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Protective Effects of Taurine Against Oxidative Stress in the Heart of MsrA Knockout Mice

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ABSTRACT

Taurine has been shown to have potent anti-oxidant properties under various pathophysiological conditions. We reported previously a cellular dysfunction and mitochondrial damage in cardiac myocytes of methionine sulfoxide reductase A (MsrA) gene knockout mice ($MsrA^{-/-}$). In the present study, we have explored the protective effects of taurine against oxidative stress in the heart of $MsrA^{-/-}$ mice with or without taurine treatment. Cardiac cell contractility and Ca^{2+} dynamics were measured using cell-based assays and in vivo cardiac function was monitored using high-resolution echocardiography in the tested animals. Our data have shown that $MsrA^{-/-}$ mice exhibited a progressive cardiac dysfunction with a significant decrease of ejection fraction (EF) and fraction shortening (FS) at age of 8 months compared to the wild type controls at the same age. However, the dysfunction was corrected in $MsrA^{-/-}$ mice treated with taurine supplement in the diet for 5 months. We further investigated the cellular mechanism underlying the protective effect of taurine in the heart. Our data indicated that cardiac myocytes from $MsrA^{-/-}$ mice treated with taurine exhibited an improved cell contraction and could tolerate oxidative stress better. Furthermore, taurine treatment reduced significantly the protein oxidation levels in mitochondria of $MsrA^{-/-}$ hearts, suggesting an anti-oxidant effect of taurine in cardiac mitochondria. Our study demonstrates that long-term treatment of taurine as a diet supplement is beneficial to a heart that is vulnerable to environmental oxidative stresses. J. Cell. Biochem. 113: 3559–3566, 2012.

KEY WORDS: TAURINE; DIET SUPPLEMENT; HEART; MITOCHONDRIA; ANTI-OXIDANT

Taurine is the most abundant intracellular sulfur containing amino acid [Bouckenooghe et al., 2006] and can be obtained from the diet, predominantly through eggs, meat, and seafood. Mediterranean diet, which is rich in seafood, has been shown to be strongly associated with a lower rate of cardiovascular disease and an improved quality of life [Martinez-Gonzalez et al., 2009; Sofi, 2009]. The benefit of taurine as a cardioprotective factor has also been observed in clinical studies [Azuma et al., 1992; Jeejeebhoy et al., 2002; Militante and Lombardini, 2002; Wojcik et al., 2012]. The majority of symptomatic patients with congestive heart failure have been shown to be significantly malnourished and supplementation of taurine improves cardiac ultrastructure, function, and contractility [Keith et al., 2001]. Taurine depletion leads to the development of a cardiomyopathy which suggests a role of taurine in the maintenance of normal cardiac function [Ito et al., 2008]. However, the mechanisms of the potential protection of taurine in the heart remain unclear.

Methionine sulfoxide reductase A (MsrA) is an enzyme that catalyzes the reduction of both free and protein-bound methionine sulfoxide (Met(O)) generated by oxidation of methionine (Met) due to reactive oxygen species (ROS). MsrA is a regulator of anti-oxidant defense in mammals and protects cells against oxidative damage by

Abbreviations used: DNPH, dinitrophenylhydrazine; EF, ejection fraction; FS, fraction shortening; MsrA, Methionine sulfoxide reductase A; SL, sarcomere length.

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G. Chen and C. Nan contributed equally to this study.

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reversing damage to proteins caused by methionine oxidation or by decreasing ROS through a scavenger mechanism [Levine et al., 1996; Weissbach et al., 2005]. Over-expression of MsrA has been proved to increase resistance to oxidative stress [Moskovitz et al., 1998; Ruan et al., 2002; Yermolaieva et al., 2004]. Enhanced repair of Met(O) by increased expression of MsrA in neonatal rat cardiac myocytes can protect the cell against hypoxia/reoxygenation induced cell death [Prentice et al., 2008]. Myristoylated MsrA protects the heart from ischemia-reperfusion injury [Zhao et al., 2011]. MsrA gene knockout mice ($MsrA^{-/-}$) have been generated to investigate the possible role of this enzyme as an anti-oxidant in mammals [Moskovitz et al., 2001; Salmon et al., 2009]. In our previous studies, we reported that deficiency of MsrA could cause cellular dysfunction and mitochondrial damage in cardiac myocytes isolated from $MsrA^{-/-}$ mice [Nan et al., 2010].

In the present study, we have explored the protective effects of taurine against oxidative stress in the heart of $MsrA^{-/-}$ mice with or without taurine treatment to test the hypothesis that anti-oxidant taurine has a protective effect in MsrA deficient hearts against oxidative stress-caused cardiac dysfunction. Our results demonstrate that long-term treatment of taurine as a diet supplement is beneficial to a heart that is vulnerable to environmental oxidative stresses.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

MsrA knockout mice, ($MsrA^{-/-}$ mice with a genetic background of C57BL/6 were a gift from Dr. Rodney Levine at NIH, Bethesda, MD) and wild type mice (C57BL/6) were used for the entire studies. This investigation conforms to the Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85–23, revised 1996) and was in accordance with the protocols approved by the Institutional Animal Care and Use Committees at Florida Atlantic University.

Taurine was added at a concentration of 0.1 mM to the drinking water as previously reported [Hanna et al., 2004]. The experimental groups of mice received the taurine-containing drinking water while the control groups were given water alone. Treatment was started in the tested animals at age of 3 months and continued without interruption for 5 months when the animals reached the age of 8 months.

IN VIVO CARDIAC ECHOCARDIOGRAPHY

Echocardiography measurements on mice were performed using a Vevo 770 High-Resolution echocardiograph (VisualSonics, Toronto, ON, Canada) as described previously [Du et al., 2006, 2008; Li et al., 2010]. All measurements were performed according to the standards established in human echocardiography [Sahn et al., 1978; Quinones et al., 2002; Feigenbaum and Ryan, 2005]. Briefly, the experimental mice were anesthetized with isoflurane at a concentration of 3% and then maintained by a facemask at concentration of 1.2% to keep heart rate higher than 450 beats/min during the whole procedure. The short-axis imaging was taken as M-mode acquisition to view the LV and RV movement during diastole and systole stages. Pulse and tissue Doppler images were collected with the apical four-chamber view to record the mitral Doppler flow spectra. All data and images were saved and analyzed by the built-in Advanced Cardiovascular Package Software for semi-automated analysis and quantification of cardiac function. Data analysis was performed offline with the use of a customized version of Vevo 770 Analytic Software. At least five mice were tested for each group.

MYOCARDIAL CELL PREPARATION AND CONTRACTILITY MEASUREMENTS

Cardiomyocyte isolation and cell mechanical property measurements using an IonOptix Myocam system (IonOptix Inc., MA) were performed as described previously [Li et al., 2010]. For physical stress tests, the stimulation frequency was varied from 0.5 to 2 Hz at 8 V. For oxidation stress tests, the cells were treated with 200 μM H_2O_2 for 10 min before measurements. Experimental data were obtained and analyzed from at least 8–10 cardiac cells per mouse and three mice per group.

INTRACELLULAR CALCIUM MEASUREMENT

Intracellular calcium transients in isolated cardiac myocytes were measured using fluorescent indicator Fura-2 as previously reported [Li et al., 2010]. The kinetics of Ca^{2+} transients was analyzed in conjunction with myocyte mechanical measurements. For oxidation stress tests, the cells were treated with 200 μM H_2O_2 for 10 min before calcium measurements. Experimental data were obtained and analyzed from at least 8–10 cardiac cells per mouse and three mice per group.

DETERMINATION OF MITOCHONDRIAL PROTEIN OXIDATION LEVELS

Freshly isolated mice hearts were perfused with ice cold Tyrode solution for 5 min using a Langendorff Perfusion Cell Isolation System to remove blood residue. Then, perfusion was resumed for an additional 20 min, with 200 uM H_2O_2 added to the Tyrode solution in the oxidative stress group while the control group received perfusion with Tyrode solution only. Isolation of mouse heart mitochondria was performed as previously reported [Jia et al., 2004]. Protein oxidation was measured as total carbonyl content that was determined by derivatization with dinitrophenylhydrazine (DNPH) according to the method described by Oliver et al., 1987 with little modification. Briefly, mitochondria proteins were extracted using T-PER Tissue Protein Extraction Reagent (Pierce, IL) following the manufacturer's protocol. The protein concentrations were determined with a BCA Protein Assay Kit (Pierce, IL). The amount of carbonyl groups was determined by measuring the OD at 370 nm and the results expressed as nmoles of carbonyl groups per mg of protein using 21.0 $mM^{-1}\,cm^{-1}$ as the absorption coefficient for the protein hydrazones. All samples were run in duplicate.

STATISTICS

All results are presented as means ± SE. ANOVA and Student's *t*-test were used to determine statistical significance. Statistic significance was set at $P < 0.05$.

RESULTS

TAURINE DIET IMPROVES CARDIAC FUNCTION IN $MsrA^{-/-}$ MICE

Cardiac function was monitored continuously using a high-resolution echocardiograph on both WT and $MsrA^{-/-}$ mice for a period of 8 months (Fig. 1; Table I). A progressive decline of cardiac

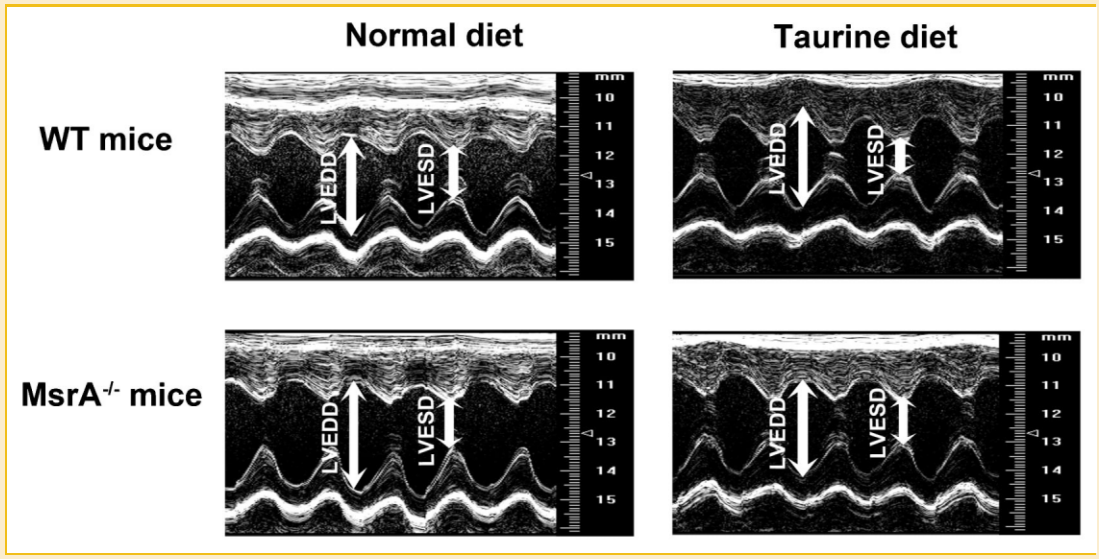

Fig. 1. Echocardiography M-mode imaging obtained from WT and MsrA$^{-/-}$ mice with or without taurine treatment. M-mode images obtained from short-axis measurements were used to evaluate LV function. The M-mode spectra were acquired from the tested mice with or without 5-month taurine treatment. Vertical arrows represent the diameters of left ventricle at diastolic or systolic stages. Three to five cardiac cycles were averaged for each experimental animal and five mice for each group. LVEDD, left ventricular end diastolic dimension; LVESD, left ventricular end systolic dimension.

function manifested as a decrease of ejection fraction (EF) and fractional shortening (FS) was observed in MsrA$^{-/-}$ mice compared to WT controls at the same age (Fig. 2A,B). The contraction parameters EF and FS showed a significant decrease in MsrA$^{-/-}$ mice at age of 8 months (Fig. 2A,B). However, the systolic dysfunction was corrected in MsrA$^{-/-}$ mice on the taurine diet. By the end of taurine treatment of 5 months, EF and FS in MsrA$^{-/-}$ mice on the taurine diet were increased significantly to the levels similar to that of the WT controls (Fig. 2C,D). Taurine also improved the cardiac function in WT mice on the taurine diet for 5 months.

TABLE I. Measurements of In Vivo Cardiac Function on WT and MsrA$^{-/-}$ Mice

	WT	MsrA$^{-/-}$	WT	MsrA$^{-/-}$
Parameters	1 month		8 month	
Body weight (g)	13.95 ± 0.50	15.26 ± 0.98	24.86 ± 0.45	31.38 ± 1.27
Heart rate (bpm)	482 ± 5	491 ± 4	481 ± 2	484 ± 4
LV end diastole				
IVS (mm)	0.57 ± 0.01	0.58 ± 0.04	0.70 ± 0.02	0.74 ± 0.02
LVEDD (mm)	3.17 ± 0.05	3.06 ± 0.10	3.64 ± 0.05	3.87 ± 0.01
LV PW (mm)	0.60 ± 0.02	0.59 ± 0.03	0.73 ± 0.01	0.74 ± 0.02
LV volume (μl)	40.21 ± 1.54	37.06 ± 2.86	57.18 ± 2.36	65.06 ± 0.45
LV end systole				
IVS (mm)	1.11 ± 0.02	1.04 ± 0.01	1.23 ± 0.02	1.18 ± 0.02
LVESD (mm)	1.52 ± 0.04	1.66 ± 0.02*	1.89 ± 0.04	2.40 ± 0.00*
LV PW (mm)	1.11 ± 0.01	1.08 ± 0.03	1.26 ± 0.03	1.26 ± 0.04
LV volume (μl)	6.42 ± 0.33	7.90 ± 0.29	11.00 ± 0.56	20.08 ± 0.05*
LV EF (%)	84.00 ± 0.32	78.40 ± 1.20*	80.28 ± 1.32	69.17 ± 0.20*
LV FS (%)	51.78 ± 0.37	45.71 ± 1.25*	48.16 ± 1.38	38.25 ± 0.17*

Values are expressed as mean ± SE from at least five mice for each group.
EDD, end diastolic dimension; ESD, end systolic dimension; LV, left ventricle; PW, posterior wall thickness of LV; IVS, Intra-ventricular septum; FS, fractional shortening of LV; EF, ejection fraction of LV.
Statistical significance was determined by ANOVA followed by post hoc Newman-Keuls (SNK) tests.
*$P < 0.05$.

However, unlike MsrA$^{-/-}$ mice, the increased EF and SF were not significant ($P > 0.05$) (Fig. 2C,D; Table II).

TAURINE PROTECTS MsrA$^{-/-}$ CARDIAC MYOCYTES FROM CONTRACTION DAMAGE CAUSED BY OXIDATIVE STRESS

Cell contractility measured as sarcomere length (SL) shortening in isolated cardiomyocytes from WT or MsrA$^{-/-}$ hearts with or without taurine treatment was performed at various electrical stimulation frequencies. In general, the sarcomere shortening velocity was reduced in MsrA$^{-/-}$ cardiac myocytes compared to WT controls (Fig. 3A). The reduced contractility was corrected in cardiac myocytes from MsrA$^{-/-}$ mice treated with taurine for 5 months (Fig. 3B). However, taurine did not cause a significant increase of cell contractility in WT controls (Fig. 3B). To further evaluate the cell contractility under oxidative stress conditions, we found that the cell contractility manifested as sarcomere shortening velocity and shortening amplitude was significantly decreased in MsrA$^{-/-}$ cardiac myocytes after exposure to H_2O_2 (Fig. 4). The injured cell contractility was corrected in cardiac myocytes from MsrA$^{-/-}$ mice treated with taurine (Fig. 4E,F). Wild type cardiac myocytes tolerated H_2O_2 treatment better than that of MsrA$^{-/-}$ cells and taurine improved the cell contractility in WT cardiac myocytes as well after exposure to H_2O_2 (Fig. 4B,E). However, taurine did not have a significant effect on intracellular Ca^{2+} dynamics as measured by Ca^{2+} transients in either WT or MsrA$^{-/-}$ cardiac myocytes exposed to H_2O_2 (Fig. 5).

TAURINE PROTECTS MITOCHONDRIA FROM PROTEIN OXIDATION CAUSED BY OXIDATIVE STRESS

Under various conditions of oxidative stress, lysine residues of proteins can be converted to carbonyl derivatives. Accordingly, the level of protein carbonyls is generally accepted as one marker of

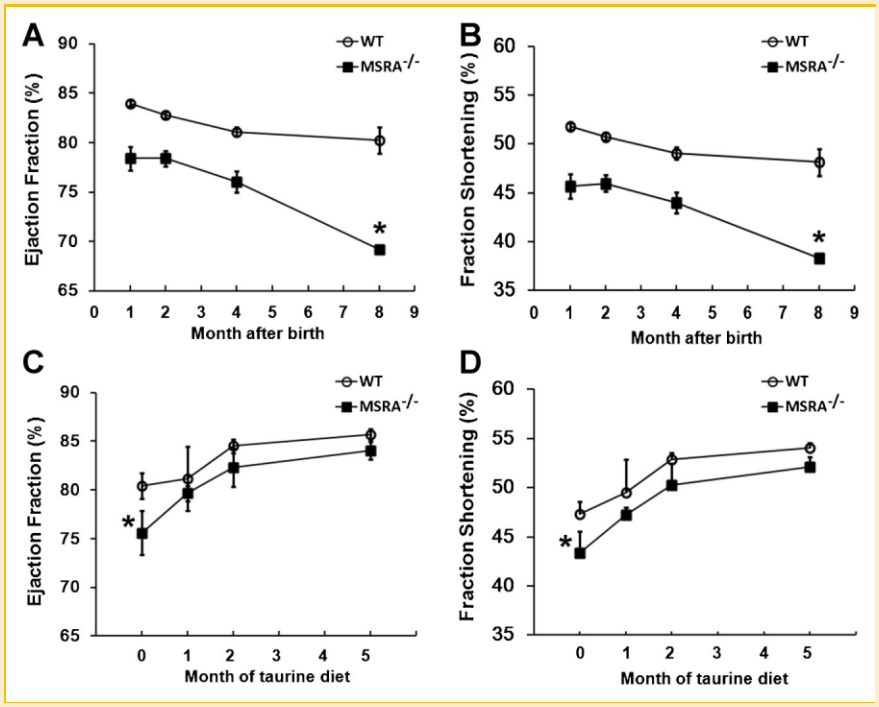

Fig. 2. Summary of echocardiography measurements on WT and MsrA$^{-/-}$ mice. A and B: Left ventricular ejection fraction (EF) and fractional shortening (FS) obtained from WT and MsrA$^{-/-}$ mice at ages of 1, 2, 4 and 8 months. Three to five cardiac cycles were averaged for each tested animal. Data are presented as means ± SE from at least five mice per group, $^{*}P < 0.05$. C and D: Left ventricular EF and FS measured with echocardiography in WT or MsrA$^{-/-}$ mice before and after taurine treatment for 1, 2, 5 months. $^{*}P < 0.05$.

oxidative stress [Levine et al., 1996; Moskovitz et al., 2001]. Protein carbonyl assays were performed using mitochondria isolated from WT and MsrA$^{-/-}$ hearts with or without taurine treatment. Figure 6 indicates that the protein oxidation levels in mitochondria isolated from taurine treated WT or MsrA$^{-/-}$ hearts had no significant difference compared with that without taurine treatment ($P > 0.05$) (Fig. 6A). However, when exposed to 200 μM H_2O_2, the protein carbonyl level in WT mitochondria did not show significant change, while the protein carbonyl level in mitochondria isolated from MsrA$^{-/-}$ hearts was increased significantly (Fig. 6B). Taurine treatment reversed the increased mitochondrial protein oxidation caused by H_2O_2 exposure in MsrA$^{-/-}$ hearts (Fig. 6B). These data indicate that mitochondrial proteins are vulnerable targets for oxidative stress in cardiac myocytes lacking MsrA and taurine protects these cells from protein oxidation caused by oxidative stress.

TABLE II. Measurement of Cardiac Function on Mice With and Without Taurine Diet

	WT mice		MsrA$^{-/-}$ mice	
Parameters	Control	Taurine diet	Control	Taurine diet
Body weight (g)	24.86 ± 0.45	23.53 ± 3.24	31.38 ± 1.27	24.10 ± 2.64
Heart rate (bpm)	481 ± 2	476 ± 4	484 ± 4	486 ± 4
LV end diastole				
IVS (mm)	0.70 ± 0.02	0.65 ± 0.03	0.74 ± 0.02	0.68 ± 0.03
LVEDD (mm)	3.64 ± 0.05	3.47 ± 0.11	3.87 ± 0.01	3.50 ± 0.10
LV PW (mm)	0.93 ± 0.01	0.71 ± 0.06	0.74 ± 0.02	0.69 ± 0.03
LV volume (μl)	57.18 ± 2.36	50.17 ± 3.74	65.06 ± 0.45	51.00 ± 3.34
LV end systole				
IVS (mm)	1.23 ± 0.02	1.26 ± 0.05	1.18 ± 0.02	1.25 ± 0.05
LVESD (mm)	1.89 ± 0.04	1.60 ± 0.05*	2.40 ± 0.00	1.68 ± 0.07*
LV PW (mm)	1.26 ± 0.03	1.20 ± 0.10	1.26 ± 0.04	1.31 ± 0.07
LV volume (μl)	11.00 ± 0.56	7.16 ± 0.54*	20.08 ± 0.05	6.48 ± 2.52*
LV EF (%)	80.28 ± 1.32	85.73 ± 0.39	69.17 ± 0.20	84.06 ± 0.88*
LV FS (%)	48.46 ± 1.38	54.06 ± 0.52	38.25 ± 0.17	52.11 ± 1.00*

Values are expressed as mean ± SE from five mice for each group.
LA, left atrium; RA, right atrium; EDD, end diastolic dimension; ESD, end systolic dimension; LV, left ventricle; PW, posterior wall thickness of LV; IVS, Intraventricular septum; FS, fractional shortening of LV; EF, ejection fraction of LV. Statistical significance was determined by ANOVA followed by post hoc Newman–Keuls (SNK) tests.
$^{*}P < 0.05$.

DISCUSSION

Cellular oxidative stress is associated with increased levels of ROS and the molecular damage they cause. Oxidative damage has been considered an important factor in loss of cardiovascular function resulting from aging and cardiac ischemia/reperfusion [Sadek et al., 2003; Picot et al., 2006]. The major oxidation product of protein-bound methionine is methionine sulfoxide. The pathophysiological importance of this modification is reflected by the methionine sulfoxide reductases (Msr) that are present in nearly all organisms [Weissbach et al., 2005]. MsrA is a member of Msr family that plays an important role in the anti-oxidant response by reducing the *S*-epimer of methionine sulfoxide to methionine and catalytically scavenging ROS [Levine et al., 2000; Weissbach et al., 2005]. Recent studies reported that increased MsrA expression can protect the

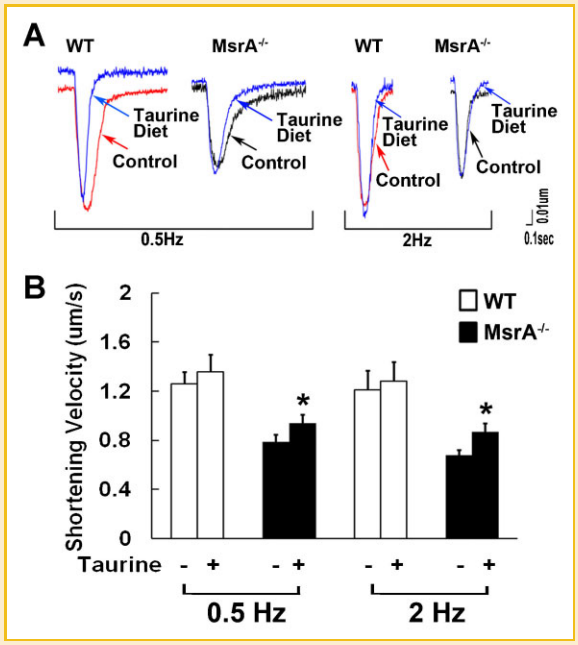

Fig. 3. Sarcomere contractility in WT and MsrA$^{-/-}$ cardiac myocytes with or without taurine treatment. Sarcomere contractility was measured in cardiac myocytes from WT or MsrA$^{-/-}$ mice with normal diet (Controls) or taurine diet for 5 months. A: Representative traces of sarcomere contraction of cells from WT or MsrA$^{-/-}$ mice under 0.5 or 2 Hz physical stimulation. B: Summary of shortening velocity measured in physically stimulated cardiac myocytes from WT or MsrA$^{-/-}$ mice with or without taurine treatment for 5 months. Values are expressed as means ± SE from at least three separate experiments (n = 25–35 cells per group). Statistical significance was determined by ANOVA followed by post hoc Newman–Keuls (SNK) tests. $^{*}P < 0.05$. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

cardiomyocytes against hypoxia/reoxygenation and ischemia-reperfusion damage [Prentice et al., 2008; Zhao et al., 2011]. Our previous study [Nan et al., 2010] demonstrated that the lack of MsrA in cardiac myocytes reduces myocardial cell's capability against physical and oxidative stresses resulting in a cellular dysfunction in the heart. In this study, we observed a gradually decrease of EF and FS in MsrA$^{-/-}$ mice starting from age of 4 months and showing a significant difference at age of 8 months compared to wild type mice of the same ages. The results are consistent with our previously reported data, suggesting that insufficiency of MsrA weakens the anti-oxidative ability in the body resulting in myocardial cell damages due to the increased ROS and oxidative stress.

Taurine, a sulfur-containing amino acids, is present in all body tissues of animals with a very high concentration in the heart and skeletal muscles [Ito et al., 2008]. It is well known that taurine is involved in various biological and physiological functions in the body, such as bile salt formation, fat digestion, anti-inflammatory, and anti-tumor function [Bouckenooghe et al., 2006; Warskulat et al., 2007]. In addition, taurine has been shown to protect various organs against damage induced by oxidative stress [Zeybek et al., 2006, 2007]. As early as in 1985, taurine was shown to have an effect on the cardiovascular system and taurine treatment significantly improved the symptoms of congestive heart failure [Azuma et al., 1985]. Several clinical trials also reported the beneficial effects of taurine in various pathophysiological conditions [Wojcik et al., 2010], although the mechanism of taurine's protective effect remain unclear. In the present study, we demonstrate that treatment of MsrA$^{-/-}$ mice with taurine for 5 months can correct cardiac dysfunction and reverse cardiac contraction parameters, EF and FS, to a level similar to that of wild type mice at the same age. Our data indicate that taurine is beneficial to the hearts vulnerable to oxidative stress due to insufficiency of the anti-oxidant enzyme, MsrA.

We further explored the cellular mechanism of taurine's protective effect in the heart. Our data indicate that MsrA$^{-/-}$ cardiac myocytes cannot tolerate oxidative stress as well as WT cardiac myocytes do. The cellular contractility of the MsrA-/- myocytes are significantly reduced in the presence of 200 μM H_2O_2 whereas the WT cardiac myocytes do not show any significant change in cellular contraction under the same conditions. After treatment of taurine for 5 months, MsrA$^{-/-}$ cardiac myocytes tolerate H_2O_2 insults much better and the cellular contractility does not change significantly when the cells are exposed to 200 μM H_2O_2, suggesting that taurine is helpful in strengthening the anti-oxidant capacity in MsrA$^{-/-}$ cardiac myocytes. Our data further demonstrate that taurine improves cardiac function by reducing the protein oxidation levels in mitochondria of MsrA$^{-/-}$ hearts. These data are consistent with our previous report that mitochondria are most vulnerable to oxidative stress when ROS and protein oxidation levels are increased in MsrA$^{-/-}$ hearts.

Myocardial mitochondria are an important organelles for energy production, intracellular calcium homeostasis regulation, and oxygen free radical generation. Myocardial mitochondria damage is considered an important trigger for the pathogenesis of heart disease [Marin-Garcia and Goldenthal, 2002]. A recent study on redox proteomics of protein-bound methionine oxidation indicates that methionine oxidation occurs in many mitochondrial proteins, which can be reduced by MsrA, including several critical ATP synthesis-related enzymes, such as mitochondrial ATP synthase subunits α, β, γ, etc. [Ghesquière et al., 2011]. Methionine-oxidation of these proteins can cause potential functional changes by preventing methylation and phosphorylation modification of the proteins. It is not surprising that the amount of protein oxidation is significantly increased in mitochondria of MsrA$^{-/-}$ hearts. Taurine has recently been proposed to have an important anti-oxidant role in mitochondria in animal cells [Hansen et al., 2006]. Taurine concentration in mitochondria isolated from hearts is as high as 30–40 μmol/g wet tissue and 30–40 mmol/L in the mitochondrial water phase [Hansen et al., 2010]. The protective effects of taurine have been linked previously to the anti-oxidant properties of mitochondria to resist oxidative stress injury [Chang et al., 2004]. Taurine supplementation has been shown to reduce oxidative stress leading to a protection of cardiac function and an improvement of arterial blood pressure in iron-overloaded mice. These mice are characterized by an increased free radical production and elevated oxidative stress as well as an altered intracellular Ca^{2+} handling in cardiac myocytes [Oudit et al., 2004]. Our results indicate that taurine can correct cardiac dysfunction in MsrA$^{-/-}$ mice probably by its anti-

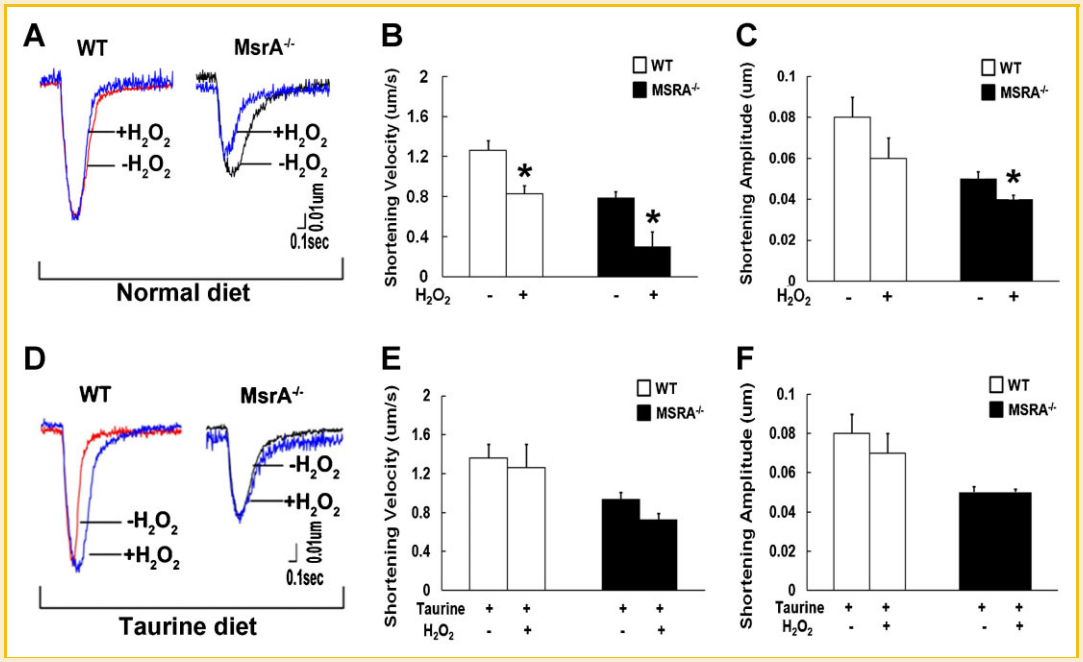

Fig. 4. Sarcomere contractility in WT and MsrA$^{-/-}$ cardiac myocytes treated with H_2O_2. A: Representative raw recordings of sarcomere contraction in normal diet WT or MsrA$^{-/-}$ mouse cardiac myocytes pre-treated with H_2O_2. Summary of shortening velocity (B) and shortening amplitude (C) in normal diet WT or MsrA$^{-/-}$ mouse cardiac cells with or without H_2O_2 pre-treatment. D: Representative raw recordings of sarcomere contraction in taurine diet WT or MsrA$^{-/-}$ mouse cardiac myocytes pre-treated with H_2O_2. Summary of shortening velocity (E) and shortening amplitude (F) of taurine diet WT or MsrA$^{-/-}$ mouse cardiac cells with or without H_2O_2 pre-treatment. The data are expressed as means ± SE from at least three separate experiments (n = 25–35 cells per group). Statistical significance was determined by ANOVA followed by post hoc Newman–Keuls (SNK) tests. $^{*}P < 0.05$. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

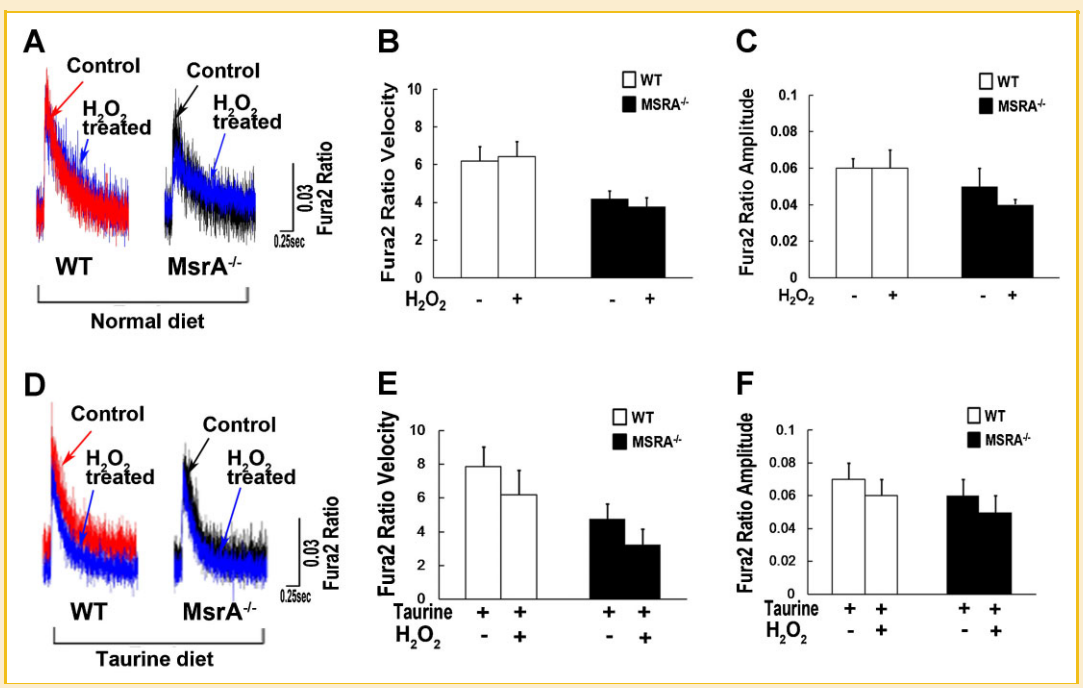

Fig. 5. Measurement of Ca^{2+} transient in cardiac myocytes from WT and MsrA$^{-/-}$ mice. A: Representative raw recordings of Ca^{2+} transients in normal diet WT (Control) or MsrA$^{-/-}$ mouse cardiac myocytes with or without H_2O_2 pre-treatment. Summary of fura-2 ratio velocity (B) and amplitude (C) in normal diet WT or MsrA$^{-/-}$ mouse cardiac myocytes pre-treated with H_2O_2. D: Representative raw recordings of Ca^{2+} transients in taurine diet WT or MsrA$^{-/-}$ mouse cardiac myocytes with or without H_2O_2 pre-treatment. Summary of fura-2 ratio velocity (E) and amplitude (F) in taurine diet WT or MsrA$^{-/-}$ mouse cardiac myocytes pre-treated with H_2O_2. The data are expressed as means ± SE from at least three separate experiments (n = 25–35 cells per group). Statistical significance was determined by ANOVA followed by post hoc Newman–Keuls (SNK) tests. $^{*}P < 0.05$. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

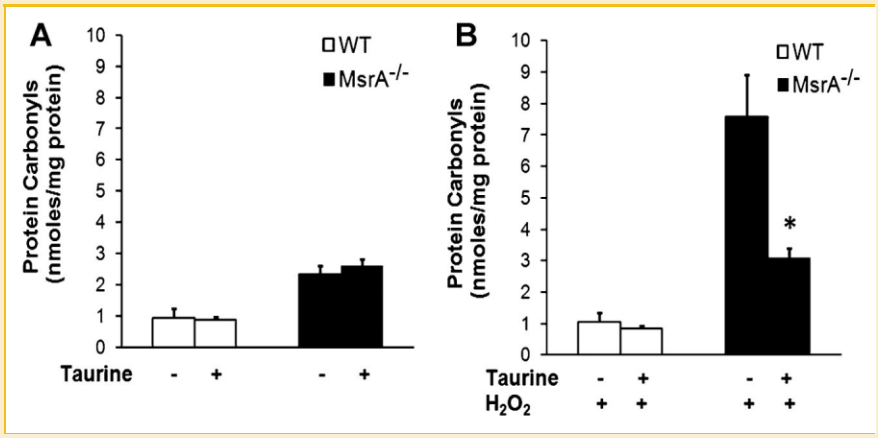

Fig. 6. Measurement of protein carbonyls contents in mitochondria isolated from WT or MsrA$^{-/-}$ hearts. A: Protein oxidation levels reflected as protein carbonyls were measured in cardiac mitochondria of WT and MsrA$^{-/-}$ mice. No significant difference in mitochondrial protein oxidation levels was observed in the tested mice with or without taurine treatment ($P > 0.05$). B: Protein oxidation levels in cardiac mithochondria exposed to H_2O_2 were significantly increased when isolated from MsrA$^{-/-}$ hearts, which were reversed by taurine treatment. Data are represented as means ± SEM from at least four separated experiments. $^{*}P < 0.05$.

oxidant effect that reverses protein oxidation levels and strengthens the anti-oxidant capacity in MsrA$^{-/-}$ cardiac myocytes.

In conclusion, our study demonstrates that taurine, when added as a dietary supplement, is beneficial to MsrA$^{-/-}$ hearts by correcting cardiac dysfunction probably through its anti-oxidant effect on mitochondria which strengthens the tolerance of cardiac myocytes against oxidative stresses.

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