Lack of Glutathione Peroxidase 1 Accelerates Cardiac-Specific Hypertrophy and Dysfunction in Angiotensin II Hypertension

Noelia Ardanaz, Xiao-Ping Yang, M. Eugenia Cifuentes, Mounir J. Haurani, Kyle W. Jackson, Tang-Dong Liao, Oscar A. Carretero, Patrick J. Pagano

Abstract—Glutathione peroxidase 1 (Gpx1) plays an important role in cellular defense by converting hydrogen peroxide and organic hydroperoxides to nonreactive products, and Gpx1−/− mice, which are characterized by reduced tissue glutathione peroxidase activity, are known to exhibit enhanced oxidative stress. Peroxides participate in tissue injury, as well as the hypertrophy of cultured cells, yet the role of Gpx1 to prevent end organ damage in cardiovascular tissue is not clear. We postulated that Gpx1 deletion would potentiate both aortic and cardiac hypertrophy, as well as mean arterial blood pressure, in response to angiotensin II (AngII). Our results show that short-term AngII markedly increased left ventricular mass, myocyte cross-sectional area, and interventricular septum thickness and decreased shortening fraction in Gpx1−/− mice as compared with wild-type animals. On the other hand, AngII resulted in a similar increase in mean arterial blood pressure in wild-type and Gpx1−/− mice. Collagen deposition increased in response to AngII, but no differences were found between strains. Vascular hypertrophy increased to the same extent in Gpx1−/− and wild-type mice. Collectively, our results indicate that Gpx1 deficiency accelerates cardiac hypertrophy and dysfunction but has no effect on vascular hypertrophy and mean arterial blood pressure and suggest a major role for Gpx1 in cardiac dysfunction in AngII-dependent hypertension. (Hypertension. 2010;55:116-123.)

Key Words: heart • angiotensin • hypertrophy • cardiac dysfunction • oxidant stress

Left ventricular hypertrophy (LVH) is an important risk factor for coronary heart disease, heart failure, and stroke.1,2 LVH involves changes in myocardial architecture consisting of myocyte hypertrophy and perivascular and myocardial fibrosis, and there is a well-established link between LVH and high blood pressure. Factors such as age, sex, race, and stimulation of the renin-angiotensin-aldosterone system play important roles in the pathogenesis of LVH.3,4 and angiotensin-converting enzyme inhibitors, as well as angiotensin II (AngII) receptor antagonists, are effective in the reduction of cardiac hypertrophy.5,6 AngII is a prototypical stimulant of hydrogen peroxide (H2O2) and other reactive oxygen species (ROS) in cardiac and vascular cells,7 and H2O2 has been shown to be a potent signaling agent in cardiomyocytes and vascular smooth muscle, promoting their hypertrophy in vitro and in vivo.7–9

Glutathione peroxidase (Gpx) 1, a ubiquitous peroxidase isoform, is a selenium-dependent enzyme that reduces cellular peroxides via their conversion to water and other nonreactive products.10 In Gpx knockout mice (Gpx1−/−) tissue, Gpx activity is markedly reduced,11 and peroxide and ROS levels are elevated, which purportedly contribute to endothelial dysfunction and cardiac matrix deposition.12 Despite a well-established role of AngII to increase H2O2 in the heart and aorta, the potential for AngII hypertension and cardiac and vascular remodeling to be accelerated in Gpx1−/− mice has not been studied. In this study, we postulated that Gpx1 deletion would potentiate aortic and cardiac hypertrophy, as well as mean arterial blood pressure (MABP) elevation, in response to AngII. The novel findings described herein illustrate that Gpx1 deletion promotes cardiac-specific hypertrophy and dysfunction without affecting vascular hypertrophy or blood pressure. These results suggest an important role for Gpx1 in initial cardiac hypertrophy and dysfunction in response to AngII.

Methods

Animals
Male Gpx1−/− mice backcrossed to the C57Bl/6J background for >10 generations were kindly provided by Dr Ye-Shih Ho (Wayne

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The authors had full access to the data and take responsibility for its integrity. All of the authors have read and agree to the article as written, correspondence to Patrick J. Pagano, Department of Pharmacology and Chemical Biology and Vascular Medicine Institute, University of Pittsburgh School of Medicine, Room 10043, BST-3, 3501 Fifth Ave, Pittsburgh, PA 15261. E-mail pagano@pitt.edu

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Radiotelemetry of MABP
Eighteen- to 20-week-old mice were instrumented with transmitters as described previously13 (an expanded Methods section can be found in the online Data Supplement, please see http://hyper.ahajournals.org). MABP and heart rate were continuously recorded and reported as 24-hour mean±SEM.

Vehicle or AngII Infusion
Mice were anesthetized with Brevital (70 mg/kg IP) to allow for the SC implantation of osmotic minipumps (Alzet 1007D). Mice were implanted with minipumps infusing either vehicle or AngII (521 ng/kg·min SC) for 7 days.

Preparation of Tissue Samples
On day 7, mice were anesthetized and the heart stopped during diastole. Mice were perfusion fixed under pressure, and hearts and thoracic aortas removed. Total heart weight:body weight (THW:BW) ratio served as parameter of cardiac hypertrophy. The left ventricular (LV) middle section and the descending thoracic aorta were processed and sectioned for analyses.

Echocardiographic Evaluation of Cardiac Morphology and Function
LV wall thickness, dimensions, and shortening fraction (SF) were evaluated with a Doppler echocardiograph equipped with a linear transducer, as described previously.14

Measurements of Interventricular Septum and LV Mass
LV mass was calculated according to the following equation14:

\[ \text{LV mass} = 1.055 \times (IVST_d + LVD_d + PWT)^3 - (LVD_d)^3, \]

where 1.055 is the specific gravity of the myocardium, IVSTd is diastolic interventricular septum thickness, LVDd is diastolic LV dimension, and PWT is diastolic posterior wall thickness.

Cardiac Chamber Dimensions
End-diastolic and end-systolic LV dimensions (LVDd and LVDs) and diastolic interventricular septum thickness were measured from the M-mode tracings.

LV Shortening Fraction
LV SF, a measure of LV systolic function, was calculated from the M-mode LV dimensions according to the following equation:

\[ \text{SF(\%) = } \frac{[(LVD_d - LVD_s)/LVD_d \times 100].} \]

Myocyte Cross-Sectional Area and Interstitial Collagen Fraction
Six-micrometer heart sections were processed in Bouin fluid over-night, washed, and incubated with 0.1% picrosirius red. Twenty-one images of each left ventricle section were captured at ×400 magnification. Myocyte cross-sectional area (CSA; MCSA) and interstitial collagen fraction (ICF) were digitally recorded.

Histological Examination of Mouse Thoracic Aortic Cross-Sections for Measurement of Vascular Remodeling
Sections were stained with Masson Trichrome Accustain, as described previously.15 Cross-sectional area (CSA), thickness of the media, and external perimeter (Pe) were digitally measured. Lumen diameter (L) was calculated according to the formula:

\[ L = 2 \times \left( \frac{P_e}{2\pi} - \frac{(CSA/\pi)}{1.055} \right)^{1/3}, \]

where 1.055 is the specific gravity of the myocardium, IVSTd is diastolic interventricular septum thickness, LVDd is diastolic LV dimension, and PWT is diastolic posterior wall thickness.

Body Weight and MABP
Mouse weights were ∼30 g and did not vary with time or among treatment groups (data not shown). Basal MABP was similar between strains and remained unchanged in vehicle groups of both strains throughout the study (Figure 1). AngII caused a significant and sustained increase in MABP. No significant difference was observed between Gpx1+/− and wild-type mice infused with AngII (Figure 1). Heart rate in beats per minute did not vary among treatment groups and strains of mice (wild-type: 705±6.3 bpm versus Gpx1+/−: 691±9.40 bpm for animals treated with vehicle, and wild-
Cardiac Hypertrophy: Ratio of Total Heart:Body Weight

Large visible differences in heart size were observed in Gpx1<sup>−/−</sup> versus wild-type mice infused with AngII, as demonstrated by differences in heart cross-sections seen in Figure 2A through 2D. Figure 2A and 2B correspond with representative heart cross-sections from wild-type mice treated with vehicle (wild-type+vehicle) and Gpx1<sup>−/−</sup> mice treated with vehicle (Gpx1<sup>−/−</sup>+vehicle), respectively, showing no significant differences between the strains. Figure 2C and 2D correspond with representative heart cross-sections from wild-type mice treated with AngII (wild-type+AngII) and Gpx1<sup>−/−</sup> mice treated with AngII (Gpx1<sup>−/−</sup>+AngII), respectively, showing LVH in the heart from Gpx1<sup>−/−</sup>+AngII mice. THW:BW ratios in vehicle-treated wild-type and Gpx1<sup>−/−</sup> mice were similar (Figure 2E). THW:BW ratios from wild-type mice treated with AngII were not significantly larger than those in wild-type mice treated with vehicle. Gpx1<sup>−/−</sup>+AngII hearts, however, were significantly larger than Gpx1<sup>−/−</sup>+vehicle (P<0.05) and wild-type+AngII (21% larger; P<0.05; Figure 2E).

Figure 2. Comparison of cardiac mass in wild-type vs Gpx1<sup>−/−</sup> mice. Hearts were harvested after 7 days treatment with vehicle or AngII (521 ng/kg · min SC). Representative cross-sections of hearts from wild-type+vehicle (A), Gpx1<sup>−/−</sup>+vehicle (B), wild-type+AngII (C), and Gpx1<sup>−/−</sup>+AngII (D) stained with picrosirius red. THW:BW ratio was tabulated for hearts from vehicle- and AngII-treated wild-type and Gpx1<sup>−/−</sup> mice (E). Data are expressed as mean±SEM (n=9 to 10).

Echocardiographic Measurement of Cardiac Hypertrophy and Function

Diastolic Interventricular Septum

IVSTd at day 0 did not vary among treatment groups or strains (Figure 3A). In addition, IVSTd in vehicle-treated wild-type and Gpx1<sup>−/−</sup> mice did not increase from day 0 to 7. AngII treatment, however, significantly elevated IVSTd in the Gpx1<sup>−/−</sup> group (day 7) versus its vehicle control. This elevation was significantly greater than that observed in the AngII-treated wild-type group (P<0.05).

Measurements of LV Mass

At day 0, LV mass was not different among the treatment groups and strains (Figure 3B). LV mass did not change significantly in vehicle-treated wild-type and Gpx1<sup>−/−</sup> mice (days 0 to 7). AngII treatment resulted in elevation in LV mass in mice from both strains (P<0.05), and this increase in LV mass by AngII was significantly greater in Gpx1<sup>−/−</sup> versus wild-type groups at day 7 (P<0.05).

Cardiac Shortening Fraction

Cardiac SF did not vary significantly at day 0 among all of the groups (Figure 3C). SF did not change comparing day 7 with day 0 in wild-type+vehicle, wild-type+AngII, or Gpx1<sup>−/−</sup> + vehicle mice. SF was significantly reduced (13%) after 7 days in AngII-treated Gpx1<sup>−/−</sup> mice (P<0.05). Moreover, at day 7 there was a significantly lower SF in AngII-treated Gpx1<sup>−/−</sup> mice compared with wild-type+AngII and both vehicle-treated groups (P<0.05).

Measurements of Cardiac Chamber Dimensions

Baseline (day 0) LVDs or LVDd did not differ among the treatment groups. At 7 days, in vehicle-treated wild-type and Gpx1<sup>−/−</sup> mice, neither LVDs (wild-type: 1.15±0.03 mm versus Gpx1<sup>−/−</sup>: 1.16±0.03 mm) nor LVDd (wild-type: 2.41±0.06 mm versus Gpx1<sup>−/−</sup>: 2.42±0.05 mm) was significantly different. AngII had no significant effect on LVDd in either strain (2.45±0.10 versus 2.46±0.11 mm for wild-type and Gpx1<sup>−/−</sup>, respectively). Likewise, AngII had no effect on LVDs in wild-type versus Gpx1<sup>−/−</sup> mice (1.17±0.05 versus 1.29±0.10 mm, respectively).

MCs and ICF

MCs did not differ between vehicle-treated wild-type and Gpx1<sup>−/−</sup> mice (Figure 4A, 4B, and 4E). Ang II significantly
increased MCSA in both strains (P<0.05; Figure 4C through 4E). Importantly, hypertrophy, as measured by MCSA, was greater in Gpx1<sup>−/−</sup> mice than in wild-type mice (P<0.05). Representative images showing interstitial collagen in each group are illustrated in Figure 5A through 5D. ICF was measured at 3.8±0.2% in wild-type+vehicle; AngII significantly increased ICF to 11.0±1.2% (P<0.001; Figure 5E). Similar collagen deposition was observed in Gpx1<sup>−/−</sup> mice. That is, ICF was 4.0±0.2% in Gpx1<sup>−/−</sup> mice, and AngII elevated it to 12.0±2.0% (P<0.05; Figure 5E).

**Assessment of Gpx Activity and Protein Levels**

Gpx activity was measured in aortas, as well as in heart homogenates. In aortas of wild-type mice, Gpx activity was 0.28±0.14 mU/mg in vehicle-treated animals, whereas it was 0.47±0.24 mU/mg in AngII-treated animals. In aortas of Gpx1<sup>−/−</sup> mice, the enzyme activity was below the detection limit of the assay under both treatment conditions. In the case of heart homogenates, Western blots were used to assess protein levels and to corroborate previously published findings showing a 10-fold decrease in Gpx1<sup>−/−</sup> compared with wild-type mice. As expected, the levels of Gpx1 in Gpx1<sup>−/−</sup> animals were undetectable compared with the robust levels in wild-type mice, both for vehicle or AngII-treated animals (Figure S1, available in the online Data Supplement). The data show a major reduction of vascular and cardiac Gpx1 from wild-type to knockout mice, corroborating previous findings by Ho et al, which demonstrated a 10-fold decrease in Gpx1 in Gpx1<sup>−/−</sup> mice compared with wild-type mice.

**Discussion**

Gpx1 is suggested to play an important role in moderating H<sub>2</sub>O<sub>2</sub> under pathological conditions. In nonstressed heterozygous Gpx1 knockout mice, elevated ROS and oxidative stress in cardiovascular tissue are associated with ROS-mediated endothelial dysfunction. Because AngII is a prototype stimulant of H<sub>2</sub>O<sub>2</sub> in the heart and aorta, we hypothesized that, in AngII-infused mice, deletion of Gpx1 would potentiate blood pressure elevation and both cardiac and aortic hypertrophy. Previously, the role of Gpx1 in hypertension and its effects on cardiac and vascular remodeling had not been studied. Here, using a Gpx1-knockout mouse model characterized by suppressed levels of Gpx activity and protein, we provide anatomic (heart weight), morphological (LV mass and posterior wall thickness by echocardiography), and histological (MCSA) evidence of enhanced AngII-induced LV hypertrophy in Gpx1<sup>−/−</sup> mice, despite no difference in blood pressure between AngII-treated wild-type and Gpx1<sup>−/−</sup> mice. In contrast to the heart, AngII-induced hypertrophy was not enhanced in Gpx1<sup>−/−</sup> aortas. Furthermore, preliminary data indicated a rise in ANP levels in Gpx1<sup>−/−</sup> mice consistent with enhanced hypertrophy in this strain (data not shown). Taken together, our results reveal a novel
observation of a unique cardiac hypertrophy and dysfunction in Gpx1−/− mice.

**MABP Measurements**

AngII increased MABP compared with vehicle-treated mice of both strains. In light of a reported endothelial dysfunction in Gpx1−/− mice and findings that Gpx1 deletion enhances AngII-induced impairments of vasodilatation, a greater elevation in MABP after AngII might have been predicted. However, no difference was observed between the strains, which is possibly attributable to the existence of countervailing mechanisms affecting blood pressure. Importantly, this

![Image](image_url)

**Figure 4.** LV MCSA in wild-type mice and Gpx1−/− mice. Mice were treated with AngII or vehicle, and cardiac sections were evaluated for MCSA. Panels show representative heart sections from wild-type + vehicle (A), Gpx1−/− + vehicle (B), wild-type + AngII (C), and Gpx1−/− + AngII (D) mice. Picrosirius red stain was used to demarcate myocytes as well as stain for collagen. MCSA was measured after 7 days of vehicle or Ang II treatment. MCSA (micrometers squared) was digitally measured and expressed as mean±SEM (E; n=9 to 10). Original magnification: ×200.

![Image](image_url)

**Figure 5.** LV ICF% in wild-type mice and Gpx1−/− mice. Mice were treated with AngII or vehicle, and cardiac sections were stained with picrosirius red. Panels show representative heart sections from wild-type + vehicle (A), Gpx1−/− + vehicle (B), wild-type + AngII (C), and Gpx1−/− + AngII (D) mice. ICF% of total area was digitally measured and expressed as mean±SEM (E; n=9 to 10). Original magnification: ×200.
absence of a blood pressure difference between strains highlights a critical dissociation among MABP, increased cardiac hypertrophy, and diminished function in response to AngII in Gpx1−/−. Thus, our observations challenge the notion that the effects of AngII on LV hypertrophy are simply pressor dependent.17

Measurements of Cardiac Hypertrophy and Function
THW:BW ratios did not increase significantly in wild-type animals after 7 days of AngII. This is not surprising given the short duration of AngII infusion. However, a significantly larger heart weight was observed in AngII-infused Gpx1−/− mice. This difference between strains was also clearly demonstrated histologically and echocardiographically. LV mass and MCSA were markedly higher in both AngII groups and further enhanced in Gpx1−/−. Furthermore, a significantly enhanced IVSTd was observed in Gpx1−/− mice, further supporting enhanced AngII susceptibility in Gpx1−/− versus wild-type mice.

To further test for an effect of Gpx1 knockout on cardiac function, changes in LVDs and LVDd were examined. We observed no changes in LVDd among the treatment groups. Although in wild-type mice LVDs did not change with AngII, there was a tendency for an increase in LVDs in Gpx1−/− mice. This difference between strains was also clearly demonstrated histologically and echocardiographically. LV mass and MCSA were markedly higher in both AngII groups and further enhanced in Gpx1−/−. Furthermore, a significantly enhanced IVSTd was observed in Gpx1−/− mice, further supporting enhanced AngII susceptibility in Gpx1−/− versus wild-type mice.

Two reports challenge the role of antioxidant defenses in cardiac hypertrophy and/or heart failure.18 Dieterich et al report an increased catalase activity in human end-stage heart failure. These data may be interpreted to contradict our findings that reduced antioxidant defenses contribute to cardiac dysfunction. However, as the authors point out, increased catalase activity may compensate for elevated ROS, accelerates cardiac matrix deposition and ROS-mediated collagen matrix deposition in mice for which Gpx1 was partly compromised, we did not observe enhanced collagen deposition in Gpx1−/− mice. Two possible explanations for the lack of effect of AngII in our study include the following: increased ROS did not rise to levels capable of enhancing the effect of AngII on fibrosis, and/or longer periods of elevated ROS may be necessary to sustain such an enhancement. Moreover, genetic background differences of heterozygous versus homozygous knockouts, as well as their controls, could have contributed to the discrepancy.

Gpx1−/− mice treated with AngII exhibited a significantly decreased SF compared with controls. Theoretically, this functional change might be attributed to ROS-mediated hypertrophy leading to cardiac dysfunction and failure.20 However, in the present study, the rapid development of LV dysfunction in Gpx1−/− mice is likely to have resulted from a direct adverse cardiac cell effect of H2O2. In fact, previous studies showed that ROS including H2O2 accelerate contractile dysfunction of the heart.20,21 Detrimental effects of H2O2 include intracellular acidosis and electromechanical dysfunction, alterations in cardiac action potential, and contractile force inhibition.22,23 None of the differences observed can be attributed to changes in angiotensin II type I receptor, because its levels did not vary among treatment groups (Figure S2).

Two reports challenge the role of antioxidant defenses in cardiac dysfunction and/or heart failure. Dieiterich et al report an increased catalase activity in human end-stage heart failure. These data may be interpreted to contradict our findings that reduced antioxidant defenses contribute to cardiac dysfunction. However, as the authors point out, increased catalase activity may compensate for elevated ROS at this late stage of the disease. A second study by Baumer et al reports the opposite.24 That is, catalase activity is reportedly decreased in end-stage heart failure, consistent with our hypothesis that decreased antioxidant protection leads to dysfunction. Importantly, the study did not examine catalase activity at earlier time points, and, thus, it is not possible to know whether compromised antioxidant defenses early in human disease contribute to heart failure. At this juncture, therefore, not enough evidence is available to solve this controversy. Moreover, caution should be taken in extrapo-
lating findings in the mouse to human disease, especially considering that our findings are not related to chronic heart failure.

**Lack of Effect of Gpx1 Deficiency on Vascular Remodeling**

Vascular medial hypertrophy in response to AngII is mediated, in part, by ROS. Thus, we expected that in Gpx1−/− versus wild-type mice, AngII-induced medial hypertrophy would be enhanced. However, our data showed no difference in AngII-induced hypertrophy between the strains as assessed by wall:lumen ratio and CSA. These data are noteworthy because they highlight enhanced cardiac hypertrophy in Gpx1−/− mice in the absence of a change in vascular medial hypertrophy. The data indicate an accelerated cardiac response to Gpx1 deficiency or enhanced cardiac sensitivity to smaller ROS increases compared with aorta.

In conclusion, LVH and dysfunction were accelerated in AngII-treated Gpx1−/− mice. These effects were not associated with increases in collagen deposition and were independent of blood pressure levels. No changes in vascular hypertrophy were observed, indicating a greater role for Gpx1 in cardiac versus vascular protection. Our results demonstrate that Gpx1−/− mouse hearts are more susceptible to dysfunction and further support the significance of antioxidant defense by Gpx1 in cardiac remodeling and function.

**Perspectives**

Hypertension and ventricular hypertrophy are major risk factors for cardiac dysfunction and congestive heart failure. Oxidative stress is an important mechanism involved in cardiovascular disease. ROS trigger signaling pathways that lead to cell proliferation, dysfunction, and death, as well as release of proinflammatory mediators that promote cardiovascular injury. Under physiological conditions, ROS is controlled by intrinsic antioxidant systems, including Gpx1. In this study, we demonstrated that lack of Gpx1 promotes AngII-induced LVH, dilatation, and dysfunction, supporting a pathophysiological role of ROS in the heart. The data further support the concept that antioxidant therapy may benefit the heart. Large prospective and randomized clinical trials failed to demonstrate clinical benefits of antioxidants on blood pressure and end-organ damage. Although the reasons for these outcomes are not clear, there are noteworthy limitations to those studies including the doses and forms of antioxidants chosen, preexisting cardiovascular conditions, trial design, and other medications administered to enrolled patients. Future studies will require a more focused and rational approach to targeting ROS rather than emphasizing the use of scavengers, which may not be as efficacious or discriminating in their effects.

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**Disclosures**

None.

**References**


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Lack of glutathione peroxidase-1 accelerates cardiac-specific hypertrophy and dysfunction in angiotensin II hypertension.

Ardanaz, Yang et al.: Role of Gpx1 in AngII-induced cardiac hypertrophy.

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METHODS

Animals

Male Gpx1-/- mice backcrossed to the C57Bl/6J background for greater than 10 generations were kindly provided by Dr. Y. Ho (Wayne State University, Detroit, MI) and subsequently bred at our institution. Age-matched C57BL/6J mice served as wild-type controls (wildtype) and were purchased from Jackson Laboratories (Bar Harbor, ME). Animals were housed in a temperature-controlled environment with a 12-h:12-h light-dark cycle, receiving standard mouse chow and tap water ad libitum. This study was approved by the Institutional Animal Care and Use Committee of Henry Ford Hospital and conforms to the animal care guidelines required by the National Institutes of Health.

Radio-telemetric Measurement of Mean Arterial Blood Pressure

Eighteen- to twenty-week old mice were instrumented with telemetric transmitters (PA-C10, Data Sciences International; St. Paul, MN) as previously described 1. Mice were anesthetized with pentobarbital (50 mg/kg, i.p.). Their left common carotid artery was isolated under a dissecting microscope and a transmitter catheter was introduced into the aortic arch and tied to the carotid artery and secured with tissue adhesive. The radio transmitter was placed subcutaneously along the flank between the forelimb and hind limb and the wound closed with 4-0 suture. Mice were allowed to recover for at least 1 wk. Subsequently, arterial pressure and heart rate were continuously recorded, integrated over 5 minutes and reported as 24-h means ± SEM.

Infusion of Vehicle or AngII

Mice were anesthetized with brevital (70 mg/kg, i.p.) to allow the subcutaneous implantation of osmotic minipumps (Alzet 1007D). Mice were implanted with minipumps infusing either vehicle (saline with 0.01 N acetic acid) or AngII (521 ng/kg*min, s.c.; Bachem, Torrance, CA) dissolved in vehicle for 7 days.

Echocardiographic Evaluation of Cardiac Morphology and Function

Left ventricular wall thickness, dimensions and shortening fraction (SF) were evaluated with a Doppler echocardiograph equipped with a 15-MHz linear transducer (Acuson C256) in awake mice 2. All studies were performed on awake mice before (day 0) and after AngII treatment (day 7). Echocardiographic images were traced manually and digitized by goal-directed, diagnostically-driven software within the echocardiograph. Measurements taken at three beats of the heart were averaged. Data were expressed as values taken at days 0 and 7 as well as the percent change from day 0. All measurements were done by leading edge to leading edge according to the American Society of Echocardiography guidelines.

Measurements of Interventricular Septum (IVST), Posterior Wall Thickness (PWT), and Left Ventricular (LV) Mass.

LV mass was calculated using the equation 2:

\[ \text{LV mass} = 1.055[(\text{IVST} + \text{LVDd} + \text{PWT})^3 - (\text{LVDd})^3] \]

where 1.055 is the specific gravity of the myocardium, IVSTd is diastolic interventricular septum thickness, LVDd is diastolic LV dimension, and PWT is diastolic posterior wall thickness. The derived LV mass was normalized for body weight and expressed as mg/10g body weight.
Measurements of Cardiac Chamber Dimensions
End-diastolic and end-systolic LV dimensions (LVDd and LVDs), interventricular septum and posterior wall thickness were measured from the M-mode tracings. During diastole, LV dimension and wall thickness were measured from the maximum chamber cavity; during systole they were measured during maximum anterior motion of the posterior wall.

LV Shortening Fraction
LV shortening fraction, a measure of LV systolic function, was calculated from the M-mode LV dimensions using the equation: $SF(\%) = \frac{(LVDd - LVDs)}{LVDd} \times 100$.

Preparation of Tissue Samples
On day 7 after mini-pump implantation, mice were anesthetized and the heart was stopped during diastole by injecting a 15% potassium chloride solution into the left ventricle. The animals were transcardially perfused with phosphate-buffered saline (PBS) followed by 4% formaldehyde in PBS under pressure (100 mm Hg) and hearts and thoracic aortas were removed. Hearts were weighed and total heart weight/body weight (THW/BW) ratio (mg/10g) served as parameter of cardiac hypertrophy. The LV was transversally cut into three pieces. The LV middle section and the descending thoracic aorta (5 mm below the subclavian artery) were processed, embedded in paraffin, and serially sectioned (6-μm sections) for a variety histological and morphometric analyses.

Myocyte cross-sectional area and interstitial collagen fraction
6-μm paraffin-embedded sections were de-paraffinized and re-hydrated with distilled water. Sections were soaked in Bouin’s fluid at 56°C overnight at room temperature and washed with running tap water until the rinse fluid ran colorless (about 1 hr). Sections were incubated with 0.1% picrosirius red solution for 1.5 hr at room temperature on a rotary shaker and washed twice in 0.5% acetic acid for 3-5 seconds. Sections were dehydrated in gradient alcohol, cleared in xylene and mounted in synthetic resin. Twenty one images of each left ventricle section was captured at 400 x magnification (IX81, Olympus America, Center Valley, PA) with a digital camera (DP70, Olympus America, Center Valley, PA). Myocyte cross-sectional area (MCSA) and per cent interstitial collagen fraction (ICF) was measured using an image analysis system (Microsuite Biological imaging software, Olympus America, Melville, NY) averaging per cent values of the 21 images and expressed as per cent means ± SEM.

Histological Examination of Cross-Sections of Mouse Thoracic Aorta for Measurement of Vascular Remodeling.
Sections were stained with Masson Trichrome Accustain (Sigma). Briefly, sections were de-paraffinized and hydrated, then preheated with Bouin’s solution (Sigma) at 56°C for 15 min as we previously described. The slides were cooled and washed in running water. Sections were stained in working Weigert iron hematoxylin solution for 5 min, rinsed in de-ionized water, stained in Biebrich scarlet-acid fuchsin (Sigma) for 5 min and rinsed in de-ionized water. Slides were placed in working phosphotungstic/ phosphomolybdic acid solution for 5 min and then in aniline blue solution (Sigma) for 5 min. Slides were washed in 1% acetic acid for 2 min, rinsed and dehydrated in alcohol, cleared in xylene, and mounted. Cross-sectional area (CSA), thickness of the media (Wm) and external perimeter (Pe) were digitally measured using
Microsuite Biological imaging software (Olympus America, Melville, NY). The diameter of the lumen (L) was calculated using the formula \( L = 2 \times \left( \frac{Pe}{2\pi} - \frac{CSA}{\pi} \right)^{1/2} \). Remodeling was determined among groups by comparing the ratio of medial thickness to lumen diameter.

**Measurements of Glutathione Peroxidase and in Aortic Tissue**

Tissue homogenates were prepared by homogenizing the tissues on ice in 50 mM potassium phosphate, pH 7.0 containing 1 mM EDTA. Homogenates were centrifuged at 10,000 x g for 15 min at 4°C. Supernatants were assayed for protein and stored at -80°C until the day of the assay. Glutathione peroxidase (Gpx) activity was determined using assay kit # FR 17 from Oxford Biomedical Research (Oxford, MI) following the manufacturers protocol. The assay compares sample Gpx activity to a standard curve of Gpx activity generated by purified Gpx (Sigma Aldrich). Glutathione peroxidase utilizes kit substrate tert-butyl hydroperoxide to produce oxidized glutathione. Kit reagent glutathione reductase recycles the oxidized glutathione to reduced glutathione, in turn oxidizing NADPH to NADP⁺ which results in decreased spectrophotometric absorbance at 340 nm. The rate of decrease of A340 is directly proportional to Gpx activity.

**Western Blots**

Mouse hearts were homogenized in PBS-10mmole/L EDTA, 0.086 mg/ml aprotinin, with a T-50 homogenizer for 30 sec. Homogenates were centrifuged at 16000 x g, supernatant recovered and stored at -80°C. Samples (30 μg) were run on 15 % PAGE-SDS gels, and blotted with anti-Gpx1 (Abcam), anti-AT1 antibody (Santa Cruz) and anti-β-actin. Western Blots were probed with secondary antibodies labeled with IRDye680 and 800 and analyzed by the Odyssey Imager and its software (Li-Cor Biosciences).

**Data Analysis**

Data are expressed as mean ± SEM. Comparisons between groups were made using analysis of variance, followed by Hochberg’s method for multiple comparisons. A value of \( p < 0.05 \) was considered statistically significant.

**References**


Fig. S1: Gpx1 protein levels detected by Western blot. Rat heart homogenates were run on SDS-PAGE and blotted with a rabbit antibody against Gpx 1. Western blots were analyzed using secondary antibodies labeled with IRDye 800CW, the Odyssey Imager and software from Li-COR Biosciences. Data are expressed average band intensities of Gpx 1 ± SEM (n = 2-5).
Fig. S2: AT-1 receptor levels detected by Western blot. Rat heart homogenates were run on SDS-PAGE and blotted with a rabbit antibody against AT-1 receptor and a goat antibody against β-actin. Western blots were analyzed using secondary antibodies labeled with IRDye680 and 800CW, the Odyssey Imager and software from Li-COR Biosciences. Data are expressed as the ratio of band intensities of AT-1 receptor/β-Actin ± SEM (n = 3).