Glutathione peroxidase deficiency exacerbates ischemia-reperfusion injury in male but not female myocardium: insights into antioxidant compensatory mechanisms

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IT IS WELL-RECOGNIZED THAT premenopausal women are at a lower risk for ischemic heart disease than age-matched men, and some observational studies in humans and experimental studies in animals support a beneficial role for female sex hormones in this regard. While a direct antioxidant action of female sex hormones has been implicated as the basis for this protective effect, recent large-scale clinical trials failed to show a cardioprotective benefit for postmenopausal women on estrogen/progestin replacement therapy, underscored by the need for a better understanding of the mechanisms responsible for ischemic cardioprotection in females (24, 45).

Reactive oxygen species (ROS) are known to mediate ischemia-reperfusion (I/R) injury, where endogenous enzymatic and nonenzymatic antioxidant defenses are overwhelmed by excess ROS generation, resulting in redox imbalance and oxidative stress (5, 61). Central to the detoxification of ROS are the water-soluble antioxidants, reduced glutathione (GSH) and ascorbic acid (AA) (vitamin C), which are postulated to act as a first-line defense against myocardial I/R injury (22). AA directly scavenges ROS, whereas GSH protects protein thiols and scavenges ROS, primarily by serving as an essential co-substrate for the glutathione peroxidases (GPx), selenocysteine-containing enzymes that catalyze the reduction of H2O2 and lipid peroxides via oxidation of GSH (23). Together with glutathione reductase, which catalyzes the conversion of oxidized GSH back to its reduced form, the GPx are important in regulating the glutathione cycle. Another mechanism by which oxidative stress may promote injury is by diminishing nitric oxide (NO) bioactivity (27). Nitrite (NO2−) and nitrate (NO3−) are oxidative metabolites of NO, and their measurement in blood and tissues is frequently used to monitor endogenous NO production. NO2− has recently emerged as an important signaling molecule in NO biology, and recent studies have shown that exogenous NO2− supplementation confers cardioprotective benefits in the setting of I/R injury (8, 18, 55).

Previous studies have shown sex-specific differences in antioxidant capacities in various tissues, including the heart (1, 2, 12, 29, 47, 52, 58). In fact, several studies have reported lower levels of H2O2 and higher levels of GSH and GPx activity in female mitochondria, which is postulated to confer protection from ROS-mediated damage (6, 26).

The goal of the present study was to characterize the effect of sex on posts ischemic myocardial recovery and antioxidant status and examine how deficiency of a major antioxidant enzyme, GPx1, might affect these parameters. Our results demonstrate that male, but not female, mice lacking GPx1 are more susceptible to myocardial I/R injury, characterized by poor contractile recovery and loss of redox homeostasis. The ability of female sex to compensate for the loss of GPx1 enzyme activity, by enhanced nonenzymatic ascorbate homeostasis, along with enhanced preservation of tissue NO3− concentrations, may provide a mechanism by which female sex confers cardioprotection post-I/R.
MATERIALS AND METHODS

Animals. All animals used in the study were 4–5 mo old, and all procedures were approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine and conducted in accordance with the National Institutes of Health guidelines for animal care.

Murine echocardiography. Echocardiograms were obtained in conscious mice, as previously described (25). Heart rate, and end-diastolic and end-systolic left ventricular (LV) chamber dimensions were obtained from the M-mode image (Acuson, Sequoia, CA). Fractional shortening (difference between end-diastolic and end-systolic dimensions normalized to end-diastolic dimension) was used as an index of contractile function.

Isolated mouse heart preparation. Hearts were isolated and perfused in the Langendorff mode, as previously described (20). All hearts were electrically stimulated at 420 beats/min and maintained at 37°C. Following a 20-min stabilization period, hearts were subjected to 15 min of zero-flow ischemia, followed by 30 min of reperfusion. End-diastolic pressure (EDP) and developed pressure (difference between systolic and EDP) were used to assess diastolic and contractile parameters, respectively. At the end of the protocol, hearts were immediately used for measurement of tissue glutathione, ascorbate, and NOx (nitrite + nitrate), or flash-frozen and stored at −80°C for later carbonyl analysis.

Measurement of creatine kinase release. To measure creatine kinase (CK) release in the coronary effluent, the pulmonary artery was cannulated with a thin polyethylene tube (PE-50). Samples were collected 1 min before ischemia and at 5, 10, 15, and 30 min of reperfusion. CK activity (U/ml) was determined by spectrophotometry using a CK assay kit (Pointe Scientific), and values were normalized per gram weight of heart tissue.

Measurement of protein carbonyls. Analysis of protein carbonyls in tissue homogenates was performed using the Oxyblot kit (Chemicon International). Briefly, frozen hearts were homogenized in cell lysis buffer (Cell Signaling Technology), and 20 µg of protein were reacted with dinitrophenylhydrazine for 15 min, followed by neutralization with a solution containing 2-mercaptoethanol, resolved in 12.5% SDS-PAGE, transferred to a polyvinylidene difluoride membrane, with dinitrophenylhydrazine for 15 min, followed by neutralization with a solution containing 2-mercaptoethanol, resolved in 12.5% SDS-PAGE, transferred to a polyvinylidene difluoride membrane, incubated with a rabbit anti-dinitrophenylhydrazine antibody (1:150), followed by a secondary antibody (1:300) conjugated to horseradish peroxidase. Reactive bands were detected by chemiluminescence (Pierce Chemical). The carbonyl bands were scanned, and, instead of focusing on one band for quantification, we chose to quantify all protein carbonyl bands within a given lane, as described by Judge et al. (28). Carbonyl band densities were quantified using ImageJ (National Institutes of Health) software. The optical density was determined by calculating the net optical density (sum of the background-subtracted pixel values) of all carbonylated bands within each given lane. For both male and female groups, optical density was normalized to the corresponding wild-type (WT) control.

Tissue GSH/GSSG, ascorbate, and NO2/NO3 determination. The concentrations of reduced and oxidized glutathione (GSH and GSSG), ascorbate, NO2−, and NO3− in whole heart homogenates were determined, as previously described (10). Briefly, postischemic myocardial tissues were homogenized in 100 mM sodium phosphate buffer supplemented with 5 mM EDTA (pH 7.4) in a weight-to-volume ratio of 1:5, and GSH and GSSG concentrations were determined using microplate-adapted version of the Tietze recycling assay (51). Tissue content of reduced and oxidized ascorbate was assayed as described by Carr et al. (11), with minor modifications. Total ascorbate concentration was determined by the difference in readings of the 2,6-dichlorophenol-indophenol-treated and control samples by comparison with an AA standard curve. NO2− and NO3− were quantified by ion chromatography (EN020 Analyst, Eicom, Kyoto).

Statistical analysis. Data are reported as means ± SE. Group differences of a single measure were tested by ANOVA with a Tukey post hoc test. Measures at baseline and following I/R in each group were analyzed according to the unpaired Student’s t-test.

RESULTS

No effect of GPx1 deficiency on baseline cardiac function. To confirm GPx1 deficiency, we measured enzyme activity in the myocardium and liver of WT and GPx1-deficient mice. In WT animals, total GPx activity is ~40-fold higher in the liver compared with the heart. No detectable myocardial or hepatic GPx activity was observed in GPx1(−/−) mice (data not shown). Table 1 lists baseline values for in vivo and in vitro cardiac parameters for all groups. There was no difference in LV weight-to-body weight ratio between the groups. Heart rate and fractional shortening determined by echocardiography were comparable between WT and GPx1(−/−) mice, regardless of sex. In Langendorff-perfused hearts (at a constant coronary perfusion pressure of 80 mmHg), coronary flow normalized for LV weight was not different between any of the groups. At an EDP of 10 mmHg, developed pressure was similar among all groups. Thus neither sex nor ablation of GPx1 had any effect on in vivo or in vitro baseline cardiac parameters assessed in our study.

Effect on GPx1 deficiency on myocardial function during I/R. Isolated hearts were subjected to I/R injury while perfused at constant flow. During the ischemic period, EDP gradually rose in all groups (Fig. 1A). In this isovolumic preparation, an increase in EDP reflects an increase in diastolic chamber stiffness or contracture. At end ischemia, EDP contracture values were comparable, suggesting a similar degree of ischemic injury between all groups. During the reperfusion period, however, cardiac chamber stiffness was significantly worse in the male GPx1(−/−) hearts. At end-reperfusion, EDP was greater in male GPx1(−/−) compared with WT hearts (52.8 ± 4.0 vs. 34.7 ± 3.7 mmHg, P < 0.01). This was not the case for female GPx1(−/−) hearts, as EDP during the reperfusion period was not significantly different from WT and male GPx1(−/−) hearts.

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Values are means ± SE. BW, body weight; LV/BW, left ventricular weight-to-body weight ratio; HR, heart rate; FS, fractional shortening; CF/LV, coronary flow normalized to LV weight; DevP, developed pressure; BW, LV/BW, HR, and FS were determined in 11 male and 8 female WT and GPx1(−/−) mice. CF/LV and DevP were determined in 10 male WT, 12 male GPx1(−/−), 11 female WT, and 11 female GPx1(−/−) hearts.
period in these hearts was not different compared with female and male WT hearts. Normalized developed pressure (Fig. 1B) dropped quickly at the onset of zero-flow ischemia, and, similar to diastolic performance, contractile recovery was significantly worse in male GPx1(-/-) hearts compared with the other groups during the reperfusion period. At the end of 30 min of reperfusion, recovery of contractile function was 55.5 \pm 6.1\% in male WT, 59.2 \pm 3.7\% in female WT, and 56.8 \pm 4.0\% in female GPx1(-/-) hearts compared with 40.0 \pm 3.0\% in male GPx1(-/-) hearts (P < 0.05; compared with all groups). The observed contractile and diastolic dysfunction in male GPx1(-/-) hearts was not due to impaired myocardial perfusion, as coronary flow was similar among all groups at baseline and during the reperfusion period (Fig. 2A). Thus GPx1 is essential in mediating postischemic myocardial recovery in male, but not in female, hearts.

**Myocardial injury and oxidative stress.** Total CK release from the heart, which reflects cell injury or death, was significantly increased in both male groups following I/R, peaking at 10 min into the reperfusion period (Fig. 2B). Peak CK release was significantly lower in both female groups compared with their male counterparts. At the end of reperfusion, CK release had declined to comparable levels in all groups. To determine whether GPx1 and/or sex altered oxidative protein modification, post-I/R heart homogenates were assayed for reactive carbonyl derivatives. A representative carbonyl blot is shown in Fig. 3A, and corresponding densitometry data for all protein carbonyl bands within a given lane are presented in Fig. 3B. Following I/R, there was an approximately twofold increase in carbonylated proteins in male GPx1(-/-) hearts. In contrast, GPx1 deficiency did not increase carbonyl formation in female hearts. These data suggest that GPx1 is important in protecting against oxidative protein modification in male, but not female, hearts. The data further demonstrate that the degree of cellular injury and/or necrosis following ischemia was significantly greater in male compared with female hearts, irrespective of cellular levels of GPx1.

**Effect of GPx deficiency on myocardial redox/NOx homeostasis.** To gain insight into whether GPx1 and/or sex altered redox/NOx homeostasis, we determined tissue levels of glutathione, ascorbate, as well as NO\textsubscript{2} and NO\textsubscript{3} at baseline and following I/R. The absence of GPx1 did not alter baseline levels of total glutathione or GSH (Fig. 4, A and B); however, baseline GSSG levels were lower in GPx1(-/-) hearts (Fig. 4C), which may reflect a reduced capacity of these hearts to catalyze the reduction of peroxides using GSH, thus generating...
Sex-specific antioxidant compensatory mechanisms

Sex differences in postischemic tolerance in mice lacking GPx1. Previously, our laboratory and others have shown that GPx1 is an essential antioxidant enzyme in protection against ROS-mediated myocardial and vascular injury (20, 60). In this report, we found that, contrary to these previous findings in male mice, female mice lacking GPx1 did not exhibit an enhanced susceptibility to I/R injury compared with their WT counterparts. We observed a female-associated decrease in CK enzymatic activity (Fig. 4, A and B). We also found a female-related increase in NO2− and NO3− tissue levels between the WT and female mice (Fig. 4, C and D). These changes in NO2− and NO3− could be ascribed to the absence of GPx1 in the female hearts, resulting in a significant decrease in the NO3−/NO2− ratio in female mouse hearts compared with the other groups (Fig. 6D).

At baseline, levels of total ascorbate and AA were significantly higher in male GPx1(−/−) hearts (Fig. 5, A and B), while dehydroascorbate (DHA) levels tended to be higher in male hearts (Fig. 5C). Largely owing to lower DHA levels in female compared with male hearts, the baseline AA-to-DHA ratio tended to be higher in WT females, although differences between groups did not reach statistical significance (Fig. 5D). Following I/R, total ascorbate, AA, and DHA all decreased relative to baseline in all groups (Fig. 5, A–C). In contrast to the GSH-to-GSSG ratio, the absence of GPx1 did not affect the AA-to-DHA ratio post-I/R. Female hearts exhibited a more favorable ascorbate redox homeostasis following I/R, as evidenced by a significantly increased AA-to-DHA ratio compared with male hearts (Fig. 5D).

We detected no differences in baseline NOx (sum of NO2− and NO3−), nor individual NO2− or NO3− tissue levels between groups (Fig. 6, A–C). Interestingly, the NO3−/NO2− ratio [an indicator of nitrosative stress (10)] was almost twofold higher in baseline female GPx1(−/−) hearts compared with the other groups (Fig. 6D). Following I/R, steady-state tissue levels of NO2− increased significantly relative to baseline in all groups after 30 min of reperfusion (Fig. 6B). Although not statistically significant, there was a decrease in NO3− in the female GPx1(−/−) hearts, with no change in NO3− in the other groups (Fig. 6C). These changes in NO2− and NO3− following I/R resulted in a significant decrease in the NO3−/NO2− ratio in all groups, with the largest decrease occurring in female GPx1(−/−) hearts (Fig. 6D). These data suggest that, in contrast to male mice, female mice are able to compensate for GPx1 deficiency post-I/R by improving their ascorbate redox homeostasis, perhaps via an alteration of NO2− and NO3− metabolic pathways.

**DISCUSSION**

Fig. 2. Coronary flow (CF) and creatine kinase (CK) release, post-I/R. A: CF normalized to LV weight in male WT (○, n = 10), male GPx1(−/−) (●, n = 12), female WT (●, n = 11), and female GPx1(−/−) (●, n = 11) mouse hearts at BL and 30 min of reperfusion. B: CK activity measured in the coronary effluent collected from male WT (○, n = 5), male GPx1(−/−) (●, n = 4), female WT (●, n = 6), and female GPx1(−/−) (●, n = 8) hearts at BL and 30 min of reperfusion. Shaded box represents the ischemic period. Values are means ± SE. *P < 0.05 male vs. female.

Fig. 3. Measurement of reactive carbonyl derivatives in heart homogenates, before and after I/R. A: representative carbonyl blot. B: bar graph presentation of optical densities for all of the protein carbonyl bands within a given lane. All data are normalized to male WT at BL. Values are means ± SE from 4 independent experiments in each group. *P < 0.05 vs. male WT at BL. †P < 0.05 vs. male WT after I/R.
leakage, similar to previous studies demonstrating a significant decrease in infarction and lactate dehydrogenase release following I/R in female compared with male mouse hearts (49, 56). Interestingly, while the degree of necrotic injury or cell death was sex specific in our study, it appeared to be independent of GPx1. Exacerbation of contractile dysfunction in male GPx1 supports the role of this antioxidant enzyme in protection from oxidative injury (20, 60). The increase in carbonyl formation in postischemic myocardial homogenates from these hearts is consistent with this conclusion. Our observation that CK release was not different between male GPx1 (G1/H11002) and WT hearts, despite a significantly inferior postischemic functional recovery, suggests that GPx1 deficiency rendered male hearts more vulnerable to reversible forms of cellular injury, such as myocardial stunning. Thus we speculate that male GPx1 (G1/H11002) hearts had the worst outcome because they are predisposed to both irreversible (infarction) and reversible (stunning) forms of myocardial injury.

Our finding that GPx1 deficiency did not have the same deleterious effect in female mice was not expected, based on previous literature. Borras et al. (6) have reported lower levels of H2O2 production and oxidative stress and higher GPx activity in female hepatic mitochondria, and they posit this difference as a possible explanation for increased female longevity. Similarly, mitochondrial oxidative stress and lipid peroxidation have been shown to be lower in female myocardium, whereas GPx1 activity has been shown to be higher in female compared with male myocardium following I/R injury (2, 17, 26, 47). In a large prospective study, premenopausal women had higher levels of erythrocyte GPx1 activity compared with men, and a low level of erythrocyte GPx1 activity was independently associated with an increased risk of cardiovascular events (4). This literature would seem to support the notion that GPx1 deficiency should eliminate the sex-specific cardioprotection. Our observation that female hearts lacking GPx1 were no more susceptible to I/R injury compared with WT and that glutathione redox homeostasis (GSH-to-GSSG ratio) was similar after I/R argues against the hypothesis that enhanced glutathione antioxidant capacity is the key to the relative cardioprotection of the female heart.

There are four selenocysteine GPx isoforms found in rodents (7). GPx2 is believed to be specific to the gastrointestinal tract, while GPx3 transcripts are found in the heart, but upon translation is secreted and found in extracellular fluids (41). GPx1 and GPx4 are the only two known isoforms expressed in the myocardium and preferentially scavenge soluble hydroperoxides and phospholipid hydroperoxides, respectively. It is interesting that, while GPx1 is most abundant, deficiency in GPx1

Fig. 4. Myocardial glutathione levels at BL and following I/R. A: total glutathione. B: reduced glutathione (GSH). C: oxidized glutathione (GSSG). D: ratio of GSH/GSSG at BL (open bars) and following I/R (solid bars). Values are means ± SE from 3–5 independent experiments in each group. *P < 0.05 between indicated bars.
is only associated with a mild phenotype, while GPx4 deficiency is embryonically lethal (23, 59). In two separate studies, Cheng et al. (13, 14) reported no change in GPx4 activity in hearts from either GPx1(+/−/+) or GPx1 transgenic mice compared with WT. It should be noted that, in both studies, the WT and GPx1 groups were one-half male and one-half female, and the results were pooled. While the two studies by Cheng et al. did not specifically address sex differences, their pooled data lead us to conclude that there was no compensatory increase in GPx4 activity to account for the increased cardioprotection seen in female GPx1(+/−/+) hearts. A recent study showed an increase in serum GPx3 activity in young females compared with young males, although the physiological significance of this remains to be established (46). In addition to GPx, other enzymatic systems consume GSH, resulting in formation of GSSG, including the DHA reductases: glutaredoxin and protein disulfide isomerase (PDI), which have been found in the myocardium and are believed to play an important role in the endogenous antioxidant defense mechanism, as well as maintenance of redox homeostasis (33). While we are unaware of any specific reports of the effect of sex on these enzymes in the myocardium, it is possible that increased expression of either glutaredoxin or PDI could compensate for the loss of GPx1 in female myocardium.

Role of ascorbate and NO-related metabolites in conferring tissue protection. Our report is the first to identify enhanced ascorbate redox homeostasis in female compared with male hearts following I/R injury. While total ascorbate levels following I/R injury were similar in all groups, the ratio of reduced to oxidized ascorbate (the AA-to-DHA ratio, which reflects the true ascorbate antioxidant capacity) was higher in females, regardless of GPx1 deficiency. This suggests that female ischemic hearts are more efficient at recycling the oxidized DHA back to its reduced (antioxidant) form, AA. This observation is consistent with prior studies in ischemic brain tissue showing a greater preservation of ascorbate in females compared with males (19, 31). Ascorbate is known to have a multiplicity of antioxidant properties, including the ability to act as a reducing agent to scavenge free radicals directly, and sparing other antioxidants, such as α-tocopherol (vitamin E) and GSH (35, 42). Furthermore, a previous study has shown that administration of high-dose ascorbate attenuates myocardial I/R injury and mitochondrial dysfunction, suggesting an antioxidant action localized at or near the mitochondrial compartment (40). Based on studies demonstrating similar antioxidant actions and a mutual sparing effect between GSH and ascorbate (37, 57), there appears to be some functional redundancy between these two antioxidant systems. Thus the shift in
cellular redox balance toward more reduced ascorbate redox status in females may compensate in part for the disrupted glutathione redox cycling and confer cardioprotection from I/R-induced oxidative stress.

The recycling of AA can occur via a nonenzymatic reaction with GSH (36), which is consistent with the higher levels of GSH observed in female hearts following I/R, which we predict will enhance recycling of AA. Recycling of AA can also be accomplished enzymatically via the DHA reductases: glutaredoxin and PDI; both use GSH as reductant (33). The activity of glutaredoxin is constitutively higher in female brain tissue and has been shown to confer protection against neurotoxin-mediated injury (16, 30). Furthermore, 17ß-estradiol induces expression of glutaredoxin in cardiac H9C2 cells, which has been shown to protect H9C2 cells from H2O2-induced apoptosis (53). Using a proteomic approach, we have shown that myocardial PDI was significantly altered following short-term NO2−/NO3− exposure, although the physiological significance of this remains to be established (44). These observations lead us to speculate that GSH-dependent mechanisms in recycling DHA are more effective in females, thus accounting for the higher AA-to-DHA ratio in female hearts following I/R.

While beyond the scope of this study, future experiments are needed to clearly delineate the importance of enhanced ascorbate recycling in cardioprotection and to determine the role of the sex hormones in ascorbate recycling.

Several reports have shown sex differences in susceptibility to I/R injury that may be related to a role of the sex hormones on NO synthase (NOS) activity, and thus NO bioavailability (50, 54). A previous report has shown that endothelial NOS (eNOS) is significantly higher in female hearts, and inhibition of eNOS with L-arginine methyl ester abolished the cardioprotective effect in female hearts following I/R injury (54). NO2− and NO3− have recently been shown not merely to represent oxidative breakdown products of NO, but to act as NO reservoirs in ischemic/hypoxic tissues (15, 32). More importantly, exogenous NO2− supplementation has been shown to increase myocardial NO2− levels and protect the heart from I/R injury (8, 18, 55). NO2− may also act in an NO-independent fashion by directly modulating signaling pathways previously thought to be largely under control of NO (9). A surprising finding in our study was that tissue NO2− levels increased in all groups following I/R. Our laboratory and others have previously reported that NO2− is consumed during ischemia when

Fig. 6. Myocardial NOx (nitrite + nitrate) levels at BL and following I/R. A: total NOx. B: nitrite. C: nitrate. D: ratio of nitrate/nitrite at BL (open bars) and following I/R (solid bars). Values are means ± SE from 4–8 independent experiments in each group. *P < 0.05 between indicated bars.
NOS is inactive, and this process is associated with formation of nitroso and nitrosyl species in the heart (8, 10, 62). Our current data imply that extra NO$_3$ was generated during the reperfusion period, perhaps through enhanced NO production, as previous studies have shown an increase in NOS activity during myocardial I/R (reviewed in Ref. 48). Thus it is plausible that enhanced NOS activity during the 30-min reperfusion period resulted in increased NO production and subsequent oxidation to NO$_2$.

An alternative mechanism for greater NO$_2$ production could be enhanced conversion from NO$_3$. One metabolic pathway for NO$_3$ production is via decomposition of peroxynitrite (ONOO$^-$), generated through the reaction of NO with superoxide (O$_2^-$) (10). Our laboratory has previously shown that GPx1 deficiency resulted in enhanced lipid peroxidation and nitrosative stress, myocardial dysfunction following I/R, and impaired endothelial-dependent vasodilator function, and we attributed this to a decrease in bioavailable NO, as NO is rapidly consumed to generate ONOO$^-$ (20, 21). ONOO$^-$ has an extremely short half-life and is difficult to analyze. However, NO$_2$ is measurable, and our finding that steady-state NO$_3$ levels were modestly decreased in the GPx1(-/-) female compared with male mice suggests that less O$_2^-$ was produced in these hearts during the I/R insult. This may be due to greater scavenging of O$_2^-$ by ascorbate in female GPx1(-/-) hearts or to another compensatory antioxidant pathway not investigated in the present study.

The lower NO$_3$ levels reported in GPx1(-/-) female hearts could also represent enhanced conversion of NO$_3$ to NO$_2$. While the underlying mechanisms of conversion of NO$_3$ to NO$_2$ are not entirely understood, xanthine oxidoreductase (XOR) has been shown to be capable of reducing NO$_3$ to NO$_2$ and NO$_2$ further to NO, both in the absence of oxygen (32). XOR, in turn, has generally been implicated in cardiovascular disease via generation of damaging O$_2^-$ during I/R (3); however, recent studies suggest that, with exogenous NO$_2$ administration, XOR might actually have beneficial effects (8, 18). Webb et al. (55) reported that exogenous infusion of NO$_2$ during I/R injury or diversion from metabolic NO$_3$ production implies greater utilization of available bioactive NO reservoirs in these hearts. This observation is consistent with a recent report demonstrating that rats pretreated with an NO scavenger exhibited enhanced I/R-induced hepatic injury, which can be, in part, recapitulated in rats pretreated with allopurinol; i.e., NO protects rat liver during I/R, in part via the generation of XOR-catalyzed NO (34).

How bioactive NO ultimately mediates cardioprotection is not entirely clear, as multiple mechanisms have been reported, including activation of soluble guanylyl cyclase, decreased Ca$^{2+}$ influx through increased S-nitrosylation of L-type Ca$^{2+}$ channels, and activation of mitochondrial ATP-sensitive K$^+$ channels (reviewed in Ref. 27, 39). Clearly, more experiments are needed to fully elucidate the significance of XOR involvement and endogenous NO$_3$/NO$_2$ levels in cardioprotection during I/R injury. Taken together, our findings suggest that the increase in both ascorbate redox cycling and change in NOx utilization in female (but not male) GPx1(-/-) hearts post-I/R may act in concert to compensate for the decreased glutathione antioxidant potential and hence protect against the effects of GPx1 deficiency.

It should be noted that sex differences in cardioprotection are likely to be multifactorial, involving the pleiotropic actions of estrogen in females, as well as potentially deleterious effects of testosterone in males (43). Previously reported sex differences in genomic and nongenomic estrogenic-based signaling, including an increase in glucose oxidation, activation of the phosphatidylinositol 3-kinase pathway, and reduction of the mitochondrial ROS are all plausible contributory factors to female cardioprotection (38, 39).

Summary and conclusions. This report demonstrates that the female heart is more resistant to I/R injury than the male heart, even in the absence of GPx1 activity. The results further demonstrate clear sex differences in the mechanisms by which the heart manages oxidative stress. Glutathione oxidation is increased in both male and female hearts in the absence of GPx1, suggesting that a non-glutathione antioxidant defense system is involved in female myocardial protection. Following I/R, the female myocardium deficient in GPx1 was able to maintain higher levels of reduced ascorbate, as well as increased NOx-to-NO flux. This combined antioxidant action may compensate for a deficiency in GPx1 and thus attenuate some of the deleterious effects of I/R injury. The ability of female mice to enhance different antioxidant systems may provide a mechanism for the well-recognized cardioprotective benefit of female sex.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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