimental model.** Animals were obtained from an established colony of NHRs and HHRs, derived as reported (19). Briefly, these strains were derived from the F2 progeny of a cross between the Fischer 344 and the spontaneously hypertensive rat (SHR). Normotensive offspring were selected for either enlarged or normal heart size by echocardiography over successive generations to achieve genetic enrichment of Melbourne Animal Ethics Committee (AEC Project 06066). The project was approved by the University of Melbourne Animal Ethics Committee (AEC Project 06066).

**Isolated heart preparation.** Age-matched male and female NHRs and HHRs (12 wk) were maintained under identical conditions at the Biological Research Facility at the University of Melbourne, Australia. All animals were handled in the manner specified by the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). The project was approved by the University of Melbourne Animal Ethics Committee (AEC Project 06006).

**Immunoblotting.** Ventricles were homogenized in a HEPES-sucrose buffer (5 mM HEPES (pH 7.5), 250 mM sucrose, 0.2% sodium azide + protease inhibitors) at 4°C using an Ultra-Turrax tissue grinder (Crown Scientific, NSW, Australia). Samples were centrifuged at 3,000 g for 5 min (4°C), and the supernatant was reconstituted in sodium dodecylsulphate (SDS) sample buffer and heated at 95°C for 5 min. Sample protein concentrations were determined with a Bradford assay (595 nm) (Bio-Rad, NSW, Australia).

Equal amounts of protein were loaded onto 10% polyacrylamide gels and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using the Invitrogen XCell system (Invitrogen, VIC, Australia). Proteins were then transferred onto polyvinylidene difluoride (PVDF) membranes using the Invitrogen XCell II blotter (Invitrogen, VIC, Australia) at 30 V for 1 h. PVDF membranes were blocked with 5% skimmed milk (in Tris-buffered saline-0.1% Tween 20) for 3 h and incubated in primary antibodies overnight at 4°C. Membranes were subsequently probed with an anti-rabbit secondary antibody (Amersham ECL-HRP linked, GE Healthcare, NSW, Australia) for 1 h at room temperature and incubated in enhanced chemiluminescent reagent (Amersham ECL Plus, GE Healthcare) for 5 min. Protein bands were visualized with a Bio-Rad Chemi-XRS Imaging device, and band intensity was quantified using Quantity One imaging software (Bio-Rad).

Each membrane was initially probed with a phosphospecific antibody, the antibody subsequently stripped with Re-Blot Plus (Chemicon International, Millipore, NSW, Australia), and then reprobed with the relevant total antibody. Finally, membranes were stained with Ponceau S (Sigma-Aldrich, NSW, Australia) to quantitatively verify equal protein loading. Primary antibodies used in these studies included phospho- and total Akt (catalogue numbers: 9271 and 9272, respectively), phospho- and total ERK1/2 (9101 and 9102, respectively), and phospho- and total JNK (9251 and 9252, respectively), all purchased from Cell Signaling (Genesearch, QLD, Australia).

**Statistics.** Data are presented as means ± SE. Differences between groups were assessed using a two-way ANOVA with repeated measures where appropriate. For statistical analysis of incidence of ventricular arrhythmias, a Kruskal-Wallis test (nonparametric ANOVA) was performed with a post hoc Mann-Whitney multiple comparisons test. Differences were considered significant at P <
Table 1. Age, body weight, and heart weight of male and female NHR and HHR

<table>
<thead>
<tr>
<th></th>
<th>NHR Male</th>
<th>NHR Female</th>
<th>HHR Male</th>
<th>HHR Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, wk</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>12.1±0.0</td>
<td>12.1±0.0</td>
<td>12.1±0.1</td>
<td>12.1±0.1</td>
</tr>
<tr>
<td>Ventricular weight, g</td>
<td>258±7*</td>
<td>169±7*</td>
<td>251±8</td>
<td>159±8*</td>
</tr>
<tr>
<td>VVI, mg/g</td>
<td>4.62±0.07</td>
<td>4.80±0.10</td>
<td>5.76±0.22</td>
<td>7.82±0.49†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of rats. Ventricular weights are wet weights at the end of reperfusion. VVI, ventricular weight index (ventricular weight normalized to body weight). NHR, normal heart rat; HHR, hypertrophic heart rat. *P < 0.05, female vs. male; †P < 0.05, HHR vs. NHR.

0.05. All statistical calculations were performed using SPSS v. 13.0 (SPSS, Chicago, IL).

RESULTS

Animal and heart weights. NHRs and HHRs exhibited similar body masses with female rats significantly smaller than males in both strains (Table 1). HHR had increased ventricular wet weights compared with NHR controls in both males (22% increase in HHR hearts, P < 0.05) and females (53% increase in HHR hearts, P < 0.05) and hence a significantly increased ventricular weight index (VVI). This increase in VVI was accentuated in HHR females, although no significant interaction factor was detected in the two-way ANOVA (sex × strain) for either ventricular weight or VVI. Ventricles were rapidly dissected and weighed at the completion of the reperfusion protocol, and the values obtained incorporate variability reflecting this rapid excision method.

Basal left ventricular function in Langendorff-perfused hearts. Left ventricular function was measured in Langendorff-perfused NHR and HHR hearts after equilibration to examine basal cardiac function (Table 2). Sex-matched NHR and HHR hearts exhibited similar ex vivo function, indicating that the occurrence of primary hypertrophy did not confer a functional detriment under basal conditions in those hearts of young adults. Sex differences were observed in the basal kinetics of ventricular function, with females in both strains exhibiting reduced rates of ventricular pressure development and relaxation (dP/dt max and dP/dt min). Coronary flows were similar for all groups. The RPP, which provides an indirect measure of work performed, was also not different. Heart rate trend was lower in females, but no statistical difference was identified.

Functional recovery in I/R. To determine the impact of primary cardiac hypertrophy on perfused heart susceptibility to I/R injury, the recovery of left ventricular function (as a percentage of basal function at the end of the equilibration period) was measured throughout reperfusion. There was a considerably greater degree of cardiac dysfunction throughout reperfusion in HHR hearts compared with NHR controls in both males and females (Fig. 1), with functional recovery approximately doubled in HHR hearts compared with HHR hearts. The significantly depressed left ventricular developed pressure (Fig. 1A), RPP (Fig. 1B), and dP/dt max and dP/dt min (Fig. 1, C and D, respectively) indicate both systolic and diastolic dysfunction in HHR hearts during reperfusion. A modest reduction in coronary flow was observed in HHR (Fig. 1F). Heart rates (Fig. 1E) were not different when compared with NHR controls.

Sex differences in the extent of postischemic dysfunction were observed in NHRs, but not in HHRs. Female NHR hearts exhibited an enhanced postischemic recovery of dP/dt max (a reliable and systematically used measurement of ventricular function in both experimental and clinical settings) compared with strain-matched males throughout reperfusion (end-point percent recoveries, 95.0 ± 12.2% vs. 60.5 ± 9.4%, female vs. male). This resilience was absent in the female HHR (end-point percent recoveries, 29.0 ± 5.6% vs. 25.9 ± 6.3%, female vs. male).

Incidence of reperfusion-induced arrhythmias. The detrimental effect of hypertrophy on postischemic outcome in HHR hearts was reflected in the incidence of arrhythmias recorded during the initial 10 min of reperfusion. Figure 2 shows that the incidence of arrhythmias (percent ectopy) was significantly greater in HHRs compared with NHR controls. Further characterization of arrhythmia type occurring during this early phase of reperfusion (Table 3) indicated that incidence of ventricular tachycardia was increased in HHR. Furthermore, consistent with the higher level of postischemic function maintained by the female NHR hearts, ventricular fibrillation was not detected in female NHR hearts, in contrast to the prevalence of ventricular fibrillation in both male and female HHR, where no sex difference was observed.

LDH release in postischemic reperfusion. LDH release into the coronary effluent was measured as a marker of cell lysis and injury. A higher level of LDH was measured in the effluent collected during reperfusion (at 15- and 30-min time points) in HHR hearts compared with those of NHR controls (Fig. 3). No statistical differences in the coronary LDH content were detected between male and female NHRs or HHRs.

Protein analysis. Figure 4 shows total expression of Akt was significantly increased in female NHR hearts compared with males. This increase was not apparent in female HHR hearts, with both male and female HHR Akt expression levels closely

Table 2. Basal left ventricular function at the end of the stabilization period in male and female NHR and HHR

<table>
<thead>
<tr>
<th></th>
<th>NHR Male</th>
<th>NHR Female</th>
<th>HHR Male</th>
<th>HHR Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>113±6.2</td>
<td>138±8.4</td>
<td>128±8.1</td>
<td>124±5.9</td>
</tr>
<tr>
<td>RPP, mmHg•beats⁻¹•min⁻¹</td>
<td>25,407±804</td>
<td>24,191±1,410</td>
<td>24,904±2,411</td>
<td>20,176±3,051</td>
</tr>
<tr>
<td>dP/dt max, mmHg/s</td>
<td>4,258±152</td>
<td>4,036±171*</td>
<td>4,540±259</td>
<td>3,974±160*</td>
</tr>
<tr>
<td>dP/dt min, mmHg/s</td>
<td>−3,158±198</td>
<td>−3,522±78*</td>
<td>−3,368±320</td>
<td>−2,771±208*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>228±12.1</td>
<td>178±12.5</td>
<td>200±21.4</td>
<td>172±30.5</td>
</tr>
<tr>
<td>Coronary flow, ml•min⁻¹•g⁻¹</td>
<td>6.8±0.6</td>
<td>6.6±0.6</td>
<td>6.8±0.5</td>
<td>6.4±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of hearts. LVDP, left ventricular developed pressure; RPP, rate-pressure product (developed pressure × heart rate) dP/dt max and dP/dt min, maximum and minimum first derivative of pressure, respectively. *P < 0.05, female vs. male.
Fig. 1. Time course of left ventricular function in male and female normal heart rat (NHR) and hypertrophic heart rat (HHR) hearts. Left ventricular function was measured throughout equilibration (aerobic perfusion) and reperfusion. Data are normalized to the basal value at the end of the equilibration period (i.e., 30 min). Parameters measured included left ventricular developed pressure (LVDP; A), rate-pressure product (RPP; developed pressure × heart rate; B), maximum (dP/dt max; C) and minimum (dP/dt min; D) first derivative of pressure, heart rate (E), and coronary flow (F). Data are means ± SE; n = 8–10 hearts/group, analyzed by 2-way ANOVA with repeated measures (reperfusion only). †P < 0.05, HHR vs. NHR; #P < 0.05, strain × sex interaction.
matched to male NHR. Although the relative proportion of phosphorylated Akt at the end of reperfusion did not change between groups, the increased pool of Akt in the female NHR indicates a heightened total Akt activity in these hearts, which may be related to the protection afforded to the female NHR hearts in I/R. Similarly, a strain × sex interaction in total ERK1/2 expression was evident, with female NHR hearts expressing more ERK1/2 than in males in contrast to that seen in female and male HHR hearts (Fig. 5). Again, this is consistent with a sex-specific, protective role in I/R in female NHRs that is absent in the hypertrophied heart. No differences in the total expression of JNK or in the phosphorylated/total levels of JNK were detected (data not shown).

**DISCUSSION**

This study demonstrates significant sex-dependent differences in the ex vivo basal function and ischemic responses of hypertrophic hearts of normotensive animals. In the HHR model of primary cardiac hypertrophy, VWI was markedly increased in both males and females compared with that in nonhypertrophic control NHRs. Female hearts of both strains exhibited lower contractility under basal conditions compared with hearts of strain-matched males. The greater ischemic resilience observed in hearts of female NHRs (relative to male NHRs) was not evident in female HHR hearts (relative to male HHR hearts), although the extent of ischemic damage (assessed by LDH release) was similar for females and males of each strain. In female NHRs, cardiac expression levels of Akt and ERK were selectively elevated and ventricular fibrillation events in early reperfusion were selectively suppressed. Thus the occurrence of load-independent primary cardiac hypertrophy undermines the intrinsic resistance of female hearts to I/R insult, with the observed abrogation of endogenous cardioprotective signaling pathways consistent with a potential mechanistic role in this loss of protection.

**Female NHR and HHR hearts exhibit similar basal hypotrophy.** We have previously reported the development and establishment of the polygenic HHR strain produced by selective breeding from a second generation cross of F344 and SHR. The genetic control NHR strain was established in parallel, i.e., selecting normotensive animals with normal heart size. Our earlier studies characterized the concentric cardiac hypertrophy in adult male HHRs (19). This is the first report describing the phenotype in females, and it is particularly noteworthy that the female HHRs demonstrate a ventricular hypertrophy that is comparable with that seen in the male. This finding confirms the utility of the HHR as a disease model, with female phenotype and natural history consistent with the human disease state, appropriate for investigation of sexually dimorphic cardiac responses.

Female NHR and HHR hearts exhibit hypocontractility (reduced dP/dt indexes) relative to strain-matched males. Sex differences in basal cardiac contractility have been previously reported, and the usual (but not universal) finding of diminished contractility in females is consistent with the present finding (18, 34). There is evidence that in females, higher estrogen levels and reduced androgen levels both contribute to the lower inotropic state via mechanisms that alter cardiomyocyte Ca2+ flux during excitation-contraction coupling (12, 18, 34). In both strains, basal mechanical performance showed similar sex-dependent differences, indicating that in nonmetabolically/workload-challenged conditions, the hypertrophic hearts of both sexes are functionally well compensated.

**NHR females display intrinsic ischemic cardioprotection.** In the NHR, mechanical recovery postischemia was significantly enhanced in the female compared with the male. Some previous studies of nonhypertrophic Langendorff-perfused hearts have identified sex differences in susceptibility to I/R injury. Bae and Zhang (2) showed that young adult female rat hearts were protected from I/R injury compared with males, exhibiting increased functional recovery in reperfusion and reduced infarct size. This protection was mediated by PKCe and PI3K (2). Meldrum and colleagues (32, 42, 43) have shown that the functional recovery of female rat and mouse hearts is greater than for that in control males, with evidence suggesting a reduced inflammatory response and a modulation in the activity MAPKs.

**Table 3. Classification of arrhythmias in early reperfusion**

<table>
<thead>
<tr>
<th>Arrhythmia</th>
<th>NHR Male</th>
<th>NHR Female</th>
<th>HHR Male</th>
<th>HHR Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventricular premature beats, beats</td>
<td>144±40 (9)</td>
<td>88±33 (8)</td>
<td>131±27 (8)</td>
<td>146±34 (10)</td>
</tr>
<tr>
<td>Ventricular tachycardia, beats</td>
<td>635±196 (8)</td>
<td>226±177 (7)</td>
<td>1,772±690 (7)*</td>
<td>1,317±499 (8)*</td>
</tr>
<tr>
<td>Ventricular fibrillation, s</td>
<td>32±2 (2)</td>
<td>0 (0)†</td>
<td>70±48 (5)</td>
<td>155±86 (5)</td>
</tr>
</tbody>
</table>

Values are means ± SE (number of hearts exhibiting relevant arrhythmia is shown in parentheses); n, number of hearts. Arrhythmias were scored throughout the first 10 min of reperfusion. *P < 0.05, HHR vs. NHR; †P < 0.05, NHR female vs. HHR female and HHR male.

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Other investigators have, however, failed to detect a sex difference in the response to I/R. A series of studies by Murphy and colleagues (9–11, 21) found no sex disparity in the ischemic tolerance of male and female hearts under control conditions, although a sex difference in the I/R response was elicited in a hypercontractile environment. Indeed, an overexpression of cardiac-specific β2-adrenoceptors (β2-ARs) increased contractile function in both male and female mice yet improved posts ischemic recovery only in females (10). Similarly, isoproterenol-challenged female mouse hearts exhibited a significant improvement in both functional recovery and diminished size of infarct compared with isoproterenol-treated males (16). This protection was dependent on the presence of the β-isof orm of the estrogen receptor. The basis for these discrepancies is unclear; species/strain differences and the effects of genetic manipulations cannot be discounted. It is likely that age (and, indirectly, the state and prior duration of estrogenic priming) may play a role (44).

Hypertrophy impairs post-I/R recovery and undermines female cardioprotection. The finding that the intrinsic functional resistance to I/R in female NHR is virtually abolished in the female HHR is a novel and significant finding. Hypertrophic exacerbation of I/R damage has been previously observed in some experimental models where cardiac growth and function are modified by systemic-loading conditions. This study shows that primary hypertrophy occurring without hemodynamic compHlication impairs posts ischemic functional recovery. In the HHR (male and female), elevated levels of LDH and increased incidence of early reperfusion arrhythmia were observed, coincident with a significant strain-dependent functional deficit in posts ischemic recovery. These findings suggest that hypertrophy per se exacerbates oncotic damage in the ischemic heart.

This study provides evidence that hypertrophy undermines sex-dependent I/R tolerance in female hearts, demonstrated by the depressed contraction kinetics and occurrence of ventricular tachyarrhythmia in female HHR hearts (relative to female NHR hearts). The extent of posts ischemic dysfunction at the end of reperfusion showed a marked variation. Whereas male and female HHR recovery levels were at or beyond 25% of preischemic values, the recovery levels observed in NHR hearts were more robust (males, ~60%; and females, ~85%). This range of values indicates that 25 min was a sufficient ischemic duration to apply to all four groups, long enough to cause marked but not profound damage and appropriate to differentiate the recovery capacity of normal and hypertrophic hearts.

Differences in the susceptibility of female and male hypertrophic hearts to ischemic insult have been previously investigated (albeit not extensively), but there is no consistent finding. In the SHRs, female hearts were found to exhibit more robust functional recovery post-I/R than in male hearts (6), whereas female Dahl salt-sensitive rats were found to be more susceptible to ischemic injury (35). In both of these studies, there was no sex difference in the response of nonhypertrophic controls to I/R. Another investigation of aortic-banded animals (Sprague-Dawley) found that females and males showed similar recovery post-I/R, but surprisingly in this study, the cardiac function of male and female sham-operated animals was not different (38). These three animal models are characterized by hypertension (of varying degree), but the neurohumoral milieu and the cardiac morphological remodeling states differ and confound the interpretation of the sex-specific effects of hypertrophy per se on I/R response. In the Goto-Kakizaki rat, a model of Type 2 diabetes, aged females were found to be more susceptible to posts ischemic injury than males (13). These animals were normotensive; however, they exhibited profound endocrinologic and metabolic disturbances, and the influence of aging may have been important in determining this experimental finding.

The majority of studies investigating sex differences in animal models of I/R have used the Langendorff-perfused heart, enabling interventions to be assessed without the complicating factors associated with an intact systemic vasculature and neural control. The use of crystalloid perfusate and provi-
sion of glucose as sole energy substrate may limit the extent to which functional differences in performance of male and female hearts may be discriminated. Indeed, sex differences in the recovery of cardiac function in working-perfused hearts have been shown to be dependent on the presence of fatty acids in the perfusate (7). Thus the sex differences identified in this study reflect the specific experimental conditions employed and may be masked or enhanced under other conditions.

Mechanism of protection in females? In NHR female hearts, significant and selectively higher levels of the expression of total Akt and of ERK1/2 were observed. MAPK activation in I/R has been extensively studied. ERK1/2 has usually been identified to play a protective role, and p38 and JNK, a detrimental one, although the complex nature of the cross talk between these kinases suggests that this interpretation may be an oversimplification. Wang et al. (43) have recently shown increased phospho-ERK/total ERK expression in female mouse hearts exhibiting improved functional recovery from I/R (43). Both the increase in ERK phosphorylation and the increased recovery were abolished in female estrogen receptor-α receptor knockout mice, suggesting that ERK plays a prominent role in

Fig. 4. Akt expression in NHR and HHR hearts. Ventricular homogenates were subjected to SDS-PAGE and probed for phospho- (p) and total Akt (A). Mean band intensities for total Akt (B) and the ratio of phospho- to total Akt (C) are shown. Data are means ± SE; n = 8–10 hearts/group, analyzed by 2-way ANOVA. #P < 0.05 strain × sex interaction.

Fig. 5. ERK1/2 expression in NHR and HHR hearts. Ventricular homogenates were subjected to SDS-PAGE and probed for phospho- and total ERK1/2 (A). Mean band intensities for total ERK1/2 (B) and the ratio of phospho- to total ERK1/2 (C) are shown. Data are means ± SE; n = 8–10 hearts/group, analyzed by 2-way ANOVA. #P < 0.05 strain × sex interaction.
the estrogen receptor-mediated cardioprotection afforded to females. PI3K and its downstream target, Akt, have previously been shown to confer protection in I/R (5), and female mice exhibit a nuclear accumulation of phospho-Akt compared with males (8). Akt phosphorylation-induced inhibition of GSK-3β has also been shown to increase ischemic tolerance in hypertrophied hearts (3).

In this study, the increased total expression levels of Akt and ERK1/2 indicate the availability of a larger total pool of activated Akt and ERK1/2 in the female NHR myocardium and may constitute the signaling substrate/s for the protected status of these hearts (although the contribution of other endogenous cardioprotective substrates cannot be ruled out). No differences in the ratios of phospho-to-total expression in these kinases were observed, and this may be related to the use of a single time point of investigation at the end of reperfusion. Phosphorylation of MAPks in I/R has been previously shown to be a rapid and continuously changing process (4, 5, 14), such that 30 min of reperfusion may not necessarily be the optimal time point to detect a difference between phosphorylated and nonphosphorylated forms of these kinases in these experimental groups. No difference in the expression level or phosphorylation status of JNK was detected, suggesting that, in the female NHR, the resistance to I/R represents an enhanced level of protection rather than a reduction in damage signaling. Further work is required to dissect the temporal and sequential activation of intermediates of these signaling pathways to characterize the molecular basis of I/R resilience in the female NHR.

In summary, this study demonstrates that for relatively young adult females, where there is an underlying genetic predisposition for cardiac hypertrophy (and no coincident hemodynamic disturbance), ischemic resistance is significantly impaired, even in the context of maintained basal cardiac function. Our findings suggest that although both sexes are impaired, even in the context of maintained basal cardiac function and prevents injury after transient cardiac ischemia in vivo. The CARDIA study. Coronary Artery Risk Development in Young Adults. J Clin Invest 112: 57–64, 2003.

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