Cardiac-Specific Overexpression of Catalase Identifies Hydrogen Peroxide-Dependent and -Independent Phases of Myocardial Remodeling and Prevents the Progression to Overt Heart Failure in \( \text{G}q \)-Overexpressing Transgenic Mice

Fuzhong Qin, MD, PhD; Shannon Lennon-Edwards, PhD; Steve Lancel, PhD; Andreia Biolo, MD; Deborah A. Siwik, PhD; David R. Pimentel, MD; Gerald W. Dorn, MD; Y. James Kang, PhD; Wilson S. Colucci, MD

**Background**—Although it seems that reactive oxygen species contribute to chronic myocardial remodeling, questions remain about (1) the specific types of reactive oxygen species involved, (2) the role of reactive oxygen species in mediating specific cellular events, and (3) the cause-and-effect relationship between myocardial reactive oxygen species and the progression to heart failure. Transgenic mice with myocyte-specific overexpression of \( \text{G}q \) develop a dilated cardiomyopathy that progresses to heart failure. We used this model to examine the role of O$_2$H in mediating myocardial remodeling and the progression to failure.

**Methods and Results**—In \( \text{G}q \) myocardium, markers of oxidative stress were increased at 4 weeks and increased further at 20 weeks. \( \text{G}q \) mice were crossbred with transgenic mice having myocyte-specific overexpression of catalase. At 4 weeks of age, left ventricular end-diastolic dimension was increased and left ventricular fractional shortening decreased in \( \text{G}q \) mice and deteriorated further through 20 weeks. In \( \text{G}q \) mice, myocardial catalase overexpression had no effect on left ventricular end-diastolic dimension or fractional shortening at 4 weeks but prevented the subsequent deterioration in both. In \( \text{G}q \) mice, myocyte hypertrophy; myocyte apoptosis; interstitial fibrosis; and the progression to overt heart failure, as reflected by lung congestion and exercise intolerance, were prevented by catalase overexpression.

**Conclusion**—In \( \text{G}q \) mice, myocyte-specific overexpression of catalase had no effect on the initial phenotype of left ventricular dilation and contractile dysfunction but prevented the subsequent progressive remodeling phase leading to heart failure. Catalase prevented the cellular hallmarks of adverse remodeling (myocyte hypertrophy, myocyte apoptosis, and interstitial fibrosis) and the progression to overt heart failure. Thus, O$_2$H, associated oxidant pathways, or both play a critical role in adverse myocardial remodeling and the progression to failure. (Circ Heart Fail. 2010;3:306-313.)

**Key Words:** free radicals ■ heart failure ■ remodeling ■ apoptosis ■ hypertrophy

Several lines of evidence suggest that reactive oxygen species (ROS) contribute to adverse myocardial remodeling and the progression to failure.\(^1,2\) However, several important questions remain. First, relatively little is known about the specific types of ROS involved (eg, superoxide, hydrogen peroxide, and peroxynitrite). Second, although ROS can cause several cellular events (eg, myocyte hypertrophy, apoptosis, and fibrosis) that participate in myocardial remodeling,\(^1,3\) little is known about the specific role of ROS in mediating these events. Finally, little is known about the cause-and-effect relationship of ROS to the overall progression to myocardial failure.

The G-protein \( \text{G}q \) mediates signaling for several stimuli (eg, norepinephrine, angiotensin, and mechanical strain) that cause hypertrophy and apoptosis in cardiac myocytes in vitro.\(^4\) The relevance of \( \text{G}q \) signaling for myocardial remodeling and failure in vivo\(^5\) is supported by the demonstration that transgenic mice with cardiac myocyte-specific overexpression of \( \text{G}q \) develop myocardial remodeling that progresses to a dilated cardiomyopathy.\(^6\) Oxidative stress is increased in the myocardium of \( \text{G}q \) mice\(^7\) and has been implicated in the pathophysiology of myocardial remodeling and failure in this model.
We therefore used Gq mice to test the hypothesis that H₂O₂ mediates pathological remodeling in vivo and to delineate the mechanisms involved. Accordingly, mice with cardiac-specific overexpression of Gq were crossbred with transgenic mice with myocyte-specific overexpression of catalase,⁸,⁹ the primary enzyme responsible for detoxification of H₂O₂. To understand the temporal relationship of myocardial remodeling, left ventricular (LV) dimensions and function were assessed serially from 4 to 20 weeks of age.

Methods

Experimental Animals

Transgenic mice with cardiac-specific overexpression of Gq (Gq-40 mice, FVB/N)⁹ and wild-type (WT, FVB/N) mice were crossbred with transgenic mice having myocyte-specific overexpression of catalase (Line 742; 60X catalase activity; FVB/N).⁹ The resulting males (n = 5 to 9 in each group) were used in this study. The protocol was approved by the Institutional Animal Care and Use Committee at Boston University School of Medicine (Boston, Mass).

Echocardiographic Measurements

LV dimensions and function were measured in nonanesthetized mice using an Acuson Sequoia C-256 echocardiograph machine equipped with a 15 MHz linear transducer (model 15L8). Briefly, the heart was imaged in the 2D parasternal short-axis view, and an M-mode echocardiogram of the midventricle was recorded at the level of papillary muscles. Anterior wall thickness, posterior wall thickness, LV end-diastolic dimension (EDD), and end-systolic dimension were measured from the M-mode image. LV fractional shortening was calculated as (EDD−ESD)/EDD×100; where ESD indicates end-systolic dimension. LV relative wall thickness was calculated as (AWT+PWT)/EDD, where AWT indicates anterior wall thickness; PWT, posterior wall thickness.

Exercise Capacity

Maximal exercise capacity was tested at 20 weeks using a rodent treadmill with air-puff motivation.¹⁰,¹¹ Total exercise time was recorded as the elapsed time to exhaustion and then converted to distance. Exhaustion is defined as the point at which the animal cannot keep pace with the treadmill (within 15 seconds) despite the airflow motivator. The maximal exercise capacity was calculated as the total distance run by the animal during the exercise protocol.

Organ Weight and Histology

The mice were sacrificed at 20 weeks of age. The heart, LV with septum, lung, and liver were weighed. LV samples were fixed in 10% buffered formalin, embedded with paraffin, and sectioned. Sections were stained with hematoxylin and eosin and examined under a light microscope. Five random fields from each of 4 sections per animal were analyzed, and 60 myocytes per animal were measured. The quantification of myocardite cross-section area was determined using ImageJ software (National Institutes of Health, Bethesda, Md). To assess fibrosis, sections were stained with Masson trichrome kit and examined under a light microscope.

Immunohistochemistry for 3-Nitrotyrosine and 4-Hydroxy-2-Nonenal

LV tissue sections (4 μm) were blocked with 10% goat serum in phosphate-buffered saline, incubated with rabbit anti-3-nitrotyrosine (NY) polyclonal antibody or mouse anti-4-hydroxy-2-nonenal (HNE) monoclonal antibody and then incubated with goat biotin-conjugated antirabbit IgG or goat biotin-conjugated antimouse IgG. The sections were incubated with avidin and biotinylated horseradish peroxidase macromolecular complex and stained with 3-amin0-9-ethylcarbazole and hematoxylin. For negative control, the primary antibody was omitted instead of normal rabbit IgG or normal mouse IgG. The samples were examined under a light microscope. Ten color images of NY or HNE staining were randomly selected from 4 sections of the heart and photographed at a magnification of ×40. The area and intensity of staining were blinded to score for quantification. The scoring range was as follows: 0, no visible staining; 1, faint staining; 2, moderate staining; and 3, strong staining.

Ratio of Reduced-to-Oxidized Glutathione

The reduced-to-oxidized glutathione (GSH/GSSG) ratio was determined in LV tissue homogenate as described using an Oxis GSH/GSSG-412 kit.¹²

TUNEL Staining

Apoptosis was assessed using an in situ cell death detection fluorescein kit according to the manufacturer’s instructions. Briefly, LV sections were incubated with the reaction mixture containing terminal deoxynucleotidyl transferase and fluorescein-labeled dUTP. To identify cardiomyocytes, sections were incubated with mouse anti-α-sarcomeric actin monoclonal antibody and then incubated with goat antirabbit IgG-conjugated tetramethylrhodamine B isothiocyanate. Finally, to identify all nuclei (nonapoptotic and apoptotic), sections were stained with Hoechst 33258. The samples were analyzed under a fluorescence microscope. Four sections per animal.
were analyzed. Cardiomyocyte nuclei were determined by random counting of 10 fields per section. The number of apoptotic nuclei was calculated per 10,000 cardiomyocytes.

Statistical Analysis
Results are presented as mean±SEM. The statistical significance of differences among groups or between 2 means was determined using repeated-measures ANOVA and the Bonferroni test for multiple comparisons. The incidence of pleural effusions was tested by the Fisher exact test. P<0.05 was considered statistically significant.

Results
Age-Related Oxidative Stress in the Gαq Mouse Heart
The GSH/GSSG ratio, an index of overall cellular oxidative stress, was measured in hearts from WT and Gαq mice at 4 and 20 weeks of age. GSH was unchanged in Gαq versus WT hearts at either age. In Gαq mice, GSSG was increased at 4 weeks and increased further at 20 weeks, and likewise, the GSH/GSSG ratio was decreased at 4 weeks and decreased further at 20 weeks (Figure 1). Consistent with the observed decrease in GSH/GSSG ratio at 20 weeks, immunohistochemical staining of myocardium from Gαq mice at this age demonstrated marked increases in 2 markers of oxidative stress, NY and HNE, both of which were visualized diffusely over myocytes (Figure 2).

Catalase Overexpression Attenuates Myocardial Oxidative Stress
Based on the observed increase in oxidative stress in the Gαq mouse heart, we crossbred Gαq mice with the mice that overexpress catalase in a myocyte-specific manner that were generated by Kang and coworkers.8,9 In the Gαq/catalase mice, the intensities of both NY and HNE staining were reduced to the levels present in WT mice (Figure 2), and likewise, the GSH/GSSG ratio was normalized to a value similar to that in WT mice (Figure 2E). Thus, oxidative stress in the Gαq mouse heart is sensitive to myocyte-specific expression of catalase.

Time Course of Cardiac Remodeling and Failure
Although several studies have demonstrated the development of severe dilated cardiomyopathy in Gαq mice, relatively little is
known about the time course of remodeling. Accordingly, we performed echocardiography every 4 weeks beginning at 4 weeks of age, the first age at which these studies were feasible. At 4 weeks of age, there was already marked eccentric LV remodeling, with chamber dilation, reduced wall thickness, and decreased fractional shortening (Figure 3). Subsequently, LV size and function remained relatively stable until 8 weeks of age, after which all progressively deteriorated (Figure 3).

**Myocyte-Specific Catalase Overexpression Prevents the Progressive Remodeling Phase**

Myocyte-specific overexpression of catalase had no effect on any measure of remodeling at 4 or 8 weeks of age (Figure 3). However, beginning at 12 weeks of age, catalase-overexpressing Gq mice had significant improvements in all measures of remodeling such that progression between 4 and 20 weeks of age was almost completely halted. Thus, although the early phenotype in this genetic model is not catalase sensitive, the subsequent progressive phenotype is highly sensitive to catalase.

**Myocyte-Specific Catalase Overexpression Prevents Myocyte Hypertrophy, Apoptosis, and Interstitial Fibrosis**

At 20 weeks, LV weight in Gq mice was increased ~14%. The increase in LV weight was prevented in the Gq/catalase mice (Table). Likewise, myocyte cross-sectional area was markedly increased in Gq mice (versus WT), and the increase was attenuated but not completely prevented in Gq/catalase mice (Figure 4). Myocardial fibrosis, assessed by Masson trichrome staining, revealed increased interstitial fibrosis in Gq mice that was almost completely prevented in Gq/catalase mice (Figure 5).

Myocyte apoptosis was assessed by TUNEL staining using triple labeling to colocalize fragmented nDNA, nuclei (Hoechst 33342), and α-sarcomeric actin. Specificity of the technique to detect DNA fragmentation was documented by positive labeling of nuclei after exposure to deoxyribonuclease I (Figure 6A through 6D). DNA fragmentation was absent when the terminal deoxynucleotidyl transferase was omitted in the enzymatic reaction (data not shown). Representative images from WT,
Table. Body and Organ Weights

<table>
<thead>
<tr>
<th></th>
<th>WT (n=6)</th>
<th>CAT (n=9)</th>
<th>Gαq (n=5)</th>
<th>Gαq/CAT (n=5)</th>
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<tr>
<td>BW, g</td>
<td>31.7±1.4</td>
<td>30.9±0.8</td>
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<td>HW, mg</td>
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<td>HW/BW, mg/g</td>
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<td>4.63±0.09</td>
<td>5.57±0.18*</td>
<td>4.99±0.16†</td>
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<td>LV W, mg</td>
<td>95.9±2.6</td>
<td>96.8±2.5</td>
<td>109.5±2.7*</td>
<td>98.3±3.1†</td>
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<td>LV W/BW, mg/g</td>
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<td>3.08±0.04</td>
<td>3.21±0.02*</td>
<td>3.04±0.03†</td>
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<td>Lung W, mg</td>
<td>166.1±8.2</td>
<td>170.9±5.0</td>
<td>211.5±8.4*</td>
<td>179.5±4.5*</td>
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<td>Lung W/BW, mg/g</td>
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<td>6.22±0.26*</td>
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<td>Lung W, wet/dry</td>
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<td>0/9</td>
<td>4/5*</td>
<td>0/5†</td>
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</tbody>
</table>

Values are presented as mean±SE. BW indicates body weight; CAT, catalase; HW, heart weight; W, weight. *P<0.05 versus WT group. †P<0.05 versus Gαq group.

Gαq, and Gαq/catalase mice are shown in Figure 6E through 6P. Quantification of apoptotic myocytes demonstrated an ∼9-fold increase in Gαq mice that was almost completely prevented in Gαq/catalase mice (Figure 6Q).

Catalase Prevents the Development of Overt Heart Failure

Gαq mice had evidence of functional impairment with an ∼40% reduction in maximal exercise capacity compared with WT mice. In Gαq/catalase mice, exercise capacity was preserved and similar to that in WT mice (Figure 7). During the course of 20 weeks, 1 Gαq mouse died, whereas no WT, catalase, or Gαq/catalase mice died. Of the surviving Gαq mice, 80% had pleural effusions at sacrifice, whereas pleural effusion was not evident in any WT, catalase, or Gαq/catalase mice (Table). Likewise, at sacrifice, lung weight was increased in the Gαq mice, indicative of lung congestion, and the increase was prevented by catalase overexpression (Table). Thus, at 20 weeks of age in the Gαq mice, there was functional and anatomic evidence of overt heart failure, which was prevented by myocyte-specific expression of catalase.

Discussion

The major new finding of this study is that myocyte-specific expression of catalase has no effect on the initial abnormal myocardial phenotype in Gαq mice but largely prevents subsequent progressive LV remodeling and the development of overt heart failure. The beneficial effect of catalase is associated with marked inhibition of myocyte hypertrophy, myocyte apoptosis, and interstitial fibrosis. These findings address several fundamental, unresolved questions about the role of ROS in myocardial failure.

Mice with myocyte-specific expression of Gαq develop a severe dilated cardiomyopathy. Because most studies in the Gαq mouse have focused on a single time point, relatively...
little is known about the time course during which myocardial remodeling and progression to heart failure occur. Echocardiography at 4 weeks of age, immediately after weaning, showed marked LV dilation, eccentric hypertrophy, and systolic dysfunction. We cannot determine the time course of this early phase of adverse remodeling. Between 4 and 8 weeks of age, there was only slight worsening in LV structure and function, but by 12 weeks, there was clear deterioration with further LV dilation and systolic dysfunction that steadily worsened through 20 weeks of age. Thus, in the Gq/CAT mice, there was an initial phase (4 weeks) marked by myocardial dysfunction that was followed by a second phase (12 to 20 weeks) characterized by progressive remodeling and failure.

Increased oxidative stress in the myocardium has been observed in several animal models of heart failure, including pressure overload, myocardial infarction, and rapid pacing, and in humans with heart failure. In the Gq/CAT mouse, we found that immunohistochemical staining for 2 markers of oxidative stress, NY and HNE, was positive at 20 weeks of age. Likewise, at 20 weeks, the GSH/GSSG ratio, a measure of overall cellular oxidative stress, was markedly reduced due to an increase in GSSG. Of note, the GSH/GSSG ratio also was decreased at 4 weeks of age, the earliest age studied, but was less reduced than at 20 weeks, indicating that myocardial oxidative stress in the Gq/CAT mouse heart increases over time. These findings are consistent with those of Satoh et al, who recently showed increased levels of ROS and peroxide-derived oxygen radicals in the Gq/CAT mouse heart at 14 to 15 weeks of age. Our study thus confirms the presence of increased myocardial oxidative stress in this and other heart failure models and, further, demonstrates that oxidative stress increases with the progression of LV remodeling.

To assess the relationship between increased oxidative stress and myocardial remodeling in the Gq/CAT mouse heart, we crossbred Gq/CAT mice with mice that have myocyte-specific expression of catalase. The catalase mouse overexpresses catalase by ~60-fold and has been shown to rescue the cardiac effects of doxorubicin and ischemia and reperfusion. The concurrent overexpression of catalase in Gq/CAT mice had no effect on the initial phenotype, as assessed by echocardiography. In striking contrast, catalase abolished the subsequent progressive deterioration that occurred after 8 weeks of age.
weeks such that there were no further changes in LV diameter, wall thickness, or systolic function. In parallel, catalase restored the protein markers of oxidative stress NY and HNE to control levels observed in normal mice. One limitation of our study is that we do not know the exact time at which catalase expression increased. However, both the catalase and Gq transgenes are under the control of the α-myosin heavy chain promoter, and, therefore, the expression of both would be expected to increase rapidly in the days immediately after birth.

In cardiac myocytes in vitro, we and others have shown that ROS, including H₂O₂, can cause hypertrophy and apoptosis, hallmarks of myocardial remodeling. An important feature of the dilated cardiomyopathy in the Gq mouse is eccentric hypertrophy with severe LV wall thinning relative to chamber diameter. At the cellular level, this cardiomyopathy is associated with myocyte hypertrophy and apoptosis, both of which were essentially abolished by catalase as was progressive wall thinning. Thus, these data clearly indicate that myocyte hypertrophy and apoptosis that occur during the progression phase are sensitive to catalase and, thus, mediated by ROS. It should be noted that myocyte widening, as observed in this study, not only is more typical of concentric hypertrophy, but also may occur in eccentric hypertrophy in conjunction with myocyte lengthening. In addition, given the marked decrease in wall thickness observed by echocardiography, the finding of myocyte widening suggests that there is myocyte loss, as would be expected based on the increased rate of apoptosis observed.

Recently, Satoh et al showed in the Gq mouse that estrogen markedly improve remodeling in association with decreased levels of ROS, increased expression of the thioredoxin system, and inhibition of apoptosis-related signaling kinase-1. They concluded that estrogen ameliorated heart failure by antioxidant mechanisms that involve the upregulation of thioredoxin and inhibition of Rac1-mediated nicotinamide adenine dinucleotide phosphate oxidase activity and apoptosis signal-regulating kinase-1. Our observations support the importance of oxidative stress in this model and provide further information about the specific role of H₂O₂, associated oxidative pathways, or both in mediating myocyte apoptosis, myocyte hypertrophy, and fibrosis.

Oxidative stress is systemic in heart failure and may be involved in wide-ranging effects on the vasculature and other organs that can influence cardiac remodeling. Previous studies that have examined the role of ROS in myocardial remodeling have used the systemic administration of antioxidants such as probucol, EUK-8, dimethylthiourea, vitamins A and E, estrogen, or the generalized overexpression of an antioxidant enzyme. Thus, an important and unique feature of the myocyte-specific Gq and catalase mice used in this study is that the resulting observations allow conclusions about the role of ROS in the myocyte per se. In this regard, it is interesting that myocyte-specific catalase expression markedly suppressed interstitial fibrosis in the Gq mouse, suggesting that H₂O₂ in the myocyte may mediate fibrosis directly through the elaboration of collagen regulatory proteins, indirectly through paracrine effects on neighboring fibroblasts, or both.

Although superoxide anion is the primary type of ROS generated by mitochondria and oxidases in the heart, it is both short lived due to rapid dismutation to H₂O₂ by superoxide dismutases and membrane impermeant. By contrast, H₂O₂ is relatively longer lived and membrane permeant. H₂O₂ itself is reactive and may generate highly reactive hydroxyl radicals. Superoxide, H₂O₂, and hydroxyl radicals all have been observed to be increased in failing myocardium. Although our data are consistent with a direct role for H₂O₂ in mediating the observed events, it is also possible that some or all of these events are mediated by other ROS that are up- or downstream of H₂O₂.

In the Gq mouse, the effects of catalase distinguish the initial phenotype from the progressive remodeling that eventually results in overt heart failure and in doing so, suggest that in this model these phases involve distinct pathophysiological. The initial phenotype in the Gq mouse may be a reflection of transcriptionally determined events mediated by Gq. A consequence of this initial phenotype is the stimulation of a subsequent, ROS-dependent remodeling process. It is noteworthy that catalase prevented the progression of myocardial remodeling and the subsequent development of overt heart failure with lung congestion, pleural effusions, and exercise intolerance. In patients, the development of clinical heart failure often is the culmination of months or years of remodeling following a discrete injury to the myocardium (eg, myocardial infarction). With time, progressive myocardial remodeling leads to declining cardiovascular function and overt heart failure. In this regard, it is of interest that in the Gq mouse, the early phenotype, although associated with substantial abnormalities in LV geometry and function, was not associated with a reduction in exercise capacity. This finding might be analogous to the clinical setting in a patient with asymptomatic LV dysfunction. These observations thus raise the possibility that strategies to inhibit the ROS-dependent phase of myocardial remodeling may be of value in the prevention of heart failure in humans.

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**Disclosures**

None.

**References**


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