Altered left ventricular performance in aging physically active mice with an ankle sprain injury

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Abstract We assessed the impact of differing physical activity levels throughout the lifespan, using a musculoskeletal injury model, on the age-related changes in left ventricular (LV) parameters in active mice. Forty male mice (CBA/J) were randomly placed into one of three running wheel groups (transected CFL group, transected ATFL/CFL group, SHAM group) or a SHAM Sedentary group (SHAMSED). Before surgery and every 6 weeks after surgery, LV parameters were measured under 2.5 % isoflurane inhalation. Group effects for daily distance run was significantly greater for the SHAM and lesser for the ATFL/CFL mice ($p = 0.013$) with distance run decreasing with age for all mice ($p < 0.0001$). Beginning at 6 months of age, interaction (group $\times$ age) was noted with LV posterior wall thickness-to-radius ratios (h/r) where h/r increased with age in the ATFL/CFL and SHAMSED mice while the SHAM and CFL mice exhibited decreased h/r with

age ($p = 0.0002$). Passive filling velocity (E wave) was significantly greater in the SHAM mice and lowest for the ATFL/CFL and SHAMSED mice ($p < 0.0001$) beginning at 9 months of age. Active filling velocity (A wave) was not different between groups ($p = 0.10$). Passive-to-active filling velocity ratio (E/A ratio) was different between groups ($p < 0.0001$), with higher ratios for the SHAM mice and lower ratios for the ATFL/CFL and SHAMSED mice in response to physical activity beginning at 9 months of age. Passive-to-active filling velocity ratio decreased with age ($p < 0.0001$). Regular physical activity throughout the lifespan improved LV structure, passive filling velocity, and E/A ratio by 6 to 9 months of age and attenuated any negative alterations throughout the second half of life. The diastolic filling differences were found to be significantly related to the amount of activity performed by 9 months and at the end of the lifespan.

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Introduction

The age-related decline in left ventricular (LV) structure and performance can lead to a reduced functional capacity and result in heart failure (Kitzman 2000; Lakatta and Levy 2003; Schulman et al. 1992). Regular exercise is known to help attenuate these age-related changes

(Garber et al. 2011; Turner et al. 2013). Past work from our laboratory has shown regular activity early in life results in significant LV diastolic filling differences by the second quarter of life that are maintained through the second half of the lifespan (Turner et al. 2013). Factors hindering regular exercise participation (i.e., causing physical inactivity) could potentially promote the age-related decline in LV performance.

A lower body musculoskeletal injury, such as an ankle sprain, has been found to negatively influence physical activity early in life (Hubbard-Turner et al. 2013; Verhagen et al. 1995). Additionally, ankle sprains are the most common musculoskeletal injury suffered during physical activity and sport (Fernandez et al. 2007; Hootman et al. 2007) and the CDC has reported that lower extremity injury is a leading reason why people stop participating in lifelong physical activity (Centers for Disease Control and Prevention 2009). Although commonly viewed as an inconsequential injury, around \$4 billion dollars of medical costs annually are related to this injury (Soboroff et al. 1984) with up to 30–75 % of children and adults reporting chronic ankle problems associated with lifelong residual problems (e.g., balance deficits) (Mandarakas et al. 2014; Peters et al. 1991; Smith and Reischl 1986). Also, approximately 78 % of those with chronic ankle problems will develop ankle osteoarthritis (Hirose et al. 2004; Valderrabano et al. 2006). With these residual problems, we perceive additional long-term alterations will develop, primarily from the reduced physical activity level we commonly observe with these individuals. Therefore, utilizing an ankle sprain research model could provide insight into the long-term impact of reduced physical activity, as a result of a musculoskeletal injury, on LV structure and performance throughout the lifespan.

The significant impact of regular exercise with aging is known to result in positive changes in LV structure and performance, yet a longitudinal research model to study the relationship of differing activity levels impacting these characteristics throughout the lifespan has not been performed. Past research has indicated moderate- to high-intensity training for at least 3 months resulted in positive LV changes in older adults (Molmen et al. 2012; Takemoto et al. 1992). Also, master athletes regularly exhibit marked cardiac performance compared to age-matched controls (Forman et al. 1992). Although regular exercise is known to be beneficial, the specific influence of exercise frequency, intensity, and duration on age-related LV diastolic performance would be useful for

exercise prescription in at-risk populations. This is a compelling issue considering the expected positive impact of regular exercise in a population that appears to be at risk for low activity levels for an extended period throughout one's life. A musculoskeletal injury model allows us to monitor differing physical activity levels and LV characteristics in mice of similar genetic background and environmental conditions.

Therefore, we assessed the impact of differing physical activity levels throughout the lifespan, using a musculoskeletal injury model, on the age-related changes in LV structure and performance parameters in active mice. We hypothesized the control mice with a running wheel (SHAM) would exhibit greater daily physical activity patterns resulting in improved structure and performance, by the middle of the lifespan, compared to the mild ankle sprain mice with a running wheel (CFL) and moderate to severe ankle sprain mice with a running wheel (ATFL/CFL). Also, we hypothesized the ATFL/CFL mice would exhibit reduced daily physical activity patterns resulting in impaired performances compared to the SHAM and CFL mice but not different from control mice without a running wheel (SHAMSED) throughout the lifespan. Lastly, we expected to observe a relationship between physical activity and the diastolic filling variables by the middle of the lifespan (critical age when differences occur between groups) as well as a relationship in total lifetime physical activity with the end of life diastolic variables.

Materials and methods

Animal subjects

Forty male CBA/J male mice were purchased from Jackson Laboratory (JAX; Bar Harbor, ME) at 5 weeks of age. All mice were randomly sorted into four groups (SHAM, CFL, ATFL/CFL, and SHAMSED). Thirty of the mice (SHAM, CFL, and ATFL/CFL) were provided with running wheels in their cage while the remaining ten sedentary control mice (SHAMSED) were housed in similar cages without a running wheel. At 6 weeks of age, all mice were individually housed in the university vivarium with 12-h light/dark cycles; room temperature and humidity were standardized to 18–22 °C and 20–40 %, respectively. All mice received ad libitum water and standard chow (Harland Tekland 8604 Rodent Diet;

Madison, WI). Each mouse was monitored daily by the research team and vivarium staff from 6 weeks of age to the end of the lifespan. Maximal lifespan of a male CBA/J mouse has been noted to be approximately 75 to 85 weeks of life (Storer 1966; Tillmann et al. 2000). At 93 weeks of age, all mice were anesthetized with isoflurane gas at 4.0 % concentration in oxygen with a 800 cm³/min flow rate. Once under anesthesia, the mouse was exsanguinated by removal of the heart, where heart wet weights were recorded and then stored for histological analysis at a later date. All investigation-al procedures were in accordance with the ethical standards of the Institutional Animal Care and Use Committee (IACUC) at The University of North Carolina at Charlotte, and all applicable national and/or institutional guidelines for the care and use of animals were followed throughout the study period.

Surgical procedures

All surgical procedures have been described previously (Hubbard-Turner et al. 2013). Briefly, at 7 weeks of age, each mouse was anesthetized using isoflurane gas at 4.0 % concentration in oxygen with a 800 cm³/min flow rate. The right hind leg was shaved and prepped with alcohol and a chlorhexidine scrub, after which the mouse was moved to a sterile surgical field under a surgical microscope. A small incision was made with a sterile equipment. All mice in the SHAM and SHAMSED groups did not have any ligaments transected, the CFL group had the calcaneal fibular ligament (CFL) transected, and the ATFL/CFL group had the calcaneal fibular and anterior talofibular ligaments (ATFL/CFL) transected. After the surgical procedure, the skin was closed using two drops of formulated cyanoacrylate surgical adhesive and the mouse was removed from anesthesia and taken to a recovery area. Each mouse received a subcutaneous injection of 5.0 mg kg⁻¹ of carprofen (Rimadyl) diluted in saline and were allowed to recover under a warming lamp until moving freely. Mice were monitored every 24 h post surgery with each mouse provided with 12.5 mg of carprofen (Rimadyl) tablet ad libitum, with additional carprofen available if the 12.5-mg tablet was consumed, for the first 3 days for pain management. No additional analgesia was needed beyond the initial 12.5 mg of carprofen.

Left ventricular measurement

Transthoracic echocardiography with Doppler ultrasound techniques was used to assess left ventricular (LV) systolic and diastolic parameters and function starting at 6 weeks of age (baseline) and continuing every 6 weeks until the end of life. For all mice, left ventricular structure and function were evaluated with transthoracic two-dimensional (2-D) echocardiography in accordance to the guidelines recommended from the American Society of Echocardiography (Waggoner and Bierig 2001). Measurements were performed by an experienced researcher and repeated at the same time of day in all surviving mice throughout the study period, as previously reported (Turner et al. 2013). Mice were anesthetized using isoflurane gas at 2.5 % concentration in oxygen with a 800 cm³/min flow rate. Isoflurane is a known respiratory and cardiodepressant that can reduce resting heart rate by 10–20 % depending upon the concentration (Janssen et al. 2004). Isoflurane concentration and flow rate, along with time under anesthesia limited to 6 to 7 min, were tightly monitored to control for this cardiodepressant effect. In the supine position, the chest of the mouse was shaved with an electric trimmer and ultrasound transmission gel applied to a SONOS 15-6L ultrasound probe prior to being placed on the left chest for the transthoracic echocardiography. During cardiac imaging, each mouse was always positioned in the supine position on the left lateral decubitus in an effort to control for any effects of venous return and blood pressure. Venous return and blood pressure are known to alter preload and afterload conditions, and must be controlled to potentially negate any influence on ventricular performance (Downes et al. 1990; Kass 2002). Images were visualized on a Philips 10.5-in. monitor (Philips Industries; Saronno, Italy) attached to an echocardiograph (SONOS 5500, Agilent Technologies; Andover MD). Upon completion of Doppler ultrasound measurements, each mouse was removed from isoflurane gas and monitored to ensure the return to normal cage habits.

Two-dimensional M-mode echocardiography was used to measure interventricular septum (IVS), LV posterior wall (LVPW) at end diastole, LV end-diastolic diameter (EDD), and LV end-systolic diameter (ESD). A short-axis view of the left ventricle was used to measure parameters according to the recommended guidelines (Sahn et al. 1978). The IVS and LVPW were measured at end diastole. All measurements were performed from images for five consecutive cardiac cycles. Left ventricular mass (LV mass) was calculated based

on the following formula developed by Devereux et al. (Devereux et al. 1986): $LV\ mass = 1.05 [(IVS + EDD + LVPW)^3 - (EDD)^3]$. Left ventricular thickness-to-radius ratio (h/r), a ratio of left ventricular posterior wall thickness to the radius of the internal left ventricular chamber size during diastole, was calculated with the following formula: $h/r = LVPW/(EDD/2)$. Fractional shortening (FS) was calculated as $FS = (EDD - ESD)/EDD$.

Pulsed-wave Doppler mitral valve inflow recordings were used to measure the peak early (E-wave) and late (A-wave) mitral valve diastolic flow velocities. Measurements were made with the ultrasound probe placed inferior to the mitral valve and perpendicular to flow for LV filling. Peak E-wave and A-wave measurements were performed from five consecutive cardiac cycles. Peak E wave and A wave measurements were used to calculate the E/A ratio. All measurements were additionally corrected for resting heart rate to control for any Frank-Starling effects. Resting heart rate was determined from the extrapolation of time required for six consecutive cardiac cycles.

Physical activity measurement

Physical activity was measured as voluntary running wheel activity and has been previously performed in our laboratory with inbred mice (Knab et al. 2009; Lightfoot et al. 2004; Lightfoot et al. 2008; Turner et al. 2013; Turner et al. 2005). At 8 weeks of age, 30 mice received a solid surface running wheel (127 mm, Ware Manufacturing, Phoenix, AZ), a magnetic sensor, and a digital odometer (Sigma Sport BC600, Olney, IL) recorded the number of wheel revolutions. A plate with a magnetic sensor and a cycle computer was attached to the top of each cage, aligned with the magnet on the running wheel. Previous 24-h distance (km/day) and cumulative duration (min/day) were recorded each day from 8 weeks of age (1 week post surgery) through the end of the lifespan, and daily running speed (m/min) was calculated by dividing daily distance by duration.

Western blot analysis

Ten milligrams of heart tissues was homogenized in RIPA lysis buffer containing Halt protease and phosphatase inhibitors (Pierce et al. 2007). Protein content was determined using the BCA method (Pierce et al. 2007). Twenty micrograms of protein was resolved by SDS-PAGE using 5 % Tris-HCL pre-cast gels (Bio-

Rad). Gels were then stained in Coomassie Brilliant Blue (0.08 % Coomassie Brilliant Blue G-250; 1.6 % ortho-phosphoric acid; 8 % ammonium sulfate; 20 % methanol) overnight with gentle rocking. Following the overnight stain, gels were rinsed in deionized water 5 times for 5 min each to remove background. Gels were then scanned, and myosin heavy chain alpha and beta protein bands were quantified using Image J software.

Statistical analysis

Our hypotheses were assessed using a two-way analysis of variance (ANOVA) with repeated measures (group $\times$ age). Analyses were performed to assess any differences between groups (SHAM, CFL, ATFL/CFL, SHAMSED) throughout the lifespan for all cardiac and running activity variables. For our third hypothesis, Pearson's product correlation coefficients were performed to determine any relationships between diastolic filling parameters and physical activity variables. Comparisons were performed for each LV diastolic measurement and total physical activity performed up to 9 months of age, where differences were found between groups. Also, the week 93 (i.e., end of life) LV diastolic filling parameters were compared with total lifetime physical activity. An alpha level of 0.5 was used to denote statistical significance. Tukey's post hoc analyses were performed with an alpha level of 0.05 denoting statistical significance between groups and age periods. All statistical analyses were performed using JMP Statistical Analysis software (SAS Institute).

Results

General characteristics and physical activity

Five of the 40 mice did not survive to the study completion age of 93 weeks. There was no difference in the age of death between groups (SHAM 87.9 ± 16.1 weeks, CFL 88.2 ± 9.4 weeks, ATFL/CFL 92.4 ± 1.9 weeks, SHAMSED 93.0 ± 0.0 weeks; $p = 0.49$). Body weight was different between groups ($p = 0.03$) with a significantly greater weight in the SHAMSED group throughout the study period compared to the remaining three groups (post hoc $p = 0.005$) who all had access to a running wheel. Body weight significantly increased with age for all mice ($p < 0.0001$). Resting heart rate (HRrest) under isoflurane anesthesia was different

between the groups ($p = 0.02$) throughout the lifespan and was significantly greater in the SHAMSED and ATFL/CFL mice compared to the SHAM and CFL mice (post hoc $p = 0.01$). Additionally, HR_{rest} increased with age ($p < 0.0001$). Heart wet weight at the end of life was significantly different between groups ($p = 0.007$; Table 1) with the SHAM mice exhibiting lesser heart weight (mean $\pm$ SEM; 158.6 ± 6.5 mg) compared to the remaining groups and SHAMSED (181.7 ± 2.1 mg), and ATFL/CFL mice (174.7 ± 2.8 mg) had heavier hearts compared to the CFL (167.9 ± 3.9 mg) and SHAM mice. There was no difference between the groups when heart weight was corrected for body weight (mg/g) at the end of life ($p = 0.39$; Table 1).

For the mice with access to a running wheel, daily distance run was different between the groups ($p = 0.013$; Fig. 1a). The SHAM mice ran significantly more distance each day compared to the remaining two running groups (post hoc $p = 0.011$) while the ATFL/CFL mice ran significantly less distance each day than the remaining two running mice groups (post hoc $p = 0.009$). Post hoc analyses suggest group differences at 4–6, 9–11, 13–15, and 18 months. Daily distance run decreased significantly throughout the lifespan ($p < 0.0001$). Daily duration was different between the three running groups ($p = 0.048$; Fig. 1b). The SHAM mice ran significantly more minutes each day compared to the remaining two running groups (post hoc $p = 0.046$) while the ATFL/CFL mice ran significantly less minutes each day (post hoc $p = 0.028$). Post hoc analyses found group differences at 4, 6, 9, 13–15, 18, and 21 months. Daily duration decreased significantly throughout the lifespan ($p < 0.0001$). Average running speed was different between the groups ($p = 0.012$; Fig. 1c). The SHAM mice ran at a faster daily speed versus the remaining two groups of mice (post hoc $p = 0.019$), and the ATFL/CFL mice ran significantly slower each day compared to the CFL and SHAM mice (post hoc

$p = 0.005$). Post hoc analyses exhibited group differences at 4, 5, 9, 11, 14, and 15 months. Average daily running speed slowed down with an increase in age ($p < 0.0001$).

Structure and systolic parameters

Left ventricular EDD was significantly different between groups ($p < 0.0001$; Table 2 and Fig. 2a), with a significantly smaller EDD for the ATFL/CFL and SHAMSED groups compared to the SHAM and CFL groups (post hoc $p < 0.0001$). The ATFL/CFL mice exhibited EDD values that were not different from the SHAMSED mice (post hoc $p = 0.14$). Post hoc analyses suggest group differences from 6 months to the end of the lifespan. There was a significant increase in EDD with age ($p < 0.0001$). There were no differences between the groups with LV ESD ($p = 0.51$). However, ESD did increase with age ($p < 0.0001$; Table 2). Fractional shortening (FS) was different between groups ($p < 0.0001$; Table 2), with the SHAM and CFL mice eliciting greater FS compared to the ATFL/CFL and SHAMSED groups (post hoc $p < 0.0001$). The ATFL/CFL mice were not different from the SHAMSED mice (post hoc $p = 0.56$). Post hoc analyses suggest group differences for FS started to be evident by 7.5 months of age. There was no significant age effect with FS ($p = 0.09$). We observed a significant group effect with LVPW ($p < 0.0001$; Table 3 and Fig. 2b), with the ATFL/CFL and SHAMSED mice possessing greater posterior wall thickness compared to the SHAM and CFL mice (post hoc $p < 0.0001$). The SHAMSED mice had a greater LVPW when compared to the ATFL/CFL mice (post hoc $p = 0.016$). Post hoc evaluation suggests a significant difference between groups beginning at 6 months of age. LVPW increased significantly with age ($p < 0.0001$). A group effect was observed for LV h/r ($p < 0.0001$; Table 3 and Fig. 2c). The ATFL/CFL and SHAMSED mice developed greater LV

Table 1 Mean ($\pm$ SEM) end of life (21 months of age) resting heart rates, body weights, heart weights, and relative heart weights for the four mouse groups

	Resting heart rate (bpm)	Body weight (g)	Heart weight (mg)*	Relative heart weight (mg/g)
SHAM	523.0 ± 12.7	30.8 ± 1.3	158.6 ± 6.5	5.21 ± 0.25
CFL	489.8 ± 11.6	31.7 ± 0.9	167.9 ± 3.9	5.31 ± 0.11
ATFL/CFL	513.4 ± 7.0	31.3 ± 0.5	174.7 ± 2.8	5.58 ± 0.11
SHAMSED	510.4 ± 6.1	33.9 ± 1.2	181.7 ± 2.1	5.29 ± 0.17

*Significant group effect ($p < 0.05$)

Fig. 1 **a** Mean ($\pm$ SEM) daily distance, **b** daily duration, and **c** average running speed for the three running wheel groups across the lifespan. The asterisk denotes significant group effect with SHAM mice running more distance, duration, and faster speed compared to the remaining two groups and the ATFL/CFL running less distance, duration, and slower speed compared to the other two groups ($p < 0.05$). Post hoc analyses, as denoted by the line (—), suggest group differences at 4–6, 9–11, 13–15, and 18 months for daily distance, at 4, 6, 9, 13–15, 18, and 21 months for daily duration and at 4, 5, 9, 11, 14, and 15 months for average running speed. The dagger denotes significant age effect with the mice exhibiting a decrease in distance, duration, and average running speed ($p < 0.05$)

h/r compared to the SHAM and CFL mice (post hoc $p < 0.0001$), and the SHAMSED mice possessed greater LV *h/r* compared to the ATFL/CFL mice (post hoc $p = 0.005$). A significant difference was found between groups beginning at 6 months of age. LV *h/r* exhibited a significant age effect ($p < 0.0001$). Additionally, we observed a group $\times$ age effect ($p = 0.0002$) with the SHAM and CFL mice developing a smaller LV *h/r* with age while the ATFL/CFL and SHAMSED mice increased LV *h/r* with age. LV mass was different between groups ($p = 0.015$; Table 3), with the SHAM and CFL mice

having lesser LV mass compared to the ATFL/CFL and SHAMSED mice (post hoc $p = 0.004$). There was no observed difference in LV mass between the ATFL/CFL and SHAMSED mice (post hoc $p = 0.19$). Post hoc evaluation suggests group differences beginning at 9 months of age. LV mass increased with age ($p < 0.0001$).

Diastolic filling parameters

Passive filling velocity (*E* wave) was significantly different between groups ($p < 0.0001$; Fig. 3a), with a significantly lower passive filling velocity for the ATFL/CFL group compared to the SHAM and CFL groups (post hoc $p < 0.0001$). Also, the *E* wave filling velocity was greater for the SHAM mice compared to the other groups (post hoc $p < 0.0001$). Lastly, the passive filling velocity for the ATFL/CFL mice was not different from the SHAMSED mice (post hoc $p = 0.84$). Post hoc analyses suggest group differences from 9 months to the end of the lifespan. There was a significant increase in *E* wave with age ($p < 0.0001$). Active filling velocity (*A* wave) was not different between groups ($p = 0.10$; Fig. 3b) and significantly increased with age ($p < 0.0001$). Passive-to-active filling velocity ratio (*E/A* ratio) was different between groups ($p < 0.0001$; Fig. 3c), with a significantly higher *E/A* ratio for the SHAM group compared to the CFL and ATFL/CFL groups (post hoc $p < 0.0001$). The *E/A* ratio was lower for the ATFL/CFL mice compared to the other running groups (post hoc $p < 0.0001$). Additionally, the *E/A* ratio for the ATFL/CFL mice was not different from the SHAMSED mice across the lifespan (post hoc $p = 0.86$). Post hoc analyses suggest group differences from 4.5 months to the end of the lifespan. Passive-to-active filling velocity ratio decreased with age ($p < 0.0001$). There was a group $\times$ age interaction ($p < 0.0001$) with the *E/A* ratio, where the SHAM mice exhibited a slower decline in *E/A* with age compared to the other three groups (post hoc $p = 0.005$). The *E/A* ratio for the ATFL/CFL mice decreased at a greater rate with age compared to the SHAM and CFL groups (post hoc $p < 0.0001$). Lastly, there was a greater decline in *E/A* with age in the SHAMSED mice compared to the ATFL/CFL mice (post hoc $p < 0.0001$).

When corrected for HR_{rest}, passive filling velocities were significantly different between groups ($p < 0.0001$), with similar differences between groups as noted above. SHAM mice had greater *E*/HR_{rest} than

Table 2 Mean ($\pm$ SD) left ventricular end-diastolic diameter (EDD), end-systolic diameter (ESD), and fractional shortening (FS) for the four mouse groups at pre-surgery, 7.5 months, and end of life

	EDD (mm)* [†]			ESD (mm) [†]			FS (%)*		
	Pre	7.5 months	21 months	Pre	7.5 months	21 months	Pre	7.5 months	21 months
SHAM	3.9 $\pm$ 0.2	4.5 $\pm$ 0.2	4.8 $\pm$ 0.1	2.9 $\pm$ 0.3	3.2 $\pm$ 0.3	3.5 $\pm$ 0.4	26.6 $\pm$ 5.7	29.1 $\pm$ 5.9	25.9 $\pm$ 7.0
CFL	3.9 $\pm$ 0.1	4.5 $\pm$ 0.1	4.7 $\pm$ 0.3	2.9 $\pm$ 0.2	3.5 $\pm$ 0.4	3.6 $\pm$ 0.3	26.3 $\pm$ 5.5	22.3 $\pm$ 6.5	23.8 $\pm$ 4.3
ATFL/CFL	4.0 $\pm$ 0.4	4.3 $\pm$ 0.1	4.4 $\pm$ 0.1	3.0 $\pm$ 0.4	3.6 $\pm$ 0.4	3.4 $\pm$ 0.5	24.5 $\pm$ 3.7	17.4 $\pm$ 8.2	21.9 $\pm$ 11.1
SHAMSED	3.9 $\pm$ 0.3	4.3 $\pm$ 0.2	4.4 $\pm$ 0.1	2.8 $\pm$ 0.4	3.3 $\pm$ 0.4	3.7 $\pm$ 0.4	28.0 $\pm$ 6.0	23.5 $\pm$ 8.5	14.8 $\pm$ 10.1

The *n* for SHAM, CFL, ATFL/CFL, and SHAMSED were 10 for each group at Pre and 7.5 months, with 9, 9, 10, and 8, respectively, remaining at 21 months

*Significant group effect, [†] significant age effect ($p < 0.05$)

the CFL and ATFL/CFL groups (post hoc $p < 0.0001$), the ATFL/CFL mice had lower E/HR_{rest} compared to SHAM and CFL mice (post hoc $p < 0.0001$), and the ATFL/CFL mice were not different from the SHAMSED mice (post hoc $p = 0.29$). Passive filling velocity corrected for HR_{rest} did not change with age ($p = 0.23$). Active filling velocity corrected for HR_{rest} was not different between groups ($p = 0.56$) and significantly increased with age ($p = 0.009$). E/A ratio corrected for HR_{rest} exhibited similar findings as E/A analyses. $E/A/HR_{rest}$ was significantly different between groups, where the SHAM mice exhibited greater $E/A/HR_{rest}$ compared to the remaining groups (post hoc $p < 0.0001$); the ATFL/CFL mice were significantly lower than the SHAM and CFL mice (post hoc $p < 0.0001$); and the ATFL/CFL mice were not different from the SHAMSED mice (post hoc $p = 0.09$). Similar group $\times$ age interaction effect and post hoc findings were observed for E/A corrected for HR_{rest} ($p < 0.0001$) as observed with E/A .

Relationships between diastolic parameters and lifelong physical activity

The total distance run from week 8 to 9 months of life was significantly correlated with the 9-month passive filling velocity measurement ($p = 0.035$, $r^2 = 0.149$; Table 4). The total duration of activity from week 8 to 9 months of life was not significantly correlated with the 9-month passive filling velocity ($p = 0.117$), but average speed from week 8 to 9-months of life was significantly correlated with the 9-month E wave filling velocity ($p = 0.039$, $r^2 = 0.143$). The total distance run, total duration, and average speed from week 8 to 9 months of life were not significantly correlated with the 9-month

active filling velocity measurement ($p = 0.96$, $p = 0.77$, and $p = 0.91$, respectively). Total distance run ($p = 0.012$, $r^2 = 0.207$; Fig. 4a), total duration ($p = 0.019$, $r^2 = 0.181$), and average speed of activity ($p = 0.006$, $r^2 = 0.243$) from week 8 to 9 months of life were significantly correlated with the 9-month E/A ratio.

The total distance run throughout the lifespan was significantly correlated with the final passive filling velocity measurement at 93 weeks of age ($p = 0.005$, $r^2 = 0.268$). The total duration of activity throughout the lifespan was significantly correlated with the final passive filling velocity ($p = 0.005$, $r^2 = 0.270$), as was average speed across the lifespan ($p = 0.018$, $r^2 = 0.196$). Total distance run, total duration, and average speed throughout the lifespan were not significantly correlated with last active filling velocity measurement ($p = 0.89$, $p = 0.94$, and $p = 0.93$, respectively). Total distance run ($p = 0.007$, $r^2 = 0.250$; Fig. 4b), total duration of activity ($p = 0.009$, $r^2 = 0.235$), and average running speed ($p = 0.037$, $r^2 = 0.157$) throughout the lifespan were significantly correlated with the final E/A ratio at 93 weeks of age.

Differences in myosin heavy chain between groups

We observed no differences in both alpha and beta myosin heavy chain (MHC) between left ventricular tissue of SHAM, SHAMSED, and ATFL/CFL at the end of the lifespan (Fig. 5). No relationships were observed between any of the physical activity parameters at the end of life and alpha or beta MHC ($p > 0.05$). Additionally, no relationships were observed for total physical activity performed throughout the lifespan (duration, distance and speed) and alpha or beta MHC ($p > 0.05$).

Fig. 2 **a** Mean ($\pm$ SEM) LV end-diastolic diameter, **b** LV posterior wall, and **c** LV thickness-to-radius ratio (h/r) for the four groups across the lifespan. The asterisk denotes significant group effect with smaller EDD and greater LV PW and LV h/r for ATFL/CFL and SHAMSED compared with SHAM and CFL groups ($p < 0.05$). Post hoc analyses, as denoted by the line (-), suggest group differences from 6 months to the end of the lifespan for each of the LV variables. The dagger denotes significant age effect with EDD, LVPW, and LV h/r ($p < 0.05$). A significant interaction was noted for LV h/r ($p = 0.0002$) suggesting more positive changes with age in SHAM and CFL versus SHAMSED and ATFL/CFL mice

Discussion

We measured left ventricular (LV) parameters across the lifespan in 40 male CBA/J mice and observed a significant improvement in passive filling velocity and passive-to-active filling ratio in the mice who performed the greatest amount of physical activity. Additionally, we observed a significant interaction effect with LV posterior

wall thickness-to-radius ratio (h/r) with deleterious alterations occurring with the mice who performed the least amount of exercise throughout the lifespan. Our research laboratory utilized an ankle sprain model by transecting either one (calcaneal fibular ligament (CFL)) or two ligaments (anterior talofibular ligament and CFL (ATLF/CFL)) in two separate groups of CBA/J mice in an effort to control for the genetic background and observe if a common musculoskeletal injury would reduce daily physical activity across the lifespan and thereby negatively alter LV parameters. Previous research in our laboratory has shown genetic background to widely influence the regular physical activity patterns of mice (Lightfoot et al. 2004; Lightfoot et al. 2008; Turner et al. 2005). To our knowledge, this was the first study to longitudinally monitor the lifelong cardiac alterations that occur in response to a common musculoskeletal injury and compare to non-injured subjects.

We observed several expected alterations with LV structure associated with regular exercise and/or aging (Lakatta and Levy 2003). Of greatest significance were the noted alterations in the ATFL/CFL mice similar to what was observed in our SHAM mice. These findings suggest a lack of exercise stimulation, most likely due to a lack of exercise volume and intensity, resulting in an attenuation of alterations commonly expected in regularly exercising subjects. Others have previously noted a lack of LV alterations with regularly exercising subjects (Fujimoto et al. 2010; Gates et al. 2003). Of note, fractional shortening was different between groups throughout the lifespan (ATFL/CFL and SHAMSED vs. CFL and SHAM) primarily due to alterations to LV EDD. The greater LV EDD and FS of these mice are most likely the result of the greater exercise activities of these mice, resulting in the common central cardiovascular response of volume-overload hypertrophy to regular aerobic exercise (Ehsani et al. 2003; Rodeheffer et al. 1984; Seals et al. 1994; Wang et al. 2014). Thus, FS and LV EDD were increased in these mice, through the use of the Frank-Starling effect, suggestive of a greater stroke volume. A likely mechanism involved with the exercise-induced volume-overload cardiac hypertrophy is the IGF-1/class IA PI3K pathway (McMullen et al. 2003). Age-related declines in fractional shortening have been noted previously when observing these volume-overload alterations, yet our findings are unique in that we monitored these alterations throughout the lifespan rather than initiating an aerobic exercise training program to older subjects. Although

Table 3 Mean ($\pm$ SD) left ventricular posterior wall thickness (LVPW), thickness-to-radius ratio (h/r), and LV mass for the four mouse groups at pre-surgery, 7.5 months, and end of life

	LVPW (mm)* [†]			h/r * [†]			LV mass (μ g)* [†]		
	Pre	7.5 months	21 months	Pre	7.5 months	21 months	Pre	7.5 months	21 months
SHAM	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	0.44 $\pm$ 0.05	0.41 $\pm$ 0.04	0.40 $\pm$ 0.03	90 $\pm$ 11	120 $\pm$ 18	133 $\pm$ 10
CFL	0.9 $\pm$ 0.1	0.9 $\pm$ 0.1	1.0 $\pm$ 0.3	0.42 $\pm$ 0.04	0.41 $\pm$ 0.02	0.41 $\pm$ 0.02	87 $\pm$ 11	121 $\pm$ 9	134 $\pm$ 19
ATFL/CFL	0.8 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	0.40 $\pm$ 0.04	0.47 $\pm$ 0.02	0.51 $\pm$ 0.02	89 $\pm$ 12	124 $\pm$ 7	148 $\pm$ 6
SHAMSED	0.8 $\pm$ 0.1	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1	0.42 $\pm$ 0.04	0.47 $\pm$ 0.03	0.53 $\pm$ 0.03	83 $\pm$ 12	123 $\pm$ 15	158 $\pm$ 8

The n for SHAM, CFL, ATFL/CFL, and SHAMSED were 10 for each group at Pre and 7.5 months, with 9, 9, 10, and 8, respectively, remaining at 21 months

*Significant group effect, [†] significant age effect ($p < 0.05$)

our mice were regularly active but at significantly lesser amounts compared to our SHAM and CFL mice, potentially significant molecular alterations were occurring by 6 to 9 months of age which may accelerate the negative alterations we observed in our ATFL/CFL mice. These molecular factors are likely related to cardiomyocyte hypertrophy, loss of cardiomyocytes with age, and/or increased cardiac fibrosis (Cornelissen et al. 2010; Dutta et al. 2012). Recently, Bhella et al. (Bhella et al. 2014) provided insight into how low doses of regular physical activity do not appear to be sufficient to prevent the decreased LV compliance commonly associated with cardiovascular disease. Limiting frequency of regular exercise is difficult with our longitudinal mouse model but does suggest a necessary exercise volume threshold necessary for positive cardiovascular alterations.

Our findings of a greater passive filling velocity (E wave) and E/A ratio with the SHAM mice, who had no ligament damage, by 9 months of age are in agreement with past research in our laboratory where C57Bl/6J mice with a running wheel throughout their lifespan were found to exhibit greater diastolic characteristics by 7 to 8 months of age compared to non-exercising C57Bl/6J mice (Turner et al. 2013). When expressed relative to HR_{rest}, to control for any Frank-Starling effects, we observed similar findings between groups as was observed for absolute values with our diastolic filling variables. Regular exercise during the first 40 % of the lifespan appears to be ideal for eliciting beneficial LV diastolic filling with aging mice. Importantly, Carrick-Ranson and colleagues observed a similar age-related decline in LV diastolic filling around the middle of the lifespan in a cross-sectional study design with healthy subjects (Carrick-Ranson et al. 2012). These authors observed no improvements in LV filling

performance at similar filling pressures, heart rates, or LV mass, indicating an age-related decline in intrinsic factors with LV relaxation. Throughout the last third of the lifespan in the current study, passive filling velocity decreased in the SHAM mice, becoming similar to the CFL and ATFL/CFL mice by the end of the lifespan. This finding was also observed in our past work and continues to suggest a critical age period for the benefits of regular exercise with passive filling of the LV that does not appear to be maintained near the end of life.

The age-related decline in E/A ratio was slower in our SHAM mice compared to the remaining running mice. Although exercising regularly, our findings continue to suggest that the decline in LV diastolic filling is inevitable (Bradshaw et al. 2010; Groban et al. 2008; Lee et al. 2012; Shinmura et al. 2011; Turner et al. 2013). Yet, the benefit of regular lifelong physical activity is greater passive filling velocities for the SHAM mice throughout a majority of the lifespan. This age-related decline in passive filling velocity (Fig. 3), although still great enough to maintain a higher E/A ratio in our SHAM mice, is likely the result of various LV age-related mechanisms. Past literature has postulated a reduction in calcium handling with SERCA2 (Dai et al. 2009), increase in LV fibrosis and collagen accumulation (Emter and Baines 2010; Horn et al. 2012), or mitochondrial damage due to reactive oxygen species (Dai and Rabinovitch 2009) as possible mediators of the age-related decline in passive filling velocity.

The use of endurance exercise training to attenuate these age-related changes in LV diastolic filling regularly leads to positive results. Brenner et al. (Brenner et al. 2001) observed reductions in E wave velocity, E/A ratio, E wave deceleration slope, and an increase in E wave deceleration time in young (6 months old) and old

Fig. 3 **a** Mean ($\pm$ SEM) passive filling velocity (*E* wave), **b** active filling velocity (*A* wave), and **c** passive-to-active filling ratio (*E/A* ratio) for the four groups across the lifespan. The *asterisk* denotes significant group effect with greater *E* wave and *E/A* for SHAM vs. other three groups, also significantly lesser *E* wave and *E/A* for ATFL/CFL and SHAMSED compared with other two groups ($p < 0.05$). Post hoc analyses, as denoted by the line (—), suggest group differences from 9 months to the end of the lifespan for *E* wave and from 4.5 months to the end of the lifespan for *E/A*. The *dagger* denotes significant age effect with *E* wave and *A* wave increasing while *E/A* decreasing ($p < 0.05$)

(24 months old) Fisher 344/BNF1 rats. Following 12 weeks of treadmill running, the old rats improved, each LV diastolic parameter becoming more similar to the young rats' diastolic performance. Groban et al. (Groban et al. 2008) performed a 4-week treadmill running program with 6 and 28 months old Fischer 344 $\times$ Brown Norway rats resulting in improved mitral

annular descent (*e'*) with older rats similar to the young adult rats without improvements in filling pressures, or *E/e'* ratios. Lachance, et al. (Lachance et al. 2009) required adult rats to train at a moderate intensity for 9 months or remain sedentary and observed significant improvements in LV diastolic parameters as early as 3 months into the study period. In addition to improved LV filling characteristics, these authors noted a reduction in myocardial fibrosis. Thus, to develop significant improvements in LV diastolic filling with adult rats, one should perform aerobic exercise at a moderate to high intensity for at least 3 months.

When provided the access to a running wheel but incurring a moderate to severe ankle sprain, the ATFL/CFL mice exhibited significantly less physical activity throughout the lifespan when compared to the SHAM and CFL mice. This lesser activity level was primarily responsible for the increased LV EDD, LVPW, *h/r*, reduced passive filling velocity, and *E/A* ratio by 6 to 9 months of age. Previously our laboratory observed markedly reduced activity levels in the ATFL/CFL mice compared to the SHAM and CFL mice throughout the first 4 weeks following a surgically induced ankle sprain (Hubbard-Turner et al. 2013). Our current findings highlight the secondary impact of a musculoskeletal injury on reduced physical activity levels and the acceleration in negative alterations of LV structure and performance, where the increased LV *h/r* and decline in *E/A* ratio was accelerated for the ATFL/CFL mice compared to the SHAM group. Interestingly, the LVPW, *h/r*, along with the passive and active filling velocities for the ATFL/CFL mice were regularly observed to not differ from the sedentary control mice (SHAMSED) throughout the lifespan. However, the *E/A* ratios for the ATFL/CFL mice were observed to decline at a slower rate with age compared to the SHAMSED mice, suggesting a small amount of regular activity may slightly attenuate the age-related decline in LV diastolic function. To our knowledge, this is the first research to evaluate the impact of a musculoskeletal injury that is often erroneously considered minor on the lifelong alterations in LV structure and diastolic filling.

Lifelong regular physical activity has been shown to result in the attenuation of age-associated LV alterations. Yet, the ideal prescription of exercise and the timeframe has not been described for greatest improvements in LV structure and performance throughout the lifespan. Our findings suggest a significant improvement in passive filling velocity and *E/A* ratio will occur at approximately

Table 4 Pearson product correlation coefficients for physical activity and left ventricular diastolic filling at 9 and 21 months of age

	9 months			21 months		
	<i>E</i> wave	<i>A</i> wave	<i>E/A</i> ratio	<i>E</i> wave	<i>A</i> wave	<i>E/A</i> ratio
Distance	0.387*	0.010	0.455*	0.517*	0.027	0.500*
Duration	0.292	0.056	0.426*	0.442*	0.017	0.396*
Ave. speed	0.378*	0.021	0.493*	0.520*	0.014	0.484*

*Significant relationship ($p < 0.05$)

40 % of the lifespan for CBA/J mice who are regularly active and have not sustained a moderate to severe ankle sprain. At this age, the total distance run, which is the product of average speed and duration run, could explain 15 % of the variation in passive filling velocity and 21 % of the variation in *E/A* ratio. Specifically for passive filling velocity at 40 % of the lifespan, average running speed was found to significantly influence the improvement in *E* wave velocity ($r = 0.378$), suggesting higher intensities of endurance training would result in the greatest improvement in this diastolic variable by the middle of the lifespan. At 40 % of the lifespan, average speed ($r = 0.493$) and total duration ($r = 0.426$) of activity significantly increased *E/A* ratio. Thus, with higher intensities and long duration aerobic activity,

one would expect improvements in passive-to-active filling velocity by this age period.

Similar to the mid-life relationships with differing LV diastolic filling and physical activity, we observed relationships at the end of the lifespan. Total distance run throughout the life of our mice could explain approximately 27 % of the variance in end of life passive filling velocity and 25 % in end of life *E/A* ratio. More specifically, end of life passive filling velocity was related to lifelong average running speed ($r = 0.442$) and total duration of activity ($r = 0.520$). The end of life *E/A* ratio was significantly related to lifelong average running speed ($r = 0.396$) and total duration of activity ($r = 0.484$). These findings indicate the importance of higher intensity and, more importantly, longer duration

Fig. 4 **a** Comparison of 9 months *E/A* ratios for all running wheel mice versus total distance run by 9 months of age. There was a significant relationship for all mice with an $r^2 = 0.207$ ($p = 0.012$). **b** Comparison of 21 months (end of life) *E/A* ratios for all running wheel mice versus total distance run (km) throughout the lifespan. There was a significant relationship for all mice with an $r^2 = 0.250$ ($p = 0.007$)

Fig. 5 Mean ($\pm$ SEM) LV alpha and beta MHC content and Western blot for SHAM, SHAMSED, and ATFL/CFL (AI w exercise). No differences were observed between groups ($p > 0.05$)

activity throughout the lifespan appearing to slow the decline in LV diastolic filling with age. These findings appear in agreement with others (Forman et al. 1992; Molmen et al. 2012; Takemoto et al. 1992), where higher intensity and longer duration exercise regularly results in greater LV diastolic outcomes. Interestingly, Carrick-Ranson et al. (2014) prescribed an endurance exercise for 101 older adults and found frequency of exercise did not positively improve LV diastolic filling. Therefore, beneficial prescription regarding higher intensity aerobic exercise during the first 40 % of the lifespan will result in the greatest improvements in LV diastolic filling early in life, while higher intensity and longer duration aerobic activity throughout the lifespan results in better diastolic filling parameters near the end of life.

Age-related alterations in LV alpha and beta myosin heavy chain (MHC) have been widely investigated in differing species primarily in cross-sectional study designs, with the findings suggesting the amount of beta MHC expression does not change but becomes more indicative of fibrosis while alpha MHC declines in

expression relative to the development of heart failure (Gorza et al. 1984; Miyata et al. 2000; Pandya et al. 2006; Reiser et al. 2001; Samuel et al. 1983). Interestingly, we did not observe any differences in either left ventricular alpha or beta MHC content between a group of highly active mice (SHAM), low active but injured mice (ATFL/CFL), or sedentary mice (SHAMSED), suggesting similar end of life LV MHC content regardless of lifelong intervention. Also, our findings were not related to any of the activity parameters (duration, average speed, or distance) throughout the lifespan or during the last month of life for the SHAM and ATFL/CFL mice, suggesting a low likelihood of influence of regular physical on LV alpha or beta MHC content at the end of life. We did not determine any young compared to older LV alpha MHC content to be able to suggest a decline in LV alpha MHC content with age. Lastly, we are uncertain as to why the amount of LV alpha MHC relative to beta MHC content was greater than expected. The authors are unaware of any other investigations into end of life LV alpha or beta MHC content with a longitudinal model in exercising mice that would

allow for any comparisons to our findings. Additionally, it should be noted that past literature acknowledges the difficulty in comparing cardiac MHC, along with other myocardial parameters, with senescent animals and cautions investigators to this issue with regard to animal species, strain, gender, and experimental condition (Ren et al. 2007).

Conclusions

The structure and performance of the LV is known to worsen with age. Regular endurance exercise is commonly suggested to improve these outcomes. The current study observed greater physical activity throughout the lifespan and improved LV structure and diastolic filling characteristics in a SHAM surgery group when compared to a moderate to severe ankle sprain group (ATFL/CFL). Additionally, the ATFL/CFL group exhibited significantly reduced activity levels throughout the lifespan with similar LV posterior wall thickness-to-radius ratio and diastolic filling characteristics to a control group without access to running wheels (SHAMSED). Physical activity throughout the first 40 % of the lifespan and to the end of the lifespan was significantly correlated with LV passive filling velocity and E/A ratio at both age points. These findings suggest physical activity has a significant influence on LV structure and passive filling velocity starting near the end of the first quarter to half of the lifespan and continuing to the end of life, with the greatest impact being observed with higher intensity aerobic activity early in life and both higher intensity and longer duration activity resulting in more positive LV h/r ratios and filling characteristics at the end of life. Lastly, moderate to severe ankle sprains appear to negatively impact regular physical activity resulting in markedly reduced LV filling characteristics with aging similar to sedentary mice.

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Compliance with ethical standards

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