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CARDIAC-SPECIFIC OVEREXPRESSION OF CATALASE PROLONGS LIFESPAN AND ATTENUATES AGEING-INDUCED CARDIOMYOCYTE CONTRACTILE DYSFUNCTION AND PROTEIN DAMAGE

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SUMMARY

1. Oxidative stress plays a role in senescence-associated organ deterioration. This is supported by the beneficial effects of anti-oxidants against ageing-related organ damage, although their role in cardiac ageing has not been elucidated.

2. The aim of the present study was to examine the impact of cardiac-specific overexpression of catalase, an enzyme for H_2O_2 detoxification, on cardiac contractile function and protein damage in young (3–4 months) and old (26–28 months) male mice. Lifespan was analysed using the Kaplan–Meier survival curve. Cardiomyocyte contractile indices at various stimulus frequencies (0.1–5.0 Hz) were analysed, including peak shortening (PS), time to 90% PS, time to 90% relengthening (TR_{90}) and maximal velocity of shortening/relengthening (±dL/dt). Protein damage was assessed using protein carbonyl formation. Catalase transgenic mice showed longer lifespan than wild-type FVB mice. The catalase transgene itself did not alter bodyweight or organ weight, or myocyte function. Ageing depressed ±dL/dt and prolonged TR_{90}, but had no effect on other indices in FVB mice. Increased frequency triggered decreases in PS amplitude were exaggerated in aged FVB myocytes. Interestingly, ageing-induced mechanical defects were significantly attenuated in myocytes from catalase mice. Protein carbonyl formation was elevated in aged FVB compared with young FVB mice, which was significantly diminished in catalase mice. The proteomes of the myocardium of young or old FVB and catalase mice were compared using two-dimensional gel electrophoresis and mass spectrometry. Six proteins with differential expression between yound and old FVB groups were tentatively identified, some of which were reversed by catalase.

3. In summary, the present data suggest that catalase protects cardiomyocytes from ageing-induced contractile defects and protein damage.

Key words: ageing, catalase, myocytes, protein carbonyl, proteomics, shortening/relengthening.

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INTRODUCTION

Although ageing and ageing-associated occult diseases and sedentary life style contribute to senescence-related changes in cardiovascular function, the 'ageing process' itself has been demonstrated to be the single-most independent risk factor for deteriorated heart morphology and function.[1–3] Several hypotheses have been postulated for cardiac ageing, including prolonged action potential duration, myosin isozyme switch, altered membrane structure and permeability and accumulation of reactive oxygen species (ROS).[3–5] Nonetheless, the precise link between advanced age and altered cardiac contractile function has not been fully elucidated. The 'free radical theory of ageing' seems to draw the most attention for the mechanism of cardiac ageing.[6] However, little direct evidence is available regarding the role of oxidative stress and anti-oxidant defence on cardiac contractile function and protein damage during advanced age. The aim of the present study was to examine the effect of the anti-oxidant catalase on ageing-associated alterations in cardiomyocyte contractile function and protein damage. Catalase is a catalyst for the conversion of hydrogen peroxide (H_2O_2) into water and oxygen. Catalase has one of the highest turnover rates among all known biological enzymes (approximately 40 000 000 molecules/s), exhibiting its physiological role in the removal of H_2O_2 produced by peroxisomal aerobic dehydrogenase reaction.[7,8] Catalase is composed of four heme-containing subunits, with each heme group mediating the biological activity of catalase.[7,8] Catalase becomes extremely important in cytoprotection under conditions of anti-oxidant deficiency (e.g. glutathione depletion) to remove excessive oxidative stress as a result of superoxide and H_2O_2 accumulation.[8,9] To evaluate the role of catalase in cardiac ageing, cardiomyocyte contractile properties and cardiac protein damage were examined in young (3–4 month) and old (26–28 month) wild-type and transgenic mice with cardiac-specific overexpression of catalase.

METHODS

Cardiac catalase overexpressing transgenic mice and Kaplan–Meier survival curves

All animal procedures used in this study were approved by the Animal Care and Use Committees at the University of North Dakota (Grand Forks, ND, USA) and the University of Wyoming (Laramie, WY, USA). All animals were kept in our institutional animal facilities. Production and characterization of the cardiac-specific catalase transgenic line have been described in detail previously.[8,10] In brief, an 8 kb catalase transgene driven by a cardiac-specific myosin heavy chain (MHC) promoter containing the entire coding sequence

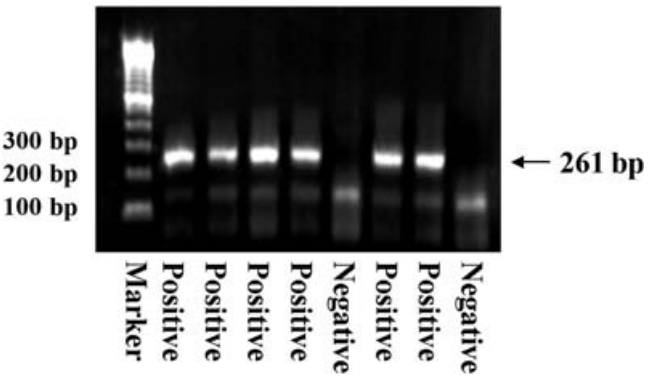

Fig. 1 Identification of catalase transgenic mice. Genomic DNA was isolated from 2 cm tail clips from 1-month-old mice. The catalase gene was identified by polymerase chain reaction using a primer pair derived from the myosin heavy chain promoter and rat catalase cDNA.

of catalase cDNA was purified on a matrix of diatemaceous earth (Prepagene; Bio-Rad Laboratories, Hercules, CA, USA) and was filtered through a 0.22 μm filter. Approximately 100 copies of purified transgene insert were microinjected into one pronucleus of each one-cell mouse embryo of the inbred albino FVB strain. The transgene transcription of catalase was controlled by the mouse *α-MHC* gene. To identify transgenic founder mice, genomic DNA was isolated from 1 cm tail clips from 4-week-old mice. The DNA was subjected to Southern and dot blot analyses, which were probed with a 550 bp *Sma*I/*Not*I fragments derived from the rat insulin II portion of the catalase transgene. This probe hybridized to an 8000 bp *Eco*RI fragment of the transgene, consistent with the presence of a unique *Eco*RI site in the catalase transgene (600 bp upstream of the MHC transcription initiation site). Founder mice were bred with mice of the same strain and transgenic offspring were identified routinely by a polymerase chain reaction (PCR) using a primer pair derived from the MHC promoter and rat catalase cDNA (Reverse: aat atc gtg ggt gac ctc aa; forward: cag atg aag cag tgg aag ga; Fig. 1).[10] Male transgenic positive mice (heterozygotes) with approximately 50-fold catalase overexpression distributed from the sarcoplasmic reticulum (SR), nucleus to peroxisomes in the atria and ventricles,[8,11] were used at young (3–4 months old) and old (26–28 months old) ages. Catalase overexpression was shown to be limited to hearts without changes in enzyme activity in the liver, lung, kidney and skeletal muscle.[8] Age- and gender (male)-matched FVB mice were used as controls. To configure the Kaplan–Meier survival curve, FVB and catalase mice were separated after weanling (< 10 mice per cage). Mortality was recorded every 3 months (excluding unexpected death, such as from fighting).

Isolation of mouse ventricular myocytes

Hearts were removed rapidly from anaesthetized (ketamine/xylazine, 3 : 5, 1.32 mg/kg) mice and mounted on a temperature-controlled (37°C) Langendorff system. After perfusion with a modified Tyrode's solution (Ca^{2+} free) for 2 min, the heart was digested with a Ca^{2+}-free Krebs'–Henseleit buffer (KHB) containing Liberase Blendzyme 4 (Hoffmann-La Roche, Indianapolis, IN, USA) for 20 min. The modified Tyrode's solution (pH 7.4) contained the following (in mmol/L): NaCl 135; KCl 4.0; $MgCl_2$ 1.0; HEPES 10; NaH_2PO_4 0.33; glucose 10; butanedione monoxime 10. The solution was gassed with 5% CO_2–95% O_2. The digested heart was then removed from the cannula and the left ventricle was cut into small pieces in the modified Tyrode's solution. Tissue pieces were agitated gently and the pellet of cells was resuspended in KHB. Extracellular Ca^{2+} was added incrementally back to 1.20 mmol/L over a period of 30 min. Isolated myocytes were used for experiments within 8 h of isolation. Only rod-shaped myocytes with clear edges were selected for mechanical studies.[12]

Cell shortening/relengthening in response to increasing stimulus frequency

Mechanical properties of cardiomyocytes were assessed using an IonOptix SoftEdge MyoCam® system (IonOptix, Milton, MA, USA).[12] In brief, cells were placed in a chamber mounted onto the stage of an inverted microscope (IX-70; Olympus, Tokyo, Japan) and superfused (approximately 1 mL/min at 25°C) with a buffer containing (in mmol/L): 131 NaCl; 4 KCl; 1 $CaCl_2$; 1 $MgCl_2$; 10 glucose; 10 HEPES (pH 7.4). Cells were field stimulated with suprathreshold voltage at frequencies of 0.1, 0.5, 1.0, 3.0 and 5.0 Hz using an FHC stimulator (Brunswick, NE, USA). Cells were allowed to reach steady state contraction (usually after the first five to six beats) at any given frequency before peak shortening (PS) was recorded. IonOptix SoftEdge software was used to capture cell shortening and relengthening. Cell shortening and relengthening were assessed using the following indices: PS, indicative of peak ventricular contractility; time to PS (TPS), indicative of contraction duration; time to 90% relengthening (TR_{90}), indicative of relaxation duration; maximal velocity of shortening (+dL/dt) and relengthening (–dL/dt), indices of maximal velocities of ventricular pressure rise/fall during contraction and relaxation, respectively.

Protein carbonyl assay

The carbonyl content of protein was determined as described previously.[13] Briefly, proteins were extracted and minced to prevent proteolytic degradation. Nucleic acids were eliminated by treating samples with 1% streptomycin sulphate for 15 min, followed by a 10 min centrifugation (6000 *g*). Protein was precipitated by adding an equal volume of 20% trichloroacetic acid (TCA) to protein (0.5 mg) and centrifuged at 11 000 *g* for 5 min at 4°C. The TCA solution was removed and the sample was resuspended in 10 mmol/L 2,4-dinitrophenylhydrazine (2,4-DNPH) solution. Samples were incubated at room temperature for 15–30 min. Following addition of 500 μL of 20% TCA, samples were centrifuged at 11 000 *g* for 3 min at room temperature. The supernatant was discarded, the pellet washed in ethanol : ethyl acetate and allowed to incubate at room temperature for 10 min. Samples were centrifuged again at 11 000 *g* for 3 min at room temperature and the ethanol : ethyl acetate steps repeated twice more. The precipitate was resuspended in 6 mol/L guanidine solution and incubated at 37°C for 60 min to dissolve pellets before being centrifuged again at 11 000 *g* for 3 min at room temperature and insoluble debris removed. The maximum absorbance (360–390 nm) of the supernatant was read against appropriate blanks and the carbonyl content was calculated using the molar absorption coefficient of 22 000 L/mol per cm.

Proteomic analysis

General protein profiles in the myocardium of young or old wild-type and catalase transgenic mice were evaluated using proteomic methods described previously.[14] In brief, frozen heart tissue taken from FVB and catalase mice was homogenized using a Polytron homogenizer in an ice-cold buffer containing 137 mmol/L NaCl, 50 mmol/L HEPES, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 1% nonidet P-40 (NP-40), 10% glycerol, 2 mmol/L phenylmethylsulphonyl fluoride, 10 mmol/L sodium pyrophosphate, 2.5 mmol/L EDTA, 10 μg/mL aprotinin, 10 μg/mL leupeptin, 2 mmol/L Na_3VO_4 and 100 mmol/L NaF, pH 7.4.[15] The homogenate (350 μg) was transferred to 600 μL lysis buffer containing 8 mol/L urea, 4% CHAPS and 20 mmol/L Tris-HCl (pH 7.4). The mixture was shaken at 4°C for 1 h and was centrifuged at 12 000 *g* for 10 min at 4°C. The supernatant, containing soluble proteins, was collected and used to rehydrate using commercially available immobilized-pH-gradient (IPG) strips (pH 3–10; 1 × 17 cm; Bio-Rad Laboratories). Rehydration of strips was conducted based on the manufacturer's instruction followed by isoelectric focusing with a maximal voltage of 8000 V to reach 100 000 voltage–h. The strip was then equilibrated in solution 1 (6 mol/L urea, 2% sodium dodecyl sulphate (SDS), 30% glycerol, 50 mmol/L Tris-HCl, pH 8.8, 1% dithiothreitol (DTT)) and solution 2 (6 mol/L urea, 2% SDS, 30% glycerol, 50 mmol/L Tris-HCl (pH 8.8), 2% iodoacetamide with 0.01% bromphenol blue) for 15 min each. The strip was loaded onto a precast

Table 1 General features of young (3–4 month) and old (24–26 month) wild-type FVB and catalase-overexpressing transgenic mice

	BW (g)	HW (mg)	HW/BW (mg/g)	LW (g)	LW/BW (mg/g)	KW (g)	KW/BW (mg/g)
Wild-type FVB mice							
Young	17.3 ± 1.2	94 ± 8	5.4 ± 0.2	0.89 ± 0.07	50.5 ± 1.5	0.24 ± 0.03	13.5 ± 0.6
Old	30.0 ± 1.0*	166 ± 9*	5.6 ± 0.3	1.55 ± 0.09*	51.2 ± 1.9	0.39 ± 0.02*	13.1 ± 0.6
Catalase-overexpressing transgenic mice							
Young	17.1 ± 1.5	91 ± 8	5.3 ± 0.1	0.88 ± 0.09	49.8 ± 1.7	0.25 ± 0.03	14.0 ± 0.8
Old	30.1 ± 0.6*	153 ± 6*	5.1 ± 0.2	1.50 ± 0.11*	49.6 ± 3.1	0.40 ± 0.02*	13.3 ± 0.4

Data are the mean±SEM (n = 15–18 mice per group). *$P < 0.05$ compared with the young FVB group.
BW, bodyweight; HW, heart weight; LW, liver weight; KW, kidneyweight.

5–20%, 200 × 200 mm,gradient gel for two-dimensional separation. To fix the strip on top of the gradient gel, low melting point agarose solution (0.188 mol/L Tris·HCl (pH 8.8), 0.1% SDS, 9% glycerol, 1% agarose, 0.01% bromophenol blue) was heated to 65°C and was then pipetted onto the IPG strip followed by two-dimensional SDS electrophoresis. Electrophoresis was conducted in electrophoresis solution (25 mmol/L Tris Base, 192 mmol/L glycine, 1 g/L SDS) at 5°C, with 10 W/gel. After the front dye line had arrived at the end of gel, gels were removed and fixed (40% ethanol, 10% acetic acid) before being stained with Coomassie blue. Following staining, gels were scanned using Image Scanner II (Amersham Biosciences, Piscataway, NJ, USA).[16]

Quantitative analysis of protein expression and in-gel trypsin digestion

Image Master (Amershan Biosciences) software was used for matching and quantitative analysis of the protein spots on the gels according to the manufacturer's instructions. Proteins with differential expression ($P \leq 0.05$) were selected for identification by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF). Briefly, protein spots with differential expression were excised, cut into 1 mm^3 pieces and washed twice with 50% acetonitrile and 50 mmol/L ammonium bicarbonate for 15 min with gentle shaking or until the Coomasie dye had been removed completely. Gel pieces were dehydrated in 100% acetonitrile for 5 min and incubated in a solution containing 50 mmol/L ammonium bicarbonate and 10 mmol/L DTT for 30 min at 56°C before being incubated in a freshly made solution containing 50 mmol/L ammonium bicarbonate and 55 mmol/L iodoacetamide for 30 min in the dark. The gel pieces were washed with acetonitrile, air dried and rehydrated with 20 μg/mL trypsin (sequencing grade; Promega, Madison, WI, USA) in 50 mmol/L ammonium bicarbonate. Then, rehydrated gel pieces with trypsin were incubated at 37°C overnight. Following centrifugation at 12 000 *g* for 5 min, the supernatant (containing tryptic peptides) was transferred to a sterile centrifuge tube. An aliquot of extraction solution (25–50 μL; compised of 60% acetonitrile and 1% trifluoroacetic acid (TFA)) was added to gel pieces and agitated gently by vortexing at the lowest setting for 10 min. The above procedure was repeated once and supernatants were pooled together. Then, supernatants were dried under vacuum and 5 μL resuspension solution (50% acetonitrile, 0.1% TFA) was added to each tube to resolve the peptides.[16]

Mass spectrometry and protein identification

Peptides were mixed with an equal volume of 10 mg/mL α-cyano-4-hydroxycinnamic acid in 65% acetonitrile/0.3% TFA and applied to the steel plate for MALDI-TOF peptide fingerprinting analysis. The instrument (Voyager DE-STR; Applied Biosystems, Foster City, CA, USA) was set at a positive ion reflector mode and the laser strength and voltage were optimized to obtain the highest signal-to-noise ratio. Protein identification was done by searching against MSDB and NCBInr protein databases through the Mascot Peptide Mass Fingerprint software, available at http://www.matrixscience.com/.

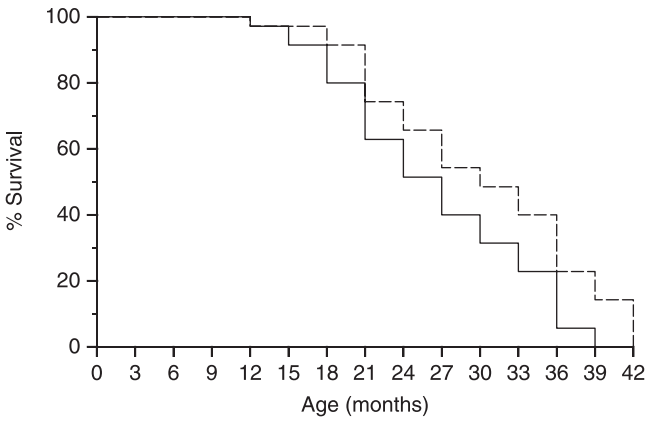

Fig. 2 Cumulative survival curve (Kaplan–Meier survival plot) of male FVB (——) and catalase-overexpressing transgenic (-----) mice. The survival rate of mice was checked every 3 months. The cumulative survival rate was plotted against time (n = 35 mice per group).

As the search parameter, the fixed carbamidomethyl modification was chosen allowing up to one missed cleavage. The peptide tolerance was set at 1.0 Da.[17,18]

Statistical analyses

Data are presented as the mean±SEM. Statistical significance ($P < 0.05$) for each variable was determined by analysis of variance (ANOVA) or *t*-test, where appropriate.

RESULTS

General information and survival rate of mice

Only mice with positive PCR catalase cDNA were included as the catalase transgenic group in the present study (Fig. 1). General features of young and old FVB and catalase transgene mice are given in Table 1; transgenic catalase overexpression did not elicit any notable effect on bodyweight and heart, liver and kidney weights compared with age-matched FVB wild-type littermates. Aged mice had heavier bodyweight and organ weights (although not organ size) compared with young counterparts, as expected. The Kaplan–Meier survival curve depicts that catalase transgenic mice have an approximate 3 month longer lifespan compared with FVB mice (Fig. 2). The two curves start to separate from each other after 15 months of age, with catalase mice exhibiting a reduced mortality rate.

Table 2 Mechanical properties of cardiomyocytes from young (3–4 month) and old (24–26 month) wild-type FVB and catalase (CAT) transgenic mice at various stimulus frequencies

	Stimulation frequency	PS (% cell length)	+dL/dt (μm/s)	–dL/dt (μm/s)	TPS (msec)	TR_{90} (msec)
Wild-type FVB mice						
Young (n = 22)	0.1 Hz	7.51 ± 0.65	154.3 ± 13.6	−135.6 ± 15.1	120 ± 18	189 ± 15
	0.5 Hz	6.88 ± 0.48	150.4 ± 11.2	−136.6 ± 11.3	100 ± 6	162 ± 16
	1.0 Hz	5.52 ± 0.48*	118.8 ± 11.2*	−102.3 ± 10.4*	99 ± 3	164 ± 12
	3.0 Hz	3.82 ± 0.36*	93.2 ± 8.5*	−75.4 ± 9.2*	78 ± 2*	125 ± 8*
	5.0 Hz	3.21 ± 0.33*	87.0 ± 10.0*	−70.1 ± 9.5*	71 ± 3*	113 ± 11*
Old (n = 26)	0.1 Hz	6.55 ± 0.50	122.0 ± 14.0†	−96.2 ± 15.9†	128 ± 10	281 ± 27†
	0.5 Hz	3.89 ± 0.42*	89.8 ± 13.5*	−69.6 ± 15.0*	119 ± 11	252 ± 24
	1.0 Hz	2.70 ± 0.38*	59.7 ± 7.7*	−49.6 ± 8.9*	117 ± 8	235 ± 20
	3.0 Hz	1.68 ± 0.30*	52.7 ± 10.5*	−46.6 ± 13.6*	82 ± 4*	125 ± 9*
	5.0 Hz	0.93 ± 0.25*	43.9 ± 9.4*	−41.2 ± 13.1*	68 ± 4*	95 ± 5*
Catalase-overexpressing transgenic mice						
Young (n = 23)	0.1 Hz	6.92 ± 0.78	139.6 ± 18.1	−119.4 ± 20.0	124 ± 13	193 ± 21
	0.5 Hz	6.28 ± 0.67	137.9 ± 18.3	−126.0 ± 16.2	114 ± 14	182 ± 25
	1.0 Hz	5.03 ± 0.65*	107.0 ± 14.6*	−102.8 ± 16.3*	112 ± 17	177 ± 23
	3.0 Hz	3.03 ± 0.40*	84.3 ± 12.0*	−63.5 ± 9.5*	81 ± 5*	115 ± 6*
	5.0 Hz	2.38 ± 0.54*	71.3 ± 18.6*	−72.0 ± 25.0*	69 ± 3*	93 ± 9*
Old (n = 23)	0.1 Hz	6.35 ± 0.58	161.4 ± 17.3	−137.0 ± 16.4	122 ± 12	217 ± 18
	0.5 Hz	4.97 ± 0.49	123.3 ± 16.0	−103.2 ± 13.2	120 ± 12	197 ± 17
	1.0 Hz	4.28 ± 0.47*	103.5 ± 10.6*	−82.8 ± 12.0*	97 ± 3	214 ± 22
	3.0 Hz	2.60 ± 0.47*	70.0 ± 10.1*	−55.0 ± 9.8*	79 ± 3*	125 ± 5*
	5.0 Hz	1.64 ± 0.30*	48.7 ± 7.9*	−43.4 ± 8.4*	66 ± 2*	88 ± 3*

Data are the mean±SEM. *P < 0.05 compared with the corresponding value at 0.1 Hz; †P < 0.05 compared with the young FVB group.
PS, peak shortening; TPS, time to PS; TR_{90}, time to 90% relengthening; ±dL/dt, maximal velocity of shortening/relengthening, respectively.

Effect of increasing stimulation frequency on myocyte shortening

To evaluate the impact of ageing on cardiac contractile function under various frequencies, we raised stimulus frequency from 0.1 to 5.0 Hz and recorded the steady state PS. Cells were initially stimulated to contract at 0.5 Hz for 5 min to ensure a steady state had been reached before commencing the frequency–response study. All recordings were normalized to PS at 0.1 Hz of the same myocyte. Figure 3a shows that cardiomyocytes from aged groups (FVB or catalase) exhibited longer resting cell length than young FVB and catalase mice. Figure 3b shows a steeper negative staircase in PS in aged FVB myocytes with increased stimulus frequency (at 0.5 Hz or higher) compared with young FVB myocytes, suggesting reduced stress intolerance with advanced ageing. Interestingly, the catalase transgene ablated this increased stimulating frequency induced stepper reduction in myocyte shortening amplitude (except at 5.0 Hz). Table 2 gives the mechanical properties obtained at the stimulus frequencies between 0.1 and 0.5 Hz for all four mouse groups. Although the baseline (at 0.1 Hz) PS and TPS were similar in myocytes among young or old FVB and catalase mice, myocytes from aged FVB mice displayed significantly reduced maximal velocity of shortening/relengthening (±dL/dt) and prolonged TR_{90} compared with myocytes from young FVB mice. Interestingly, ageing-induced baseline mechanical defects were ablated by catalase, suggesting a beneficial role of the anti-oxidant against cardiac ageing. The catalase transgene itself did not affect mechanical contractile properties in the young mouse group. Data presented in Table 2 also reveal a frequency dependent reduction of PS and ±dL/dt with a threshold between 0.5 and 1.0 Hz in young mouse groups (FVB and catalase). However, ageing shifted the threshold to the left (between 0.1 and

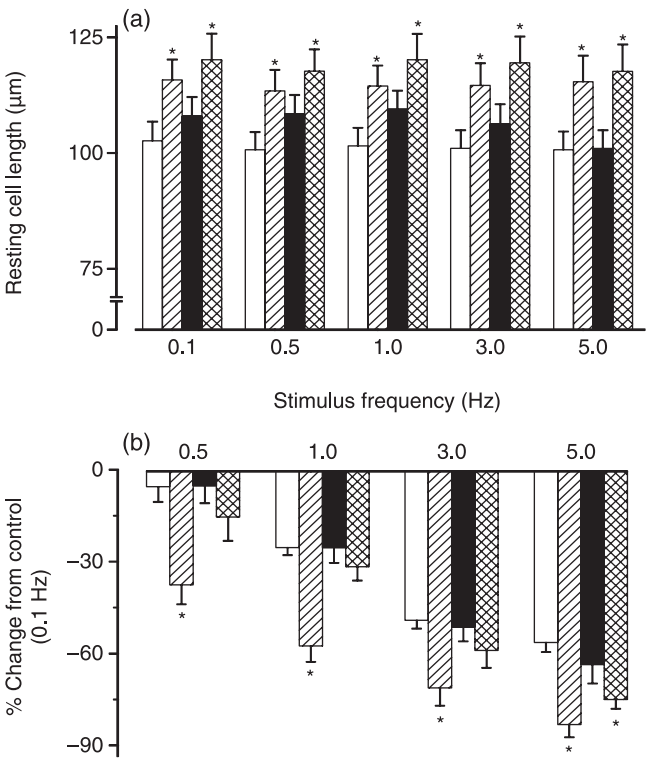

Fig. 3 (a) Resting cell length and (b) change of peak shortening of cardiomyocytes from young (□) or old (▨) FVB mice and young (■) and old (⊠) catalase-overexpressing transgenic mice at various stimulus frequencies (0.1–5.0 Hz). Peak shortening is presented as the percentage change from values obtained at 0.1 Hz in the same cell. Data are the mean±SEM (n = 22–26 cells per group). *P < 0.05 compared with the young FVB group.

0.5 Hz) in the FVB group, which was nullified by the catalase transgene. Our data further demonstrated that neither ageing nor the catalase transgene affected the threshold (between 1.0 and 3.0 Hz) for frequency dependent narrowing of TPS and TR_{90}.

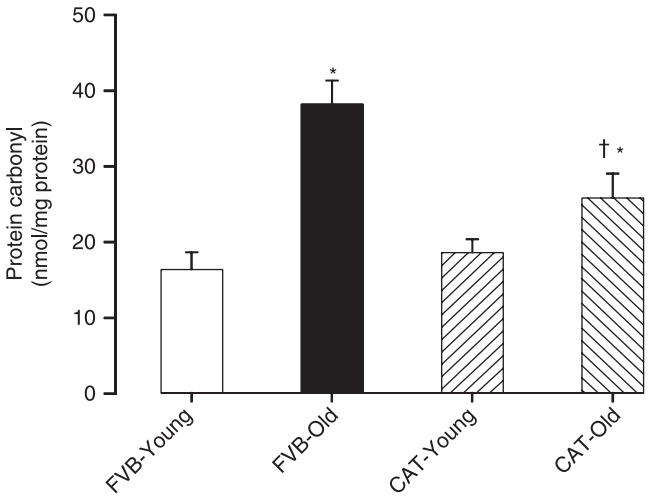

Fig. 4 Levels of cardiac protein carbonyl from young or old FVB and catalase-overexpressing transgenic mice. Data are the mean±SEM (n = 5–6 hearts per group). *$P < 0.05$ compared with the young FVB group; †$P < 0.05$ compared with the old FVB.

Effect of ageing and catalase transgene on protein carbonyl formation

Results shown in Fig. 4 indicate that protein carbonyl formation was overtly enhanced in hearts from aged FVB mice compared with young mice. Although catalase itself had little effect on cardiac protein carbonyl formation in young mice, it partially reversed ageing-induced protein carbonyl formation, suggesting a likely role of cardiac protein damage in cardiac ageing and the beneficial effect of catalase.

Two-dimensional gel analysis of cardiac proteins in young and old FVB and catalase mice

Analyses of cardiac protein profiles were performed on the 17 cm, pH 3–10 IPG strips with subsequent SDS-polyacrylamide gel electrophoresis (PAGE) and Coomassie blue staining (Fig. 5). Proteins were separated according to their respective molecular mass and isoelectric point (pI). It is evident that some highly expressed proteins found in young FVB hearts cannot be traced in old FVB hearts, whereas some proteins with low expression in young FVB hearts yielded large amounts of signal in old FVB hearts. A similar analysis was conducted to compare the protein profiles of wild-type and catalase transgenic mice. For example, some areas, especially in the basic region around apparent molecular mass 55–66 kDa and pI 7–8, show an intense spot that is expressed at a high levels in the aged FVB group compared with the young FVB group. This ageing-induced

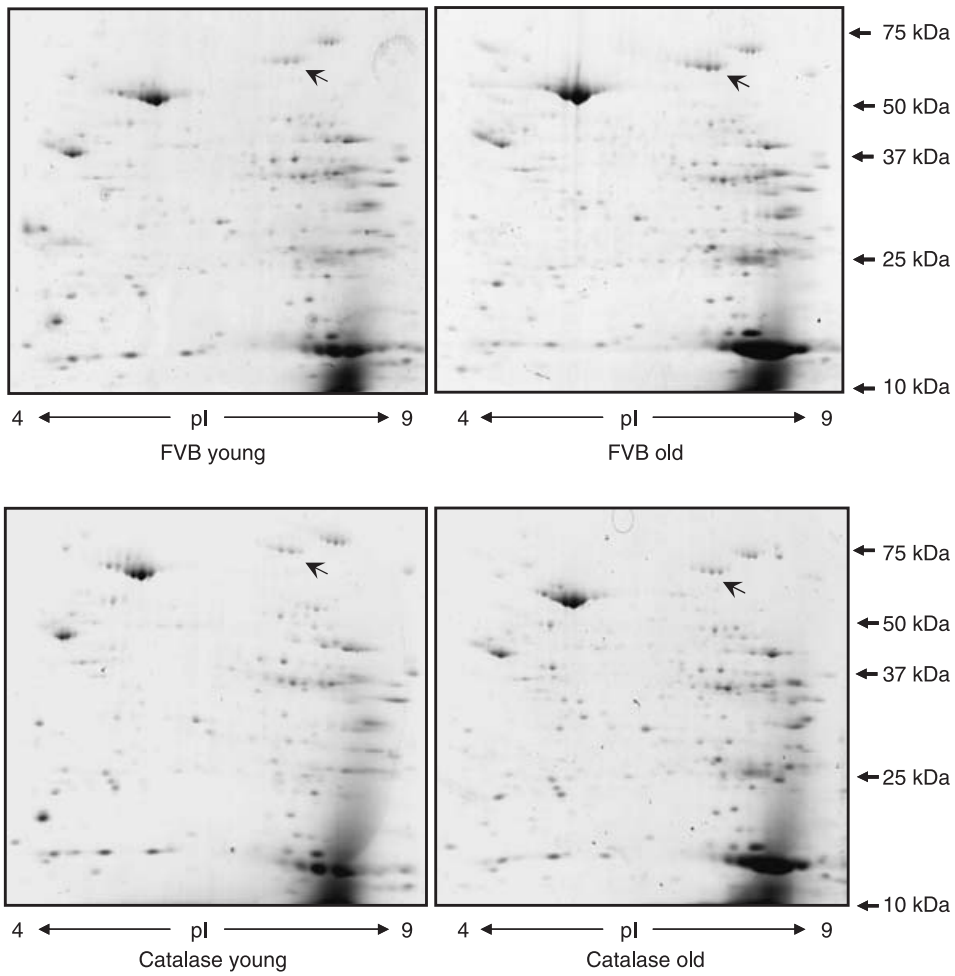

Fig. 5 Representative two-dimensional image of cardiac proteomes. Cardiac protein (350 μg) was separated on a two-dimensional system. Arrows show spots with differential expression between young and old catalase-overexpressing transgenic mice and young and old FVB mice. Four replicates were used that displayed consistent results. The pI range between 4 and 9 (strips with pI 3–10) was shown to avoid non-optimal electrofocusing owing to electric probe connection at both ends of the strip.

Table 3 Tentative identification of selected protein spots by matrix-assisted laser desorption ionization time-of-flight

Spot	Protein	pI	MW	CAT versus FVB Y versus O	*P* value
34	Conserved hypothetical protein	6.1	71	CAT-Y: FVB-Y = 1.6	< 0.05
				FVB-O: FVB-Y = 1.3	
35	Disease resistance protein	7.8	87	CAT-O: CAT-Y = 3.3	< 0.05
				CAT-O: FVB-O = 2	< 0.05
				CAT-Y: FVB-Y = 0.9	> 0.05
				FVB-O: FVB-Y = 1.7	< 0.05
40	Signal transduction histidine kinase involved in nitrogen	8.8	83		
	fixation and metabolism regulation (*Desulfovibrio desulphuricans* G20)	9.1	81		
	DNA topoisomerase (bad results)				
41	Retrotransposon hot spot protein RHS5-c	6.8	77		
48	DNA gyrase subunit B (bad result)	8.8	68		
49	Calmodulin	4.5	70		

Quantitative image analysis revealed six spots significantly altered by age and catalase (CAT).
Y, young; O, old.

differential expression was not present in the catalase mice (Fig. 5). The gel spots of interest were cut out and submitted to in-gel trypsin digestion. The tryptic peptides were analysed using MALDI-TOF mass spectrometry to identify the target proteins. Table 3 summarizes the proteins putatively identified by using Mascot software, including conserved hypothetical protein and disease resistance protein.

DISCUSSION

The results of the present study provide evidence that cardiac specific-catalase overexpression may prolong lifespan and attenuate ageing-induced cardiac mechanical, as well as protein, abnormalities. Because the catalase transgene itself did not alter cardiac mechanical and protein properties in young mice, its protective effects against ageing-induced cardiac dysfunction support the beneficial role of this enzyme in delaying the ageing process and minimizing senescence-associated mortality, as reported elsewhere.[7,19] Mounting evidence has indicated compromised cardiac function in senescence.[1,5,20] The present results revealed reduced maximal velocity of contraction and relaxation, prolongation of relaxation and reduced stress tolerance (leftward shift in the threshold of the frequency–response curve) in aged FVB cardiomyocytes. The present data demonstrated that the catalase transgene attenuated ageing-induced cardiac mechanical dysfunction and protein carbonyl formation. Our observation of improved cardiac contractile function in aged catalase mice is consistent with the recent finding that median and maximum lifespans were increased (by approximately 5 and 5.5 months, respectively) in transgenic mice overexpressing catalase localized to the peroxisome, nucleus or mitochondria of multiple organs, including hearts, brain and skeletal muscle.[19] It was demonstrated that systemic catalase-overexpressing (targeted at nucleus and mitochondrion) transgenic mice exhibit delayed cardiac pathology, reduced oxidative damage, H_2O_2 production and H_2O_2-induced aconitase inactivation, as well as diminished onset of mitochondrial deletion.[19] These data support our observation of improved cardiac contractile function and protein integrity in the aged cardiac-specific catalase mice. Our data further indicated the potential significance of hearts in the systemic overexpression of catalase-associated prolongation of lifespan and reduction of cardiac pathology.[19]

Although the mechanism(s) of action behind the catalase-elicited protective effect on altered cardiomyocyte contractile function under ageing remains obscure, it may be speculated that enhanced endogenous anti-oxidant capacity with the 50-fold overexpression of catalase may alleviate or rescue the compromised intracellular Ca^{2+} handling and protein function under ageing. It has been demonstrated that H_2O_2 impairs sarcoplasmic reticulum Ca^{2+} release and uptake through reaction with S-H groups of the ryanodine receptor.[21] In addition, H_2O_2 may alter Ca^{2+} release from mitochondria and trigger DNA damage.[22] Enhanced superoxide and H_2O_2 production has been demonstrated in ageing hearts, in conjunction with accumulation of oxidant-induced damage and age-related alterations in myocardial function.[23] By catalysing the conversion of H_2O_2 to oxygen and water, catalase should alleviate or abrogate the detrimental effects of H_2O_2 on intracellular Ca^{2+} handling and mitochondrial and protein function.

To identify potential proteins that may contribute to ageing-induced cardiac defects and catalase-elicited protective effects, we performed proteomic analysis on total cardiac proteins from young and aged FVB and catalase transgenic mice. Our analysis revealed several proteins with differential expression between young and old mice, which were tentatively identified as conserved hypothetical protein, disease resistance protein and signal transduction histidine kinase involved in nitrogen fixation and metabolism regulation (*Desulfovibrio desulphuricans* G20). Although it is possible that catalase may benefit cardiac function through regulation of one or more of these proteins, a major limitation of the present study is that many of the proteins identified in Table 3 do not have an immediate link to either ageing or heart function. The field of proteomics is still in its infancy and proteomic data should be used with caution. Further study is warranted to better understand the role of these proteins or other proteins not identified in the present study in ageing and cardiac function and whether altered expression of these proteins plays any role in catalase-elicited protection against cardiac ageing.

In conclusion, the present study revealed that cardiac-specific overexpression of catalase prolongs lifespan and rescues ageing-induced mechanical dysfunction in cardiomyocytes, possibly through reduced protein damage. These data, in conjunction with the prolonged lifespan in catalase-overexpressing mice,[19] have convincingly

demonstrated the clinical potential of catalase in the prevention and treatment of ageing-associated cardiac dysfunction.

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