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Exercise training initiated in late middle age attenuates cardiac fibrosis and advanced glycation end-product accumulation in senescent rats

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ABSTRACT

While it has long been postulated that exercise training attenuates the age-related decline in heart function normally associated with increased fibrosis and collagen cross-linking, the potential benefits associated with exercise training initiated later in life are currently unclear. To address this question, Fischer 344 × Brown Norway F1 rats underwent treadmill-based exercise training starting in late middle age and continued into senescence (35 mo) and were compared with age-matched sedentary rats. Hearts were examined for fibrosis and advanced glycation end-products in the subendocardial layer of left ventricular cross-sections. Genes for collagen synthesis and degradation were assessed by polymerase chain reaction, and matrix metalloproteinase (MMP) activity was assessed by EnzChek® Gelatinase/Collagenase Assay Kit. Exercise training of late middle-aged rats attenuated fibrosis and collagen cross-linking, while also reducing age-related mortality between late middle age and senescence. This training was also associated with an attenuated advanced glycation end-product (AGE) accumulation with aging, suggesting a decrease in collagen cross-linking. Conversely, tissue inhibitor of matrix metalloproteinase-1 (TIMP1) gene expression, TIMP and MMP1 protein expression, and MMP activity increased with age but were not significantly impacted by exercise training. While our results demonstrate that exercise training in late middle age attenuates age-related mortality and cardiac fibrosis and is accompanied by attenuated AGE accumulation indicative of less collagen cross-linking, the mechanisms explaining this attenuated replacement fibrosis did not appear to involve altered TIMP1 expression, or MMP protein and activity.
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1. Introduction

Heart failure is strongly associated with advanced age (Chen, 2009; Thomas and Rich, 2007) and represents the most common reason for the hospitalization of patients older than 65 years of age (DeFrances et al., 2007). People 65 years or older currently represent 13% of the population, a percentage which will double in the next 30 years; placing increased stress on cardiovascular care systems (Bales and Ritchie, 2002; Jacobsen et al., 2011). The decline in diastolic heart function, which occurs with age from 30 years old and onwards in humans, is observed as a decrease in the rate and volume of early (passive) diastolic filling, which when severe can lead to diastolic heart failure (Andren et al., 1995; Benjamin et al., 1992; Brenner et al., 2001; Downes et al., 1989; Grossman, 1991; Kitzman, 2002; Lakatta, 1987; Lakatta and Yin, 1982; Lye and Wisniacki, 2000; Mantero et al., 1995). Aging is also associated with fibrotic cardiac remodeling in the extracellular matrix (ECM) including accumulation of advanced glycation end-products (AGEs) and subsequent collagen cross-linking. There is mounting evidence that collagen cross-links are the major determinant of ventricular stiffness and are thus a key contributor to diastolic dysfunction (Aronson, 2003; Badenhorst et al., 2003; Brower et al., 2006; Choi

Abbreviations: AGE, advanced glycation end-product; ANOVA, analysis of variance; %BF, percent body fat; DXA, dual energy X-ray absorptiometry; ECM, extracellular matrix; ET, exercise training; F344BNF1, Fischer 344 Brown Norway F1 hybrid rat; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LV, left ventricle; MMP, matrix metalloproteinase; RT-PCR, reverse transcription polymerase chain reaction; TIMP, tissue inhibitor of matrix metalloproteinase; TTBS, Tris-buffered saline (+0.5% Tween); WGA, wheat germ agglutinin.

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et al., 2009; Jyothirmayi et al., 1998; Kass et al., 2004; Norton et al., 1997; Willemsen et al., 2011; Woodiwiss et al., 2001).

Using a diabetic rat model, Norton et al. (1996) showed that left ventricular (LV) stiffness was associated with higher quantities of AGEs. Administration of aminoguanidine, an AGE-formation inhibitor, resulted in an improved diastolic functioning associated with decreased AGEs (Norton et al., 1996). Subsequently, Avendano et al. (1999) demonstrated that dogs with glucose intolerance have increased AGEs with diastolic dysfunction. Similar to Norton et al. (1996), treatment with aminoguanidine had a beneficial effect on diastolic functioning associated with decreased AGEs (Avendano et al., 1999). Other studies using the AGE cross-link breaker ALT-711 in dogs (Asif et al., 2000), primates (Vaitkevicius et al., 2001), and humans (Little et al., 2005) have shown that administration of this drug results in improvements in diastolic functioning. In a recent study by Willemsen et al. (2011), increased levels of tissue AGEs as measured by skin autofluorescence were significantly correlated with decreased diastolic functioning in association with heart failure. Their group proposed that because AGEs form cross-links, this could be a significant contributor to the decrease seen in diastolic function.

Although some controversy remains in human subjects (Fleg et al., 1995), most studies agree that endurance exercise training (ET) can improve or even reverse the age-associated decline in passive diastolic function (Arbab-Zadeh et al., 2004; Choi et al., 2009; Levy et al., 1993; Takemoto et al., 1992). The mechanism(s) by which endurance exercise improves passive diastolic filling is currently unknown; however, an exercise-induced alteration of the ECM resulting in decreased stiffness through reduced collagen accumulation and/or cross-linking has been suggested (Choi et al., 2009; Kwak et al., 2006, 2011; Thomas et al., 1992, 2000, 2001). Further to the above points, it is currently unknown if this training effect can be induced later in life and continue into senescence where diastolic function becomes more severe and risk of heart failure becomes more prevalent. To address this question, we undertook a study where an endurance exercise program was started in late-middle aged male Fischer 344 × Brown Norway F1 (F344BNF1) rats and continued into senescence to evaluate the impact of endurance exercise initiated late in life on the accumulation of collagen and collagen cross-linking in the aging myocardium.

2. Methods

2.1. Animals

Male pathogen-free F344BNF1 rats aged 7 mo old (young adult) and 29 mo old (late middle aged) were obtained from the National Institute of Aging colony maintained by Harlan (Indianapolis, IN). Animals were randomly assigned to the exercise training group (35T, n = 12), with the remaining 29 mo old rats acting as age-matched sedentary controls (35C, n = 12). In addition, a cohort of 7 mo old adult rats acting as a young adult sedentary control group were included (7C, n = 10). The control groups had only normal cage activity. Animals were housed 2–3 per cage with a 12/12-h light/dark cycle, and given food and water *ad libitum*. Aged animals were 34–36 mo old at the time of sacrifice. The 50% survival rate for this rat strain is 33 mo (Turturro et al., 1999), meaning that the older animals were within the senescent period at the time of sacrifice. All experimental protocols were approved by the University of Calgary Animal Care Committee (protocol number BI 09R-11), and conformed with the Canadian Council on Animal Care.

2.2. Endurance training exercise protocol

The 35T group initially ran on the treadmill 5 days per week using a similar protocol to that described in a previous study (Betik and Hepple, 2008). The exercise protocol was designed to gradually acclimate the rats to exercising on the treadmill. Duration of exercise was increased progressively so that by the third week of training, the animals were running for 60 min per day at a grade of 10%, as this was shown to yield significant benefits for skeletal muscle and whole body function in late middle aged animals (Betik and Hepple, 2008). Each training session consisted of 6 × 10 min of continuous running with 2 min of rest in between repetitions. Each 10 min segment began with 8 min at a base velocity, and ended with 2 min at a higher velocity. Over the first three weeks of training, the base velocity was gradually increased from 5 m/min to 7 m/min. From the third to ninth weeks of training the base velocity was increased in increments of 0.5 m/min each week. By the ninth week the animals could no longer tolerate any increases in velocity. From the eighth week of training onwards, the frequency of training was reduced to 4 days per week to maintain any adaptations that occurred during the first eight weeks of training, and to allow more recovery time as the animals aged into senescence (Betik et al., 2009). We have previously shown that our ET program is effective in inducing exercise-mediated physiological adaptations in the exact same rat model and training program commenced at the same age (28 mo) (Betik et al., 2008). Two markers of oxidative capacity, citrate synthase activity and complex IV activity, in both plantaris and gastrocnemius muscle significantly increased after 7 weeks of ET in comparison to the age-matched sedentary controls. The last training session occurred at least 48 h before the rats were sacrificed to avoid the acute effects of exercise.

2.3. Tissue collection

Animals were weighed and anesthetized with sodium-pentobarbitol (50–65 mg/kg ip). Percent body fat (%BF) was determined *via* dual energy X-ray absorptiometry (DXA). Hearts were removed and trimmed of excess fat and vessels. The hearts were then weighed and mid-ventricular cross sections were taken from the heart for histochemistry, mounted on cork, and rapidly frozen in liquid nitrogen cooled isopentane. Samples were stored at −80 °C until sectioned for staining. The remainder of the heart tissue was snap-frozen in liquid nitrogen and then ground into a powder using a liquid nitrogen cooled ceramic mortar and pestle and stored at −80 °C.

2.4. Van Gieson's stain for collagen

To stain for collagen, 10 μm thick cross-sections were cut at −20 °C from the mid-ventricle of the heart and put onto glass slides which were kept frozen at −80 °C until use. Tissue sections were stained with Van Gieson's stain as described by Bancroft and Stevens (1982). Since exercise training has its greatest impact on the subendocardial region of the heart (Derumeaux et al., 2008), after staining, the slides were allowed to dry and mounted with a coverslip for imaging of the lateral, anterior and posterior portions of the subendocardium of the free wall of the left ventricle (LV) with a Nikon Coolpix 990 digital camera mounted on a Nikon Eclipse E400 stage (Nikon, Mississauga, ON). Analysis was done by overlaying a 100-point grid on each image and counting which points fell on cardiomyocyte, collagen, nothing, or other. Based upon these counts, the relative proportions of each tissue region were calculated and expressed as a percentage of the whole tissue area.

2.5. RNA isolation

RNA from the powdered heart samples was extracted using the TRIzol® (Invitrogen, Carlsbad, CA) method according to the manufacturer's instructions. Briefly, 90 mg of the powdered heart samples was homogenized in 1 mL of TRIzol® and allowed to sit for 5 min before the addition 200 μL of chloroform and shaking by hand for 15 s. Samples were allowed to rest for 2 min before being centrifuged at 14 000 rpm for 15 min to induce phase separation. The clear supernatant was pipetted into fresh tubes where 1 mL of isopropanol was added and to each sample and incorporated *via* inversion. After resting for 10 min the samples were centrifuged again as indicated above to pellet the RNA, which was subsequently washed with 1 mL of 75% ethanol and centrifuged again.

After carefully removing the supernatant, the pellets were allowed to air dry and then re-dissolved in 100 μL of sterile nuclease-free water.

2.6. Quality and quantity of RNA

The isolated RNA was assessed for quality through agarose gel electrophoresis and subsequent imaging. The quantity of RNA was measured according to manufacturer's instructions using the Quant-iT™ RiboGreen® RNA Assay Kit (Invitrogen, Carlsbad, CA). A FL_X-800 Microplate Fluorescence Reader (Bio-Tek Instruments, Inc., Winooski, VT) was used in conjunction with the software program KC4™ Kineticalc 3.03 (Bio-Tek Instruments, Inc., Winooski, VT) to read the fluorescence of the samples and standard at wavelengths between 485 nm and 530 nm. The quantity of RNA in each sample (ng/μL) was determined against a standard curve.

2.7. Reverse transcription of mRNA

Reverse transcription was then performed on the RNA samples to transcribe the RNA back into DNA fragments (cDNA) using SuperScript® First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA), as per manufacturer's instructions.

2.8. Quantitative real time PCR

Messenger RNA expression levels were quantified by RT-PCR using primer pairs ordered from QIAGEN (Germantown, MD) and a number of primers synthesized by the University of Calgary DNA Services Laboratory (University of Calgary, AB). Sequences for the synthesized primers are shown in Table 1. QuantiTect SYBR Green PCR Master Mix and 10 μM primer of interest were combined in a 5:1 ratio to get a final volume sufficient for 15 μL per well, and this was combined with 10 μL of the cDNA sample mixture for a total volume of 25 μL. Messenger RNA expression levels were calculated using the delta–delta-CT method and measured against the 18 s mRNA housekeeping gene.

2.9. Immunohistochemistry for advanced glycation end-products

To examine the hearts for AGEs 7 μm thick cross-sections were sliced at the mid-ventricle of the heart and put onto glass slides which were kept frozen at −80 °C until use. Slides were fixed in acetone, washed in 1× PBS solution for 5 min at room temperature, and then placed in the permeabilization solution (0.1% Triton® X-100, Sigma, St. Louis, MO; in 1× PBS) for 15 min at room temperature while rocking slowly. Slides were then washed in 3 changes of 1× PBS for 5 min in each wash at room temperature. Slides were blocked (10% goat serum and 1% BSA in 1× PBS) at room temperature for 30 min before incubating overnight at 4 °C with primary antibody (AGE antibody, ab23722, Abcam, MA) at a 1:200 dilution. Slides were washed with 3 changes of 1× PBS, blocked again, then incubated at room temperature in the dark for 1 h with a secondary antibody (Alexa Fluor® 488 goat anti-rabbit IgG (H + L), Invitrogen, Carlsbad, CA) at a 1:1000 dilution. Slides were then washed with 2 changes of 1× PBS, then incubated with DAPI (300 nM in 1× PBS, Invitrogen, Carlsbad, CA) for 5 min at room temperature, and washed again with 3 changes of 1× PBS. Wheat germ agglutinin (WGA), Alexa Fluor® 594 conjugate (Invitrogen, Carlsbad, CA) 1:200 diluted in 1× PBS was then pipetted onto the tissue, incubated for 10 min at room temperature, and then washed with 3 changes of 1× PBS. Slides were mounted and coverslips were applied with ProLong® Gold antifade reagent (Invitrogen, Carlsbad, CA).

Images were taken of the anterior, lateral, and posterior regions of the subendocardium and myocardium of the free wall of the LV with an ArcturusXT™ Laser Capture Microdissection System (Life Technologies Corporation, Carlsbad, CA) at a 100× magnification. A separate image of each of the WGA and AGE signals was taken at each location. Images were analyzed using ImageJ 1.44p (NIH, USA) software so that only the ECM portion was analyzed for AGE fluorescence (represented by the intensity of color signal).

Table 1
Forward and reverse primers designed by Primer3plus and synthesized by University Core DNA services (University of Calgary)[a].

Gene	Forward	Reverse
MMP1	acagtttccccgtgtttcag	cccacacctaggtttcctca
MMP2	agctcccggaaaagattgat	tccagttaaaggcagcgtct
TIMP4	acctccggaaggagtacgtt	tgacaggttgtgagctggag

[a] TIMP1 and 18s RNA designed and supplied by QIAGEN.

2.10. MMP biochemical activity

Samples were homogenized in a buffer containing 100 mM Tris (hydroxymethyl) aminomethane; 200 mM NaCl; 0.1% Triton X-100; pH 7.4 (Cha and Purslow, 2010). Samples were centrifuged for 20 min at 10,000 *g* at 4 °C, with the resulting supernatant aspirated off and stored. The protein content of a 1:10 dilution of the supernatant was determined in duplicate *via* Bradford assay (Bradford, 1976).

MMP activity of the supernatant of the samples was measured using EnzChek® Gelatinase/Collagenase Assay Kit (Molecular Probes®, Invitrogen, Carlsbad, CA), as per manufacturers' instructions. Values were normalized to the amount of protein in each sample, and plotted against time to yield the rate of MMP activity.

2.11. Western blotting

For each sample, 1 mL of solution B (1 M Tris (Base), 2.5 M NaCl, 0.2 M EDTA, Triton X-100, 0.5% deoxycholate, 0.04% beta-mercaptoethanol (#161-0710, Biorad, Hercules, CA) in double distilled H_2O, pH 7.4), 10 μL each of Protease Inhibitor Cocktail (Cat# P8340, Sigma-Aldrich, Oakville, ON) and Phosphatase Inhibitor Cocktail 3 (Cat# P0044, Sigma-Alrich, Oakville, ON) was added to approximately 30 mg of frozen powdered tissue. Samples were homogenized for 15–20 s, on ice then centrifuged for 5 min at 10 000 rpm at 4 °C. The supernatant was retained for analysis.

For Western blotting, 15-well, 12.5% Acrylamide/Bis gels were made for electrophoresis. For each sample, 20 μL of sample solution containing 50 μg protein and 5 μL NuPage® LDS sample buffer (4×) (Invitrogen, Carlsbad, CA) was denatured at 90 °C for 10 min, and then loaded into each well. 2 μL of PageRuler Prestained Protein Ladder 10–170 kDa (Thermo Scientific, Nepan, ON) was added to an adjacent blank lane. Gels were run at 200 V for 30–50 min and stopped before samples had reached the end of the gel. Gels were then transferred onto polyvinylidene difluoride membranes at 100 V for 1 h. The membranes were blocked for 1 h in 5% non-fat milk powder in Tris-buffered saline (+0.5% Tween) (TTBS), and then exposed to the following primary antibodies for 2 h: Rabbit Anti-MMP-1 (Cat# 444209, EMD Inc., Mississauga, ON), Rabbit Anti-MMP2 (Cat# ab37150, Abcam, Toronto, ON), Rabbit anti-GAPDH (FL-335): sc-25778 (SCB-Rabbit) (Santa Cruz Biotechnology Inc., Santa Cruz, CA). The TIMP1 and TIMP2 antibodies used in this study were a kind gift from Dr. Voon Wee Young (University of Calgary). The membranes were washed in TTBS and then exposed to the following secondary antibodies for 2 h: Goat Anti-Mouse IgG HRP conjugate (Fisher Scientific, Nepan, ON) for TIMP1, and for the other three proteins, Goat Anti-Rabbit IgG HRP conjugate (Fisher Scientific, Nepan, ON). Membranes were washed in TTBS again, then Super Signal West Dura Chemiluminescent Substrate (Thermo Scientific, Nepan, ON) was applied to the membrane for 3 min. Bands were imaged using a Fujifilm Las-3000 Mini with Image Reader Las-3000 Mini Version 2.2 software (Fuji Photo Film Co., LTD.). Band intensities were analyzed using ImageJ 1.44p (NIH, USA) and normalized to GAPDH.

2.12. Statistics

For analysis of MMP activity, a two-way analysis of variance (ANOVA) was used with group (7C, 35C, and 35T) and time (hours) as the two factors, and a post-hoc Holm–Sidak multiple comparison test. For analysis of survival curves, a Kaplan–Meier Survival Analysis Log Rank test was used to compare the 35C and 35T groups. For all other tests, a one-way ANOVA was used to compare all three groups (7C, 35C and 35T), with a Holm–Sidak post hoc multiple comparison test. The significance level was set at 0.05 ($p < 0.05$). Values are expressed as means ± standard error of the mean. Where equal variance tests failed, the non-parametric Kruskal–Wallis ANOVA on Ranks test was employed using the Dunn's Test as the post-hoc test if a significant difference was found. When only two groups were compared, a Students *t*-test was applied. Statistical analysis was done using SigmaPlot version 11 (Systat Software Inc. Chicago, IL).

3. Results

3.1. Physiological characteristics

Body mass, heart mass, heart:body mass ratio, and %BF for the various groups are presented in Table 2, while the Kaplan–Meier survival curves for the older animals are shown in Fig. 1. As reported previously by our group (Betik et al., 2009; Thomas et al., 2011), there was a significantly higher rate of survival between 29 mo and 35 mo of age in the trained animals verses the sedentary control animals ($p < 0.05$). This difference in survival was also reflected in the physical characteristics of the older rat groups where body mass and the %BF was significantly lower in the trained group (Table 2), as reported previously (Betik et al., 2009). In fact, the values obtained for the 35T group were strikingly similar to those obtained from the much younger 7C control rats. Heart mass was larger in the older rats relative to the younger controls, however here again we observed that ET resulted in a smaller heart mass relative to the older sedentary rats. Heart:body mass ratio was higher in the older rats relative to the younger controls, with the trained rats having a further significant increase in the ratio as compared to the sedentary age-matched controls due to their markedly lower body mass.

3.2. Collagen quantity

Point counting in Van Geison's stained tissue cross-sections revealed a significantly greater percent of connective tissue of all three regions (anterior, lateral, and posterior) combined in hearts of the senescent control animals (55 ± 4%) *versus* the young controls (5 ± 1%) (see Fig. 2). This increased connective tissue content with aging was attenuated by regular endurance exercise to 43 ± 5% in the 35T group. Representative images of the Van Gieson's stained cardiac cross-sections are shown in Fig. 2B, where insets demonstrate the regions that were examined.

Table 2
Mean body mass, percent body fat, and heart mass of 7C, 35C, and 35T groups.

Measurement	7C	35C	35 T
Body mass (g)	435 ± 6.9	514 ± 11.9[a]	437 ± 11.8
Heart mass (mg)	909 ± 28.2	1260 ± 34.1[a]	1150 ± 26.0[b]
Heart:Body mass	2.09 ± 0.05	2.42 ± 0.06[a]	2.73 ± 0.06[b]
% BF	14 ± 0.5	25 ± 0.9[a]	15 ± 1.4

Values are ± SEM.
[a] Denotes significant difference between 35C and other groups ($p < 0.05$).
[b] Denotes significant difference between 35T and other groups ($p < 0.05$).

3.3. Immunohistochemistry

ET in the aged group resulted in significantly less AGE fluorescence than the sedentary age-matched controls in all regions of the myocardium analyzed, combined (see Fig. 3) ($p < 0.05$), indicating that ET attenuated AGE accumulation with aging. As the amount of AGEs is proportional to the amount of cross-linking in the ECM (Bakris et al., 2004), a higher amount of AGEs corresponds to a higher amount of cross-linking, and a lower amount of AGEs corresponds to a lower amount of cross-linking. Based on these criteria, we observed that the trained animals had less cross-linking than their sedentary counterparts. Furthermore, no significant difference was observed between the aged groups when AGE levels were expressed relative to the connective tissue content.

3.4. MMP activity

An aging effect was observed in the MMP activity with significantly higher rates observed in both aged groups compared to the young control animals ($p < 0.05$). There was no difference in the rate of MMP activity between the aged groups (see Fig. 4).

3.5. Gene expression

TIMP1 gene expression was significantly greater in both aged groups compared to the young control animals ($p < 0.05$) (see Fig. 5). Although some trends were observed for aged animals to have greater MMP 1 & 2 expression, these did not reach significance ($p = 0.05$–0.09). Similarly, there were no significant effects of ET on TIMP4 expression in the aged animals.

3.6. Protein expression

When examining protein expression *via* Western blot, we observed an increased expression ($p < 0.05$) of MMP1, TIMP1 and TIMP2 in the older groups relative to the 7C group (Fig. 6); however, this effect was not significant for MMP2 ($p > 0.05$). Despite this difference in expression between the young and the older groups, we did not see a significant effect in the older groups that was associated with training.

4. Discussion

Diastolic function declines with age, which is mostly observed as a decrease in the rate and volume of early (passive) diastolic filling and is a likely cause of the exponentially increased incidence of diastolic heart failure with aging (Aronson, 2003; Kitzman, 2002; Lye and Wisniacki, 2000). The purpose of our study was to investigate what benefits ET could have when initiating the training at a later age where detrimental changes within the heart have already amassed and when the heart may exhibit a blunted ability to make positive adaptations, as has been demonstrated by our group recently in skeletal muscles (Betik et al., 2009; Thomas et al., 2010, 2011). We hypothesized that endurance exercise training initiated in late middle age would attenuate the accumulation of collagen and collagen cross-linking in the myocardium of male F344BNF1 rats between late middle age and senescence. Amongst the novel and key strengths of our study is the advanced age of the animals, a feature facilitated by the use of the F344BNF1 rat model. Specifically, the F344BNF1 rat strain lives long enough to experience cardiac age-related changes similar to humans, including diastolic and systolic dysfunction; and exhibits alterations in structure involving increased fibrosis and loss of cardiomyocyte density (Hacker et al., 2006; Turturro et al., 1999; Walker et al., 2006). The 50% survival rate for this rat strain is 33 mo old (Turturro et al., 1999), meaning that the 34–36 mo old animals of this study were within the period of senescence and corresponded approximately to an age of ≥80 years for humans based upon survival curve comparisons (Masoro and Austad, 2006; Turturro et al., 1999). Whereas the majority of previous studies

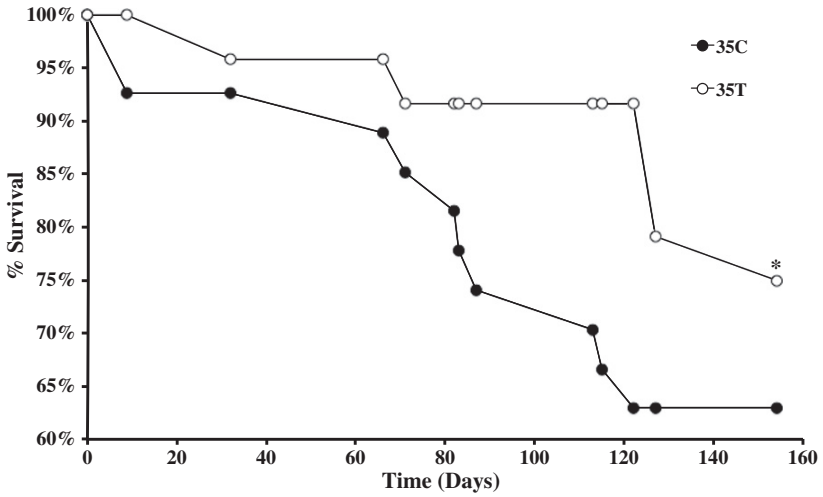

Fig. 1. Percentage of aged animals surviving in each group. Day zero indicates the beginning of the experiment when animals were 29 mo of age. For this figure only, n = 27 for the 35C group and n = 24 for the 35T group. Asterisk denotes significant difference in 35T compared to 35C ($p < 0.05$).

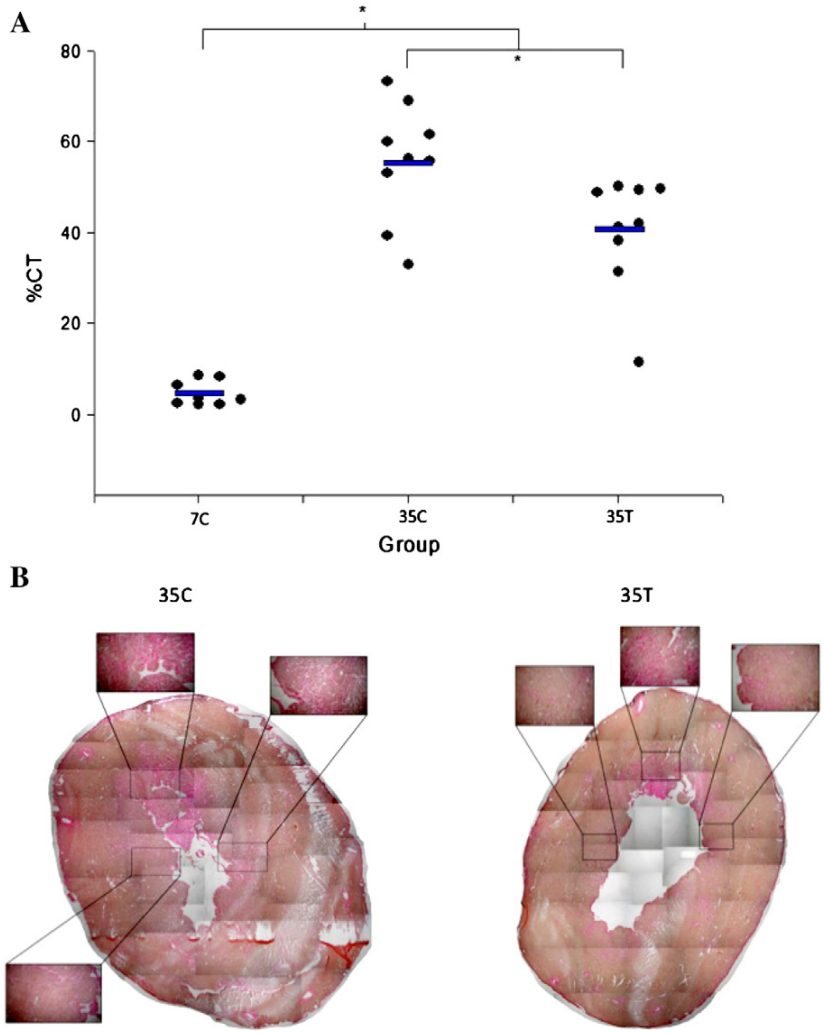

Fig. 2. A. Mean percent connective tissue (%CT) of combined anterior, lateral, and posterior regions of free wall of left ventricle. Dots denote individual animal values, bar denotes mean of group. Asterisk denotes significant difference between groups as indicated ($p < 0.05$). Fig. 2B. Mid-ventricular cross-section of a 35C (left) and 35T (right) heart using the Van Gieson's stain. Highlighted regions on each sample (clockwise, starting from lower-left) are posterior, lateral, and anterior.

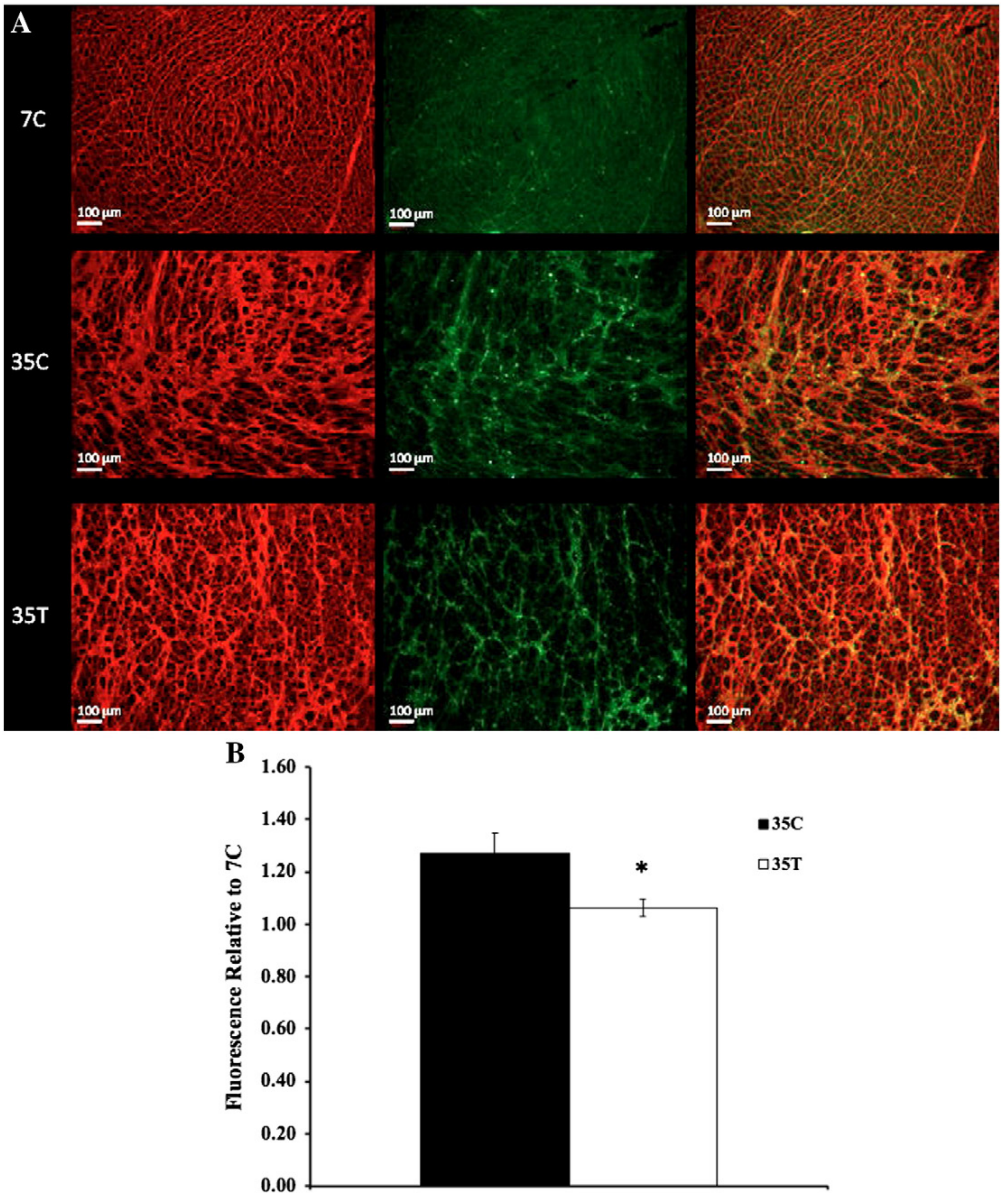

Fig. 3. A. A Sample image each from 7C, 35C, and 35T groups. First column (red) is WGA signal, second column (green) is AGE signal, third column is a merged image of the first and second columns. Fig. 3B. AGE fluorescence of 35C and 35T expressed relative to the 7C group. Asterisk denotes significant difference between 35C and 35T groups ($p < 0.05$).

have focused on ages where aging-related cardiac deterioration was modest, very little is known about the effects of endurance exercise training on hearts that have already experienced significant detrimental age-related changes, including prominent diastolic dysfunction (Burlew, 2004; Hacker et al., 2006), and how this might impact later changes in advanced age where cardiac dysfunction is much more severe. To the best of our knowledge, this study is the first time AGE fluorescence and collagen cross-linking have been tested with an endurance exercise training program initiated in late middle aged and carried through into senescence, an intervention which approximates training between the ages of 65 years and 80 years in humans. As mentioned above, at the age of training initiation detrimental age-associated changes in the heart had already begun to accumulate, such as significant fibrosis and collagen cross-linking (Choi et al., 2009; Thomas et al., 2001). Despite this fact, as we reported previously (Betik et al., 2009), we found that ET was associated with an increased rate of survival and attenuated the age-related increase in fibrosis and cross-linking of the collagen in aged myocardium. Furthermore, this was not associated with modulation of enzymatic activity, gene, or protein expression of collagen degrading enzymes or their inhibitors. Although the results did not reveal these exercise benefits to be the consequence of altered enzymatic regulation of collagen quantity and quality, the lower levels of AGE accumulation (implying lower levels of collagen cross-linking) in the trained animals is indicative of a structurally superior myocardium in regards to preserved diastolic function by attenuating the age-related increase in the stiffness of the LV.

4.1. Survival rate

As reported previously (Betik et al., 2009), the senescent endurance trained group had a significantly greater survival rate than their sedentary age-matched controls (see Fig. 1). This is in agreement with other studies demonstrating that voluntary exercise with rats running in

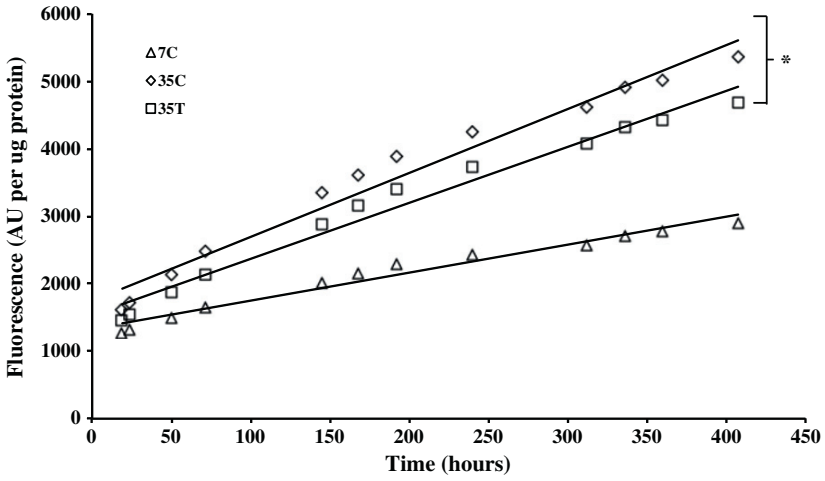

Fig. 4. MMP activity of the three groups. Asterisk denotes significant difference between 7C and aged groups ($p < 0.05$).

wheels results in an increased rate of survival (Holloszy, 1993; Holloszy and Schechtman, 1991; Holloszy et al., 1985); however, a significant difference with the current study is that the exercise training was not initiated until late in the lifespan. While this increase in survival rate could be due to a number of different factors, with regard to the heart, the lesser survival of the senescent sedentary group compared to the trained group could be due to a decrease in diastolic functioning that occurs with aging, as this decline is thought to be a key cause of the exponentially increased incidence of diastolic heart failure and thus mortality with age (Benjamin et al., 1992; Brenner et al., 2001; Downes et al., 1989; Grossman, 1991; Lakatta, 1987; Lakatta and Yin, 1982). Although no measurements of cardiac function from these animals were obtained, the trained group had a higher exercise capacity than their sedentary age-matched controls (Betik et al., 2009), which given the importance of cardiac output to exercise capacity supports superior cardiac function in the ET group. Furthermore, superior cardiac function in the ET group can also be inferred from the fact that the increase in the proportion of myocardial collagen coupled with the increase in collagen cross-linking are major contributors to the age-associated increase in ventricular stiffness, which in turn leads to impaired diastolic function (Avendano et al., 1999; Badenhorst et al., 2003; Norton et al., 1997; Woodiwiss et al., 2001). The significant attenuation of the accumulation of collagen and AGEs, and thus cross-linking, in the aged hearts of the exercise trained group could attenuate the increased stiffness of the aging myocardium and better preserve the compliance of the LV, thereby helping preserve early LV filling during diastole to some degree. Collectively, therefore, to the extent that lifespan may be limited by deteriorating cardiac function, the positive effects of ET on the aging heart could have contributed to the superior lifespan in this group.

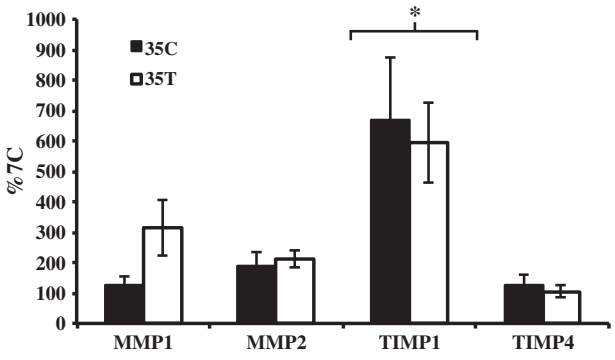

Fig. 5. Gene expression of 35C and 35T expressed as a percentage of 7C group. Asterisk denotes significant difference between 7C and aged groups ($p < 0.05$).

4.2. Collagen cross-linking

In the current study, the aged trained group had significantly less AGE fluorescence than their sedentary counterparts. Indeed, the aged trained group had very similar AGE fluorescence to levels observed at young adulthood. This attenuation in AGE fluorescence caused by endurance exercise training is in agreement with previous studies investigating changes in collagen cross-linking in older endurance exercise trained animals (Choi et al., 2009; Thomas et al., 2001). For example, in a study by Thomas et al. (2001), 10 weeks of endurance exercise training in 26 mo old F344 rats completely reversed age-related increases in collagen cross-linking. Furthermore, in a study by Choi et al. (2009), a 12-wk endurance exercise training program in 25 mo old F344 rats resulted in an attenuation of collagen cross-linking in trained rats to levels observed in young rats. In this same study (Choi et al., 2009), the attenuation of collagen cross-linking was observed with a concomitant increase in myocardial contractility. Although at 25 mo old Fischer 344 rats are considered chronologically senescent (Turturro et al., 1999), the F344BNF1 rat exhibits fewer age-related pathologies and thus exhibits a healthier aging profile (Lipman et al., 1996). Thus, the novelty of the current study lies in the study of a healthy aging model that spans the period when cardiac structural and functional changes become severe and thus, clinically relevant, the age at which the exercise intervention was initiated (starting after deleterious age-related changes in the heart had already begun to occur), and the methodology used (*in situ* labeling permitted spatial localization of the AGE signals).

It has been shown previously that the age of the animals in the current study at the onset of training is associated with a significantly elevated cardiac fibrosis and cross-linking *versus* healthy young adult (Choi et al., 2009; Hacker et al., 2006; Thomas et al., 2001). Thus, the near youthful levels of AGE fluorescence in the trained group suggests the ET not only prevented further damage with increasing age but actually reversed some of the age-related increases in cardiac remodeling that had already occurred by late middle age. That exercise training can still have a significant positive impact on the heart at advanced ages through into senescence is clinically relevant and suggests that this may be an effective way of improving health in older adults and promoting superior longevity. To the best of our knowledge, no group has previously reported AGEs in heart using immunohistochemistry *in situ*, an approach that facilitates a compartment-specific evaluation of AGE accumulation. The reduced quantity of fibrosis in the trained group could also account for the reduction of AGEs, and thus cross-

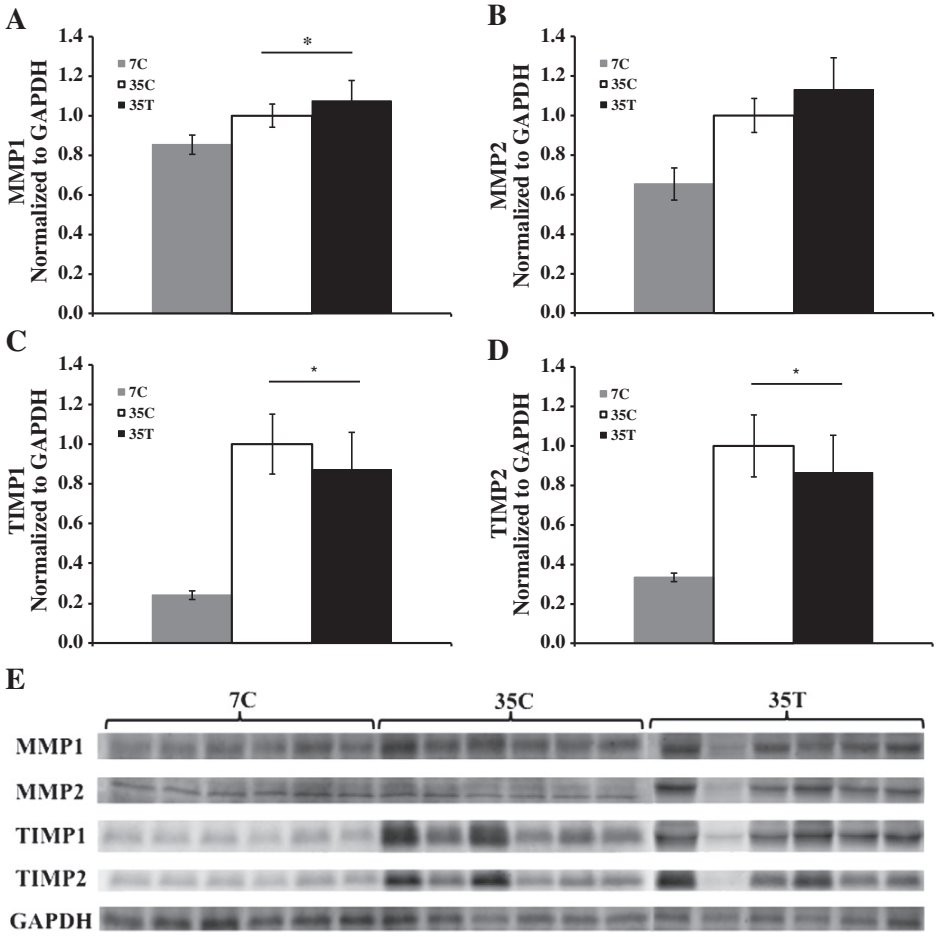

Fig. 6. Protein expression of A) MMP1, B) MMP2, C) TIMP1, & D) TIMP2. E) Images of bands on Western Blots. 7C and 35C were run on one blot while the 35T group was run on a separate blot with 35C. Asterisk denotes significant difference between sedentary groups ($p < 0.05$).

linking, in the collagen of the ECM. Besides this being the first report of ET being able to improve the quality of the collagen in late middle age where detrimental age-associated changes in the myocardium had already begun to accumulate, our findings are also in accordance with the beneficial results of ET in younger animals seen in the literature (Choi et al., 2009; Thomas et al., 1992).

Interestingly, recent evidence by Willemsen et al. (2011) has shown an association in late middle aged humans between tissue AGEs and both early diastolic functioning and aerobic exercise capacity (VO_{2peak}). Their group proposed that the higher levels of skin AGEs in aging humans corresponded to declining exercise capacity because the skin AGEs likely reflected an increase in AGEs in the myocardium which would reduce cardiac function. The current study supports the recent work of Willemsen et al. (2011) by showing directly that ET can reduce AGE accumulation in the aging heart. Lower levels of AGEs are associated with increased diastolic functioning as described by Willemsen's group, and thus could help to explain the increased health and survival of the endurance exercise trained animals in our study.

4.3. Fibrosis

The observations of the current study support the proposition of prior studies that endurance exercise training can attenuate the age-related increase in fibrosis (Kwak et al., 2006, 2011). Indeed, we found a 21% lower collagen content in the ECM with endurance exercise training compared to the sedentary controls in the subendocardium of the free wall of the LV of the heart. The decrease in the percentage of connective tissue seen in the trained group must either be due to a decreased synthesis or increased degradation of collagen in the ECM of the myocardium. Although our hypothesis proposed that the latter mechanism would be prominent, the results of both gene and protein expression yielded no significant differences between the aged groups regarding gene or protein expression of any of the collagen-degrading MMPs or their inhibitors. In this regard, our results contrast with a previous study (Kwak et al., 2011) which observed elevated MMPs in their active and pro-form, as well as in soluble (cytosolic) and ECM fractions following 12 wks of treadmill endurance exercise training in 31 mo old F344BNF1 rats at 10.5 m/min for 45 min/d, 5 d/wk at a 12% incline. Since collagen cross-linking impairs collagen breakdown (Brower et al., 2006; Vater et al., 1979), the fact that the AGE level when expressed per unit of collagen was not different between the 35C and 35T groups suggests the increased levels of AGE per unit of collagen with aging is unlikely to be the root cause of collagen accumulation in the aging heart. Because our results do not support enhanced collagen degradation as a mechanism of reducing collagen content during ET, it remains to be elucidated if ET affects collagen synthesis in late middle aged through to senescent myocardium.

5. Conclusions

Our study examined whether exercise training, initiated at an age where deleterious alterations in the heart had already begun, could

promote an attenuation of cardiac fibrosis in advanced age, where reduced cardiac compliance and impaired diastolic function are important contributors to morbidity and mortality. Furthermore, we sought to understand whether any benefits of exercise were due to an enhanced breakdown of collagen in the aging heart. Importantly, our results demonstrate that exercise training initiated at more advanced age is effective in reducing cardiac fibrosis and promoting superior longevity. This attenuated fibrosis was not accompanied by an increased enzymatic capacity for collagen degradation, nevertheless our results suggest that endurance exercise decreases net fibrosis and collagen cross-link accumulation concurrently, and thus results in a more structurally favorable myocardium. In making inferences about the applicability of our results in a rat model to a clinical setting it is important to realize that the current study in rats translates into approximately a 12–15 year training program in the human lifespan, based upon a 25–30 fold greater lifespan in humans. Whilst conducting such a study in humans would thus be extremely challenging, our observations suggest that endurance exercise training for the older individual could be of considerable clinical importance by providing an effective low-cost and low-risk intervention to attenuate the normal age-related decline in diastolic dysfunction.

Conflict of interest

The authors have no conflicts of interests.

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References

Andren, B., Lind, L., Hedenstierna, G., Lithell, H., 1995. Left ventricular hypertrophy and a decreased E-A ratio are common echocardiographic findings even in apparently healthy 70-year-old men. Cardiol. Elder. 3, 367–371.

Arbab-Zadeh, A., Dijk, E., Prasad, A., Fu, Q., Torres, P., Zhang, R., Thomas, J.D., Palmer, D., Levine, B.D., 2004. Effect of aging and physical activity on left ventricular compliance. Circulation 110, 1799–1805.

Aronson, D., 2003. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. J. Hypertens. 21, 3–12.

Asif, M., Egan, J., Vasan, S., Jyothirmayi, G.N., Masurekar, M.R., Lopez, S., Williams, C., Torres, R.L., Wagle, D., Ulrich, P., Cerami, A., Brines, M., Regan, T.J., 2000. An advanced glycation endproduct cross-link breaker can reverse age-related increases in myocardial stiffness. Proc. Natl. Acad. Sci. U.S.A. 97, 2809–2813.

Avendano, G.F., Agarwal, R.K., Bashey, R.I., Lyons, M.M., Soni, B.J., Jyothirmayi, G.N., Regan, T.J., 1999. Effects of glucose intolerance on myocardial function and collagen-linked glycation. Diabetes 48, 1443–1447.

Badenhorst, D., Maseko, M., Tsotetsi, O.J., Naidoo, A., Brooksbank, R., Norton, G.R., Woodiwiss, A.J., 2003. Cross-linking influences the impact of quantitative changes in myocardial collagen on cardiac stiffness and remodelling in hypertension in rats. Cardiovasc. Res. 57, 632–641.

Bakris, G.L., Bank, A.J., Kass, D.A., Neutel, J.M., Preston, R.A., Oparil, S., 2004. Advanced glycation end-product cross-link breakers. A novel approach to cardiovascular pathologies related to the aging process. Am. J. Hypertens. 17, 23S–30S.

Bales, C.W., Ritchie, C.S., 2002. Sarcopenia, weight loss, and nutritional frailty in the elderly. Annu. Rev. Nutr. 22, 309–323.

Bancroft, J.D., Stevens, A., 1982. Theory and Practice of Histological Techniques. Churchill Livingstone, New York.

Benjamin, E.J., Levy, D., Anderson, K.M., Wolf, P.A., Plehn, J.F., Evans, J.C., Comai, K., Fuller, D.L., Sutton, M.S., 1992. Determinants of Doppler indexes of left ventricular diastolic function in normal subjects (the Framingham Heart Study). Am. J. Cardiol. 70, 508–515.

Betik, A.C., Hepple, R.T., 2008. Determinants of VO2 max decline with aging: an integrated perspective. Appl. Physiol. Nutr. Metab. 33, 130–140.

Betik, A.C., Baker, D.J., Krause, D.J., McConkey, M.J., Hepple, R.T., 2008. Exercise training in late middle-aged male Fischer 344 × Brown Norway F1-hybrid rats improves skeletal muscle aerobic function. Exp. Physiol. 93, 863–871.

Betik, A.C., Thomas, M.M., Wright, K.J., Riel, C.D., Hepple, R.T., 2009. Exercise training from late middle age until senescence does not attenuate the declines in skeletal muscle aerobic function. Am. J. Physiol. Regul. Integr. Comp. Physiol. 297, R744–R755.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Brenner, D.A., Apstein, C.S., Saupe, K.W., 2001. Exercise training attenuates age-associated diastolic dysfunction in rats. Circulation 104, 221–226.

Brower, G.L., Gardner, J.D., Forman, M.F., Murray, D.B., Voloshenyuk, T., Levick, S.P., Janicki, J.S., 2006. The relationship between myocardial extracellular matrix remodeling and ventricular function. Eur. J. Cardiothorac. Surg. 30, 604–610.

Burlew, B.S., 2004. Diastolic dysfunction in the elderly—the interstitial issue. Am. J. Geriatr. Cardiol. 13, 29–38.

Cha, M.C., Purslow, P.P., 2010. Matrix metalloproteinases are less essential for the in-situ gelatinolytic activity in heart muscle than in skeletal muscle. Comp. Biochem. Physiol. A: Mol. Integr. Physiol. 156, 518–522.

Chen, M.A., 2009. Heart failure with preserved ejection fraction in older adults. Am. J. Med. 122, 713–723.

Choi, S.-Y., Chang, H.-J., Choi, S.-I., Il Kim, K., Cho, Y.-S., Youn, T.-J., Chung, W.-Y., Chae, I.-H., Choi, D.-J., Kim, H.-S., Kim, C.-H., Oh, B.-H., Kim, M.-H., 2009. Long-term exercise training attenuates age-related diastolic dysfunction: association of myocardial collagen cross-linking. J. Korean Med. Sci. 24, 32–39.

DeFrances, C.J., Cullen, K.A., Kozak, L.J., 2007. National hospital discharge survey: 2005 annual summary with detailed diagnosis and procedure data. Vital and Health Statistics Series 13, Data from the National Health Survey, 165 1–209.

Derumeaux, G., Ichinose, F., Raher, M.J., Morgan, J.G., Coman, T., Lee, C., Cuesta, J.M., Thibault, H., Bloch, K.D., Picard, M.H., Scherrer-Crosbie, M., 2008. Myocardial alterations in senescent mice and effect of exercise training a strain rate imaging study. Circ. Cardiovasc. Imaging 1, 227–234.

Downes, T.R., Nomeir, A.M., Smith, K.M., Stewart, K.P., Little, W.C., 1989. Mechanism of altered pattern of left ventricular filling with aging in subjects without cardiac disease. Am. J. Cardiol. 64, 523–527.

Fleg, J.L., Shapiro, E.P., O'Connor, F., Taube, J., Goldberg, A.P., Lakatta, E.G., 1995. Left ventricular diastolic filling performance in older male athletes. JAMA 273, 1371–1375.

Grossman, W., 1991. Diastolic dysfunction in congestive heart failure. N. Engl. J. Med. 325, 1557–1564.

Hacker, T.A., McKiernan, S.H., Douglas, P.S., Wanagat, J., Aiken, J.M., 2006. Age-related changes in cardiac structure and function in Fischer 344 × Brown Norway hybrid rats. Am. J. Physiol. Heart Circ. Physiol. 290, H304–H311.

Holloszy, J.O., 1993. Exercise increases average longevity of female rats despite increased food intake and no growth retardation. J. Gerontol. 48, B97–B100.

Holloszy, J.O., Schechtman, K.B., 1991. Interaction between exercise and food restriction: effects on longevity of male rats. J. Appl. Physiol. 70, 1529–1535.

Holloszy, J.O., Smith, E.K., Vining, M., Adams, S., 1985. Effect of voluntary exercise on longevity of rats. J. Appl. Physiol. 59, 826–831.

Jacobsen, L.A., Kent, M., Lee, M., 2011. America's aging population. Popul. Bull. 66.

Jyothirmayi, G.N., Soni, B.J., Masurekar, M., Lyons, M., Regan, T.J., 1998. Effects of metformin on collagen glycation and diastolic dysfunction in diabetic myocardium. J. Cardiovasc. Pharmacol. Ther. 3, 319–326.

Kass, D.A., Bronzwaer, J.G.F., Paulus, W.J., 2004. What mechanisms underlie diastolic dysfunction in heart failure? Circ. Res. 94, 1533–1542.

Kitzman, D.W., 2002. Diastolic heart failure in the elderly. Heart Fail. Rev. 7, 17–27.

Kwak, H.J., Park, M.J., Cho, H., Park, C.M., Moon, S.I., Lee, H.C., Park, I.C., Kim, M.S., Rhee, C.H., Hong, S.I., 2006. Transforming growth factor-beta1 induces tissue inhibitor of metalloproteinase-1 expression via activation of extracellular signal-regulated kinase and Sp1 in human fibrosarcoma cells. Mol. Cancer Res. 4, 209–220.

Kwak, H.B., Kim, J.H., Joshi, K., Yeh, A., Martinez, D.A., Lawler, J.M., 2011. Exercise training reduces fibrosis and matrix metalloproteinase dysregulation in the aging rat heart. FASEB J. 25, 1106–1117.

Lakatta, E.G., 1987. Do hypertension and aging have a similar effect on the myocardium? Circulation 75, I69–I77.

Lakatta, E.G., Yin, F.C., 1982. Myocardial aging: functional alterations and related cellular mechanisms. Am. J. Physiol. 242, H927–H941.

Levy, W.C., Cerqueira, M.D., Abrass, I.B., Schwartz, R.S., Stratton, J.R., 1993. Endurance exercise training augments diastolic filling at rest and during exercise in healthy young and older men. Circulation 88, 116–126.

Lipman, R.D., Chrisp, C.E., Hazzard, D.G., Bronson, R.T., 1996. Pathologic characterization of brown Norway, brown Norway × Fischer 344, and Fischer 344 × Brown Norway rats with relation to age. J. Gerontol. Ser. A Biol. Sci. Med. Sci. 51, B54–B59.

Little, W.C., Zile, M.R., Kitzman, D.W., Hundley, W.G., O'Brien, T.X., Degroof, R.C., 2005. The effect of alagebrium chloride (ALT-711), a novel glucose cross-link breaker, in the treatment of elderly patients with diastolic heart failure. J. Card. Fail. 11, 191–195.

Lye, M., Wisniacki, N., 2000. Heart failure in the elderly: a diastolic problem? Eur. J. Heart Fail. 2, 133–136.

Mantero, A., Gentile, F., Gualtierotti, C., Azzollini, M., Barbier, P., Beretta, L., Casazza, F., Corno, R., Giagnoni, E., Lippolis, A., 1995. Left ventricular diastolic parameters in 288 normal subjects from 20 to 80 years old. Eur. Heart J. 16, 94–105.

Masoro, E.J., Austad, S.N., 2006. Handbook of the Biology of Aging. Elsevier Academic Press.

Norton, G.R., Candy, G., Woodiwiss, A.J., 1996. Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. Circulation 93, 1905–1912.

Norton, G.R., Tsotetsi, J., Trifunovic, B., Hartford, C., Candy, G.P., Woodiwiss, A.J., 1997. Myocardial stiffness is attributed to alterations in cross-linked collagen rather than total collagen or phenotypes in spontaneously hypertensive rats. Circulation 96, 1991–1998.

Takemoto, K.A., Bernstein, L., Lopez, J.F., Marshak, D., Rahimtoola, S.H., Chandraratna, P.A.N., 1992. Abnormalities of diastolic filling of the left-ventricle associated with aging are less pronounced in exercise-trained individuals. Am. Heart J. 124, 143–148.
Thomas, S., Rich, M.W., 2007. Epidemiology, pathophysiology, and prognosis of heart failure in the elderly. Clin. Geriatr. Med. 23, 1–10.
Thomas, D.P., McCormick, R.J., Zimmerman, S.D., Vadlamudi, R.K., Gosselin, L.E., 1992. Aging-induced and training-induced alterations in collagen characteristics of rat left-ventricle and papillary-muscle. Am. J. Physiol. 263, H778–H783.
Thomas, D.P., Zimmerman, S.D., Hansen, T.R., Martin, D.T., McCormick, R.J., 2000. Collagen gene expression in rat left ventricle: interactive effect of age and exercise training. J. Appl. Physiol. 89, 1462–1468.
Thomas, D.P., Cotter, T.A., Li, X., McCormick, R.J., Gosselin, L.E., 2001. Exercise training attenuates aging-associated increases in collagen and collagen crosslinking of the left but not the right ventricle in the rat. Eur. J. Appl. Physiol. 85, 164–169.
Thomas, M.M., Vigna, C., Betik, A.C., Tupling, A.R., Hepple, R.T., 2010. Initiating treadmill training in late middle age offers modest adaptations in Ca2 + handling but enhances oxidative damage in senescent rat skeletal muscle. Am. J. Physiol. Regul. Integr. Comp. Physiol. 298, R1269–R1278.
Thomas, M.M., Vigna, C., Betik, A.C., Tupling, A.R., Hepple, R.T., 2011. Cardiac calcium pump inactivation and nitrosylation in senescent rat myocardium are not attenuated by long-term treadmill training. Exp. Gerontol. 46, 803–810.
Turturro, A., Witt, W.W., Lewis, S., Hass, B.S., Lipman, R.D., Hart, R.W., 1999. Growth curves and survival characteristics of the animals used in the Biomarkers of Aging Program. J. Gerontol. A. Biol. Sci. Med. Sci. 54, B492–B501.
Vaitkevicius, P.V., Lane, M., Spurgeon, H., Ingram, D.K., Roth, G.S., Egan, J.J., Vasan, S., Wagle, D.R., Ulrich, P., Brines, M., Wuerth, J.P., Cerami, A., Lakatta, E.G., 2001. A cross-link breaker has sustained effects on arterial and ventricular properties in older rhesus monkeys. Proc. Natl. Acad. Sci. U.S.A. 98, 1171–1175.
Vater, C.A., Harris Jr., E.D., Siegel, R.C., 1979. Native cross-links in collagen fibrils induce resistance to human synovial collagenase. Biochem. J. 181, 639–645.
Walker Jr., E.M., Nillas, M.S., Mangiarua, E.I., Cansino, S., Morrison, R.G., Perdue, R.R., Triest, W.E., Wright, G.L., Studeny, M., Wehner, P., Rice, K.M., Blough, E.R., 2006. Age-associated changes in hearts of male Fischer 344/Brown Norway F1 rats. Ann. Clin. Lab. Sci. 36, 427–438.
Willemsen, S., Hartog, J.W., Hummel, Y.M., van Ruijven, M.H., van der Horst, I.C., van Veldhuisen, D.J., Voors, A.A., 2011. Tissue advanced glycation end products are associated with diastolic function and aerobic exercise capacity in diabetic heart failure patients. Eur. J. Heart Fail. 13, 76–82.
Woodiwiss, A.J., Tsotetsi, O.J., Sprott, S., Lancaster, E.J., Mela, T., Chung, E.S., Meyer, T.E., Norton, G.R., 2001. Reduction in myocardial collagen cross-linking parallels left ventricular dilatation in rat models of systolic chamber dysfunction. Circulation 103, 155–160.