**N-acetylcysteine reverses diastolic dysfunction and hypertrophy in familial hypertrophic cardiomyopathy**

Tanganyika Wilder, David M. Ryba, David F. Wieczorek, Beata M. Wolska, and R. John Solaro

1Department of Physiology and Biophysics and the Center for Cardiovascular Research, College of Medicine, University of Illinois at Chicago, Chicago, Illinois; 2Department of Biology, College of Science and Technology, Florida A & M University, Tallahassee, Florida; 3Department of Medicine, Section of Cardiology, University of Illinois at Chicago, Chicago, Illinois; and 4Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio

Submitted 11 May 2015; accepted in final form 25 September 2015

N-acetylcysteine reverses diastolic dysfunction and hypertrophy in familial hypertrophic cardiomyopathy. Am J Physiol Heart Circ Physiol 309: H1720–H1730, 2015. First published October 2, 2015; doi:10.1152/ajpheart.00339.2015—S-glutathionylation of cardiac myosin-binding protein C (cMyBP-C) induces Ca\(^{2+}\) sensitization and a slowing of cross-bridge kinetics as a result of increased oxidative signaling. Although there is evidence for a role of oxidative stress in disorders associated with hypertrophic cardiomyopathy (HCM), this mechanism is not well understood. We investigated whether oxidative myofilament modifications may be in part responsible for diastolic dysfunction in HCM. We administered N-acetylcysteine (NAC) for 30 days to 1-mo-old wild-type mice and to transgenic mice expressing a mutant tropomyosin (Tm-E180G) and nontransgenic littermates. Tm-E180G hearts demonstrate a phenotype similar to human HCM. After NAC administration, the morphology and diastolic function of Tm-E180G mice was not significantly different from controls, indicating that NAC had reversed baseline diastolic dysfunction and hypertrophy in our model. NAC administration also increased sarco(end)oplasmic reticulum Ca\(^{2+}\) ATPase protein expression, reduced extracellular signal-related kinase 1/2 phosphorylation, and normalized phosphorylation of phospholamban, as assessed by Western blot. Detergent-extracted fiber bundles from NAC-administered Tm-E180G mice showed nearly nontransgenic (NTG) myofilament Ca\(^{2+}\) sensitivity. Additionally, we found that NAC increased tension cost and rate of cross-bridge reattachment. Tm-E180G myofilaments were found to have a significant increase in S-glutathionylation of cMyBP-C, which was returned to NTG levels upon NAC administration. Taken together, our results indicate that oxidative myofilament modifications are an important mediator in diastolic dysfunction, and by relieving this modification we were able to reverse established diastolic dysfunction and hypertrophy in HCM.

Hypertrophic Cardiomyopathy (HCM) often progresses to heart failure with preserved ejection fraction (HFP EF), an increasingly common diagnosis characterized by severely impaired diastolic function with little or no effect on systolic cardiac function. The etiology of HFP EF remains unclear, but with many risk factors that range from genetic predisposition, as seen in familial HCM, to common acquired conditions, such as hypertension and hyperlipidemia, HFP EF-related diagnoses are likely to rise in the coming years. However, there are few treatments targeted to relieve the diastolic dysfunction observed in HFP EF.

Despite strong implications for a role of redox-related modifications in HCM linked to mutations in sarcomeric proteins, there have been no studies investigating the potential role of reactive oxygen species (ROS)-induced exacerbation of the HCM phenotype at the level of oxidative modification of the myofilament proteins. Our previous studies indicate that ROS-related posttranslational modifications in sarcomeric proteins are likely to be a major mechanism in the diastolic abnormalities associated with familial HCM. These studies reported that ROS, associated with a hypertensive model of HFP EF, induced S-glutathionylation of cardiac myosin-binding protein C (cMyBP-C), which slowed cross-bridge kinetics and increased Ca\(^{2+}\) sensitivity. Ventricular myocytes from the model demonstrated diastolic abnormalities with no change in the Ca\(^{2+}\) transients, suggesting that the diastolic dysfunction was attributed to dysfunction at the level of the sarcosome. Restoring redox balance in this model by relieving NOS uncoupling restored diastolic function to control levels in correlation with a reduction of cMyBP-C S-glutathionylation to basal levels (5, 12).

In experiments reported here, we assessed the redox state and function of a transgenic (Tg) mouse model of familial HCM expressing a mutation in tropomyosin (Tm), where glutamic acid at residue 180 has been exchanged for a glycine (Tm-E180G). Tm-E180G mice develop severe diastolic dysfunction, hypertrophy, and left atrial dilation by 2 wk of age (1). Our previous studies reported that, compared with controls, this model demonstrates a significant increase in myofilament response to Ca\(^{2+}\) and diastolic dysfunction that is likely to trigger maladaptive remodeling (21, 26). Here, our current data show that Tm-E180G hearts display early signs of oxidative stress in the form of increased oxidative modifications of cMyBP-C and activation of the MAPK signaling cascade. Therefore, we hypothesized that treatment with the glutathione precursor N-acetylcysteine (NAC) may reverse the oxidative stress in our model and improve hypertrophy and diastolic...
dysfunction. NAC administration, beginning at 1 mo of age, reversed established cardiac hypertrophy and diastolic dysfunction in Tm-E180G mice while hastening cross-bridge kinetics and desensitizing the myofilaments to Ca\(^{2+}\) in both NTG and Tm-E180G mice. Our data support the hypothesis that altered redox state is a significant contributor to the familial HCM phenotype and that interventions restoring redox balance may be a viable therapeutic approach.

**MATERIALS AND METHODS**

All protocols and procedures involving the use of animals were given prior approval by the Institutional Animal Care and Use Committee of the University of Illinois in Chicago (with Association for Assessment and Accreditation of Laboratory Animal Care International accreditation). Animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (8th ed., revised 2011) published by the National Institutes of Health.

**Tm-E180G transgenic mice.** We employed a transgenic mouse model in a FVB/N background that displayed a phenotype similar to human HCM, in which 65% of wild-type α-Tm is replaced with α-Tm-E180G (21). Previous studies have reported that from 2 wk of age Tg mice begin to exhibit cardiac hypertrophy, myocyte disarray, interstitial fibrosis, and diastolic dysfunction with preserved systolic function (1). Controls were non-Tg (NTg) FVB/N littermates.

**Study design and NAC administration.** One-month-old male and female NTG and Tm-E180G littermates were administered regular drinking water or NAC (Sigma, St. Louis, MO) dissolved in drinking water administered at 250 mg·kg\(^{-1}\)·day\(^{-1}\), based on prior data (14), for 4 wk. All mice were subjected to echocardiography before and after the 1-mo treatment. After 1 mo of NAC administration, mice were euthanized using a ketamine-xylazine mix (200 and 100 mg/kg, respectively), and hearts were extracted for functional and biochemical assessment.

**Echocardiography.** Echocardiographic measurements were performed using a high-resolution transducer (Vevo 770 High Resolution Imaging System with a scan head center frequency of 30 MHz) in isoflurane-anesthetized 2-mo-old mice, as described previously (22). M-mode images of the left ventricle (LV), left ventricle outflow tract (LVOT), and left atrium (LA) were taken from the left parasternal long-axis view. The parasternal short-axis view at the level of the papillary muscles was used to measure the LV internal dimension, and LV diameters were measured at three points along the fiber bundle. Force and displacement motor using aluminum T-clips, and the sarcomere diameter were measured automatically using methods and a previously described experimental apparatus (33). The fiber bundles were mounted between a force transducer and displacement motor using aluminum T-clips, and the sarcomere length set to 2.2 μm using He-Ne laser diffraction. The width and diameter were measured at three points along the fiber bundle. Force per cross-sectional area was used to determine tension. The fiber was contracted initially at a saturating Ca\(^{2+}\) concentration (pCa 4.5), and sarcomere length was again adjusted to 2.2 μm. Sarcomere length remained constant throughout the rest of the experiment. ATPase activity was measured at 20°C, as described previously, and calibrated with rapid injections of ADP (0.5 nmol) with a motor-controlled syringe. The fiber was placed in relaxing solution for 2 min and then in the preactivation solution for 2–3 min each time before being placed in the activating solution for 1–2 min (until stabilization of force). The fiber bundle was then quickly returned to the relaxing solution. Various contraction-relaxation cycles were carried out using different ratios of total Ca\(^{2+}\) concentration to total EGTA concentration. The final contraction was again carried out at a saturating Ca\(^{2+}\) concentration. All experiments were carried out at 20°C. The relation between Ca\(^{2+}\) and tension or ATPase activity was fitted using a modified Hill equation (7, 28). We determined pCa values at half-maximum ATPase activity and force generation from mean data normalized to maximum activity. In all experiments, only fiber bundles retaining >80% of their initial maximum tension were included in the analysis.
**RESULTS**

*N*-acetylcysteine improves cardiac morphology and diastolic function in hypertrophic cardiomyopathy. Beginning at 1 mo of age, after HCM phenotypic changes were evident, NAC was administered to NTg and Tm-E180G mice (1). We conducted a noninvasive assessment of cardiac morphology and function in Tm-E180G and NTg mice, as summarized in Table 1. M-mode echocardiography demonstrated that Tm-E180G mice have significant left atrial dilation, which was reduced to NTG levels with NAC administration for 1 mo. Hearts of Tm-E180G mice demonstrated an increase in left ventricular mass with no change in internal diameter at diastole, which was characteristic of concentric hypertrophy. Treatment with NAC returned LV mass to NTG levels. Measurements of diastolic function and E/A and E/Em ratios were also decreased to NTG levels. Systolic function was unchanged in Tm-E180G mice.

Activation of the MAPK pathway is implicated in the development of both physiological and pathological hypertrophy (6). Additionally, these pathways are activated by elevated ROS (15). Compared with controls, there was an increase in ERK1/2 phosphorylation in hearts from Tm-E180G mice, which returned to NTG levels with NAC administration (Fig. 1, A and B). Interestingly, this reversal of MAPK signaling and hypertrophy did not decrease levels of expression of the neonatal myosin heavy-chain isoform β-myosin heavy chain observed in our model (Fig. 1, C and D).

*N*-acetylcysteine affects SERCA2 expression and phosphorylation of PLN. We have reported previously that despite changes in phosphorylation of PLN and expression of SERCA2 in ventricular myocytes isolated from Tm-E180G hearts compared with NTG controls, there were no changes in Ca^{2+} transients (26). However, early adenoviral-mediated overexpression of SERCA2a prevented the development of hypertrophy and fibrosis in Tm-E180G mice (26). Therefore, we tested for NAC-induced alterations in Ca^{2+} regulatory proteins PLN and SERCA2. NAC treatment increased the expression of total cardiac SERCA2 protein expression in both NTG and Tm-E180G hearts (Fig. 2, A and D). NAC treatment induced phosphorylation of PLN at Ser^{16} in Tm-E180G hearts to a level close to NTG-levels, but the values failed to reach statistical significance; however, PLN phosphorylation at Thr^{17} was significantly elevated in the hearts of Tm-E180G mice and returned to control levels following NAC treatment (Fig. 2, B–D).

**N*-acetylcysteine decreases Ca^{2+} sensitivity and improves cross-bridge kinetics in detergent-extracted fiber bundles.** We employed detergent-extracted (skinned) fiber bundles from NTG and Tm-E180G hearts to determine the effect of antioxidant treatment on Ca^{2+} dependence of isometric tension, actomyosin ATPase rate, and the kinetics of tension redevelopment (k_{tr}), a measurement of the rate of entry of cross-bridges into the force-generating state. Results are summarized in Table 2. Consistent with previous reports (21), there was a significant increase in Ca^{2+} sensitivity of tension, as determined from the pCa at half-maximal tension, in Tm-E180G skinned fiber bundles compared with NTG controls. After 1 mo of NAC administration, fiber bundles from both NTG and Tm-E180G hearts demonstrated a decrease in Ca^{2+} sensitivity of tension and ATPase. Maximum tension and ATPase rate were unchanged in fibers from both NTG and Tg hearts treated with NAC (Fig. 3A). Compared with NTG controls, the Ca^{2+} sensitivity of ATPase rate was increased significantly in Tm-E180G fibers, and NAC administration significantly reduced the Ca^{2+} sensitivity of the rate of ATP hydrolysis in both groups (Fig. 3B). NAC administration also resulted in a significant increase in k_{tr}, the rate of cross-bridge reattachment, measured as a slope of a linear regression of k_{tr} values plotted against relative tension (P/P_0). Measurements of k_{tr} at minimum P/P_0 revealed no changes between the groups. However, k_{tr} at maximum P/P_0 revealed significant changes in NAC-administered NTG and Tm-E180G fibers, suggesting that as tension generation increases, NAC increases the rate of cross-bridge reattachment. The slope of the relation between tension and ATPase rate is a measurement of tension cost, is related to

**Table 1. NAC improves morphology and diastolic function in hypertrophic cardiomyopathy as assessed by echocardiography**

<table>
<thead>
<tr>
<th>Parameter (Sample Size)</th>
<th>NTg (n = 6)</th>
<th>NTg NAC (n = 9)</th>
<th>Tm-E180G (n = 6)</th>
<th>Tm-E180G NAC (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/females (n)</td>
<td>3/3</td>
<td>3/6</td>
<td>3/3</td>
<td>5/4</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>23.0 ± 1.73</td>
<td>21.7 ± 0.60</td>
<td>25.1 ± 1.57</td>
<td>21.8 ± 0.95</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>500 ± 51</td>
<td>473 ± 15</td>
<td>428 ± 8</td>
<td>542 ± 21</td>
</tr>
<tr>
<td>LA size, mm</td>
<td>1.26 ± 0.06</td>
<td>1.36 ± 0.08</td>
<td>2.19 ± 0.10***††</td>
<td>1.58 ± 0.06§</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>43.90 ± 5.27</td>
<td>49.17 ± 5.27</td>
<td>65.69 ± 5.21††</td>
<td>51.39 ± 3.95</td>
</tr>
<tr>
<td>LVID d, mm</td>
<td>3.73 ± 0.09</td>
<td>3.69 ± 0.11</td>
<td>3.75 ± 0.14</td>
<td>3.47 ± 0.10</td>
</tr>
<tr>
<td>LVID s, mm</td>
<td>2.28 ± 0.09</td>
<td>2.46 ± 0.10</td>
<td>2.26 ± 0.08</td>
<td>2.19 ± 0.10</td>
</tr>
<tr>
<td>Relative wall thickness</td>
<td>0.34 ± 0.03</td>
<td>0.29 ± 0.03</td>
<td>0.37 ± 0.03</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>69.95 ± 1.44</td>
<td>63.77 ± 2.61</td>
<td>70.98 ± 0.56</td>
<td>71.01 ± 1.47</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>38.89 ± 1.22</td>
<td>34.33 ± 1.94</td>
<td>39.65 ± 0.45</td>
<td>39.72 ± 1.18</td>
</tr>
<tr>
<td>E-wave, mm/s</td>
<td>774 ± 34</td>
<td>779 ± 38</td>
<td>831 ± 81</td>
<td>845 ± 71</td>
</tr>
<tr>
<td>A-wave, mm/s</td>
<td>516 ± 32</td>
<td>490 ± 26</td>
<td>308 ± 64††</td>
<td>540 ± 35§§</td>
</tr>
<tr>
<td>E-wave, mm/s</td>
<td>26.23 ± 2.77</td>
<td>26.74 ± 2.24</td>
<td>20.34 ± 1.15††</td>
<td>35.85 ± 4.50††</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.54 ± 1.14</td>
<td>1.63 ± 0.09</td>
<td>3.03 ± 0.32‡‡‡</td>
<td>1.64 ± 0.15§</td>
</tr>
<tr>
<td>E/Em ratio</td>
<td>30.94 ± 3.10</td>
<td>30.40 ± 2.20</td>
<td>41.44 ± 2.49††</td>
<td>28.05 ± 3.30§</td>
</tr>
</tbody>
</table>

Values are means ± SE. NAC, N-acetylcysteine; NTg, nontransgenic; LA, left atrium; LV, left ventricular; LVID d, LV internal dimension at diastole; LVID s, LV internal dimension at systole. *P < 0.05 and **P < 0.001 vs. NTG; $P < 0.05 and §§P < 0.0001 vs. Tm-E180G; †P < 0.01 and ††P < 0.001 vs. NTg NAC.
the rate of cross-bridge detachment, and followed the same trend as $k_d$ values, where tension cost was increased in fibers excised from NAC-administered mice. (Fig. 4, A and B). Thus, NAC administration results in desensitization of the myofilaments to Ca\textsuperscript{2+} and increase in cross-bridge kinetics.

**N-acetylcysteine alters phosphorylation and oxidative modifications of myofilament proteins.** Regulatory redox mechanisms that involve posttranslational modifications may account for the alterations in the cross-bridge kinetics found in Tm-E180G hearts. Therefore, we assessed the phosphorylation and S-glutathionylation levels of myofilament proteins (5, 12). Previously, we identified three S-glutathionylation sites on cMyBP-C (19).

At 2 mo of age, phosphorylation of cMyBP-C Ser\textsuperscript{282} was increased in Tm-E180G mice, which was reduced upon NAC administration (Fig. 5, B and C). Phosphorylation of cTnI at Ser\textsuperscript{23/24} was decreased in Tm-E180G mice compared with NTG NAC-administered mice. NAC was associated with increased cTnI phosphorylation in Tm-E180G mice administered NAC; however, this value was not significant from controls (Fig. 5, A and C). No other differences in myofilament protein phosphorylation were found using ProQ diamond phosphoprotein stain (data not shown).

Functionally, S-glutathionylation results in increased Ca\textsuperscript{2+} sensitivity with depressed cross-bridge kinetics. Therefore, we investigated whether S-glutathionylation of cMyBP-C contributes to diastolic dysfunction in HCM (5, 12). We found that Tm-E180G mice exhibit a significant increase in S-glutathionylation of cMyBP-C. NAC reduced the levels of S-glutathionylation of cMyBP-C to NTg control levels (Fig. 6). Further analysis revealed that S-glutathionylation of Tm was significantly reduced in NAC-administered NTG and Tm-E180G mice. Additionally, we performed dinitrophenol (DNP) derivatization to determine the extent of carbonylation of myofilament proteins. Western blot analysis revealed that there was a significant increase in carbonylation of myosin heavy chain proteins.
DISCUSSION

Our data are the first to report that NAC administration is able to reverse an increase in myofilament Ca\(^{2+}\) sensitivity and diastolic dysfunction associated with S-glutathionylation of cMyBP-C in HCM. Moreover, we report novel evidence that administration of NAC alters expression of SERCA2 as well as phosphorylation of PLN. Previous studies reported that exogenous NAC treatment of models with HCM linked to a cTnT mutation is able to decrease fibrosis and hypertrophy (5, 12, 14). Additional studies were completed in rabbits containing an HCM-linked mutation on cardiac myosin heavy chain, where NAC administration was shown to have similar beneficial effects as in the cTnT murine model (10). Our studies significantly extend these findings in that in addition to reversing the hypertrophy and increase in left atrial size, we report evidence for a mechanistic basis for the reversal of diastolic abnormalities in HCM at the level of the myofilaments. The improvement of the diastolic abnormality was associated with the effects of NAC treatment on reducing the elevated Ca\(^{2+}\) sensitivity of Tm-E180G skinned fiber bundles and the hastening of cross-bridge kinetics.

A novel finding related to the improvement of diastolic function is a reversal of S-glutathionylation of cMyBP-C. Our previous studies reported a strong correlation of increased cMyBP-C S-glutathionylation with slowing of cross-bridge kinetics and an increase in myofilament response to Ca\(^{2+}\) in a model of diastolic heart failure (5, 12). In the present study, we

Table 2. NAC decreases Ca\(^{2+}\) sensitivity while hastening cross-bridge kinetics

<table>
<thead>
<tr>
<th>Parameter (Sample Size, Fibers)</th>
<th>NTg (n = 7)</th>
<th>NTg NAC (n = 6)</th>
<th>Tm-E180G (n = 7)</th>
<th>Tm-E180G NAC (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum tension, mN/mm(^2)</td>
<td>36.07 ± 1.84</td>
<td>27.78 ± 4.18</td>
<td>33.06 ± 2.27</td>
<td>31.03 ± 3.54</td>
</tr>
<tr>
<td>pCa(_{50}) of tension</td>
<td>5.78 ± 0.01</td>
<td>5.55 ± 0.06*</td>
<td>6.01 ± 0.05**,††</td>
<td>5.84 ± 0.05†,§</td>
</tr>
<tr>
<td>Max ATPase, pmol·s(^{-1})·mg(^{-1})</td>
<td>3.79 ± 0.07</td>
<td>3.64 ± 1.402</td>
<td>3.51 ± 12.91</td>
<td>377 ± 0.71</td>
</tr>
<tr>
<td>pCa(_{50}) of ATPase</td>
<td>5.73 ± 0.05</td>
<td>5.68 ± 0.03</td>
<td>6.17 ± 0.07**,††</td>
<td>5.86 ± 0.09§</td>
</tr>
<tr>
<td>Tension cost</td>
<td>9.78 ± 0.74</td>
<td>12.63 ± 0.80**</td>
<td>9.41 ± 0.74††</td>
<td>11.13 ± 0.53***,§§</td>
</tr>
<tr>
<td>k(<em>{s}) Slope, s(^{-1}/(P/P</em>{0}))</td>
<td>14.57 ± 0.81</td>
<td>22.98 ± 2.54**</td>
<td>14.15 ± 0.91†</td>
<td>23.47 ± 1.21**,§§</td>
</tr>
<tr>
<td>k(<em>{o}) (s(^{-1})) at minimum P/P(</em>{0})</td>
<td>3.76 ± 0.30</td>
<td>3.62 ± 0.88</td>
<td>3.17 ± 0.50</td>
<td>4.90 ± 1.02</td>
</tr>
<tr>
<td>k(<em>{o}) (s(^{-1})) at maximum P/P(</em>{0})</td>
<td>17.63 ± 0.74</td>
<td>28.20 ± 4.55*</td>
<td>13.77 ± 0.92††</td>
<td>27.93 ± 1.77**,§§</td>
</tr>
</tbody>
</table>

Values are means ± SE. pCa\(_{50}\), pCa at half-maximal tension; k\(_{o}\), kinetics of tension redevelopment. *P < 0.05 and **P < 0.01 vs. NTg; §P < 0.05 and §§P < 0.001 vs. Tm-E180G; †P < 0.05 and ††P < 0.001 vs. NTg NAC.
did not find a significant decrease in $k_r$ in skinned fiber bundles from Tm-E180G mice compared with NTg controls. We did find that NAC administration hastened $k_r$ and increased tension cost in both NTg and Tm-E180G skinned fiber bundles, suggesting that the improvement in diastolic function is related to a reduction in oxidative stress and an associated increase in cross-bridge kinetics.

The functional significance of cMyBP-C has generally been considered as a “brake” on the reaction of cross-bridges with the thin filament and as a desensitizer of the myofilaments to Ca$^{2+}$ (23). In Tm-E180G mice, we report differential phosphorylation of cMyBP-C, where phosphorylation of Ser$^{282}$ is increased. Although we observed a trend toward normalization in phosphorylation of Ser$^{282}$ in Tm-E180G mice, it was not significant. Additionally, this Ca$^{2+}$-desensitizing phosphorylation of cMyBP-C is present despite the constitutive increase in myofilament Ca$^{2+}$ responsiveness we observed, suggesting that $S$-glutathionylation of cMyBP-C is dominant in its Ca$^{2+}$-
sensitizing effect. Previously, we have identified that S-glutathionylation of cMyBP-C occurs at cysteine residues (C479, C627, C655) in the C3, C4, and C5 domains, whereas phosphorylation is known to occur at the M domain of the COOH terminus (19). Similar to phosphorylation, S-glutathionylation imparts a negative charge (27). Phosphorylation is thought to induce a Ca^{2+}-desensitizing effect by inducing steric hindrance of the myosin SI catalytic unit (18). S-glutathionylation may exert its dominant effect by performing the opposite, modifying the radial disposition of cMyBP-C toward the thick filament, where the C5 domain has been shown to interact away from myosin (19). A direct effect may be exerted by the increase in MHC carbonylation in Tg mice that was reversed with NAC administration. Reports have suggested MHC carbonylation is involved in the desensitization of the myofilament to Ca^{2+} and depression in cardiac function (25). However, there is no consensus as to the functional effects of myofilament protein carbonylation, and we note that many studies are not extended to other oxidative modifications of myofilament proteins. Combinational control of cross-bridge

![Graphs showing ATPase and tension data for NTg and Tm-E180G with and without NAC treatment.](http://ajpheart.physiology.org/)

Fig. 4. NAC increases cross-bridge kinetics in NTg and Tm-E180G myofilaments. A: in untreated NTg and Tm-E180G mice, there is no difference in tension cost. Upon NAC administration, there was a significant increase in tension cost of both NTg and Tm-E180G mice. B: NAC increases the rate of tension redevelopment (k_{tr}) in both NTg and Tm-E180G mice. Data are represented as means ± SE; n = 6–7 fibers, 3–5 hearts, per group.
kinetics and Ca\textsuperscript{2+} sensitivity by various posttranslational modifications is likely to play an effect, as it has been demonstrated in other thin filament proteins under scenarios of phosphorylation (17). Such an investigation in oxidative modifications is likely to be difficult, as approaches involving mutagenesis to mimic these posttranslational modifications are not available. Ultimately, future studies will shed light on the structural/functional changes due to cMyBP-C and myosin posttranslational modification.

Less clear is the effect of S-glutathionylation on tropomyosin. Previously, we have shown that Tm is able to be S-glutathionylated after in vitro incubation with oxidized glutathione (19). To our knowledge, this work is the first to suggest S-glutathionylation of Tm in vivo. The Tm-E180G mutation results in decreased thermal stability and increased flexibility, and it has been suggested that this could contribute to the increase in Ca\textsuperscript{2+} sensitivity by allowing the interaction of strongly bound cross-bridges with actin in the absence of Ca\textsuperscript{2+} (9, 11, 13). Tm contains one cysteine at residue 190, which is important for the maintenance of the coiled-coil structure by stabilizing ionic interactions throughout the molecule. Experiments performed in vitro where 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) was used to disrupt the disulfide bond created at C190, which is important in maintaining the coiled-coil structure of Tm, result in a decreased affinity of Tm for actin. The structural consequences of this “cross-linking”, or disruption of
the disulfide bond, induce partial unfolding of the molecule at physiological temperatures and increased regional flexibility, most likely through a destabilization of the structure, similar to and in the same region as the Tm-E180G mutation (31). S-glutathionylation at C190 may also disrupt the disulfide bond, resulting in the same effect as DTNB, causing a local destabilization of the molecule and promoting Ca\(^{2+}\)/H\(_{\text{11001}}\) sensitivity.

This hypothesis could explain the decrease in Ca\(^{2+}\) sensitivity observed in NTg NAC-administered mice, as we report a significant decrease in S-glutathionylation of NTg controls, although unlike the transgenic groups, no change was seen in S-glutathionylation of cMyBP-C. The same effect on S-glutathionylation of Tm was seen in Tm-E180G NAC mice.

Peña et al. (20) showed that early adenoviral overexpression of SERCA2a is able to delay the development of fibrosis and hypertrophy in Tm-E180G mice. However, SERCA2a overexpression was unable to reverse myofilament modifications and baseline diastolic dysfunction in this model. Additionally, mRNA levels of heart failure markers ANP, BNP, and β-myosin heavy chain were unchanged as the SERCA2a-overexpressing Tm-E180G mice aged. Although we also found that β-myosin heavy chain expression was unchanged with NAC, in our present work we found both a functional and morphological improvement with NAC administration. Unexpectedly, we found a significant increase in SERCA2 protein expression in both groups treated with NAC along with reversal of hypertrophy in Tm-E180G mice. The mechanism by which NAC increases SERCA2 expression is unclear. Supporting our findings, in a model of inflammatory signaling-mediated diastolic dysfunction, SERCA2a expression was reduced. The authors who made this observation attributed this reduction in SERCA2a to activation of NF-κB. Treating cells and rats with NF-κB blockers, as well as statins, improved SERCA2a expression in these models (30). Additionally, NAC administration prevented the activation of NF-κB in the hearts of brain-dead Ba-Ma miniature pigs (34).

In summary, we have demonstrated that antioxidant administration improves diastolic dysfunction, hypertrophy, Ca\(^{2+}\) regulatory protein expression, cross-bridge cycling kinetics, and posttranslational modification of myofilament proteins to relieve maladaptation in HCM. Although much work has been done toward generating drugs acting as Ca\(^{2+}\) sensitizers or sarcomere activators, no Ca\(^{2+}\)-desensitizing therapies exist (3, 29). Our work provides novel evidence for a Ca\(^{2+}\)-desensitizing therapy of an approved drug. We hypothesize that desensitization of the myofilaments to Ca\(^{2+}\) and improvement of diastolic dysfunction can be attributed to the reduction in

Fig. 7. NAC decreases myosin heavy chain carbonylation. Myosin heavy chain carbonylation was increased significantly in Tm-E180G mice, which was reduced to NTg control and NTg NAC levels after NAC administration. Actin, troponin T, and Tm carbonylation were unchanged. Data were analyzed by 2-way ANOVA followed by Tukey’s post hoc test for multiple comparisons and represented as means ± SE; n = 3 hearts/group. *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.
S-glutathionylation of myofilament proteins. Therefore, our work suggests that reducing the oxidative load on the cell may be an effective treatment for HCM. We look forward to the results of a clinical trial that is underway investigating the effect of NAC on human populations with HCM induced by sarcameric mutations (clinical trial identifier NCT01537926).

ACKNOWLEDGMENTS
We thank Richard L. Moss for the generous gift of total cMyBP-C antibody. We are grateful to Paul T. Mungai for helpful comments and conversation.

GRANTS
Our work was supported by National Institute of Health Grants PO1-HL-62426 (Project 1; to R. J. Solaro and B. M. Wolska) and T32-HL-007692, an American Physiological Society Porter Fellowship (to T. Wilder), and American Heart Association Grant 15PRE22180010 (to D. M. Ryba).

DISCLOSURES
The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

REFERENCES
7. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pelllikka PA, Picard MH, Roman MJ, Seward JB, Shawansie JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ; Chamber Quantification Writing Group; American Society of Echocardiography’s Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography’s Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 18: 1440–1463, 2005.


