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Original Article

Selectively breeding for high voluntary physical activity in female mice does not bestow inherent characteristics that resemble eccentric remodeling of the heart, but the mini-muscle phenotype does

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ABSTRACT

Physical activity engagement results in a variety of positive health outcomes, including a reduction in cardiovascular disease risk partially due to eccentric remodeling of the heart. The purpose of this investigation was to determine if four replicate lines of High Runner mice that have been selectively bred for voluntary exercise on wheels have a cardiac phenotype that resembles the outcome of eccentric remodeling. Adult females (average age 55 days) from the 4 High Runner and 4 non-selected control lines were anaesthetized via vaporized isoflurane, then echocardiographic images were collected and analyzed for structural and functional differences. High Runner mice in general had lower ejection fractions compared to control mice lines (2-tailed $p = 0.023\,6$) and tended to have thicker walls of the anterior portion of the left ventricle ($p = 0.065$). However, a subset of the High Runner individuals, termed mini-muscle mice, had greater ejection fraction ($p = 0.000\,6$), fractional shortening percentage ($p < 0.000\,1$), and ventricular mass at dissection ($p < 0.002\,7$ with body mass as a covariate) compared to non-mini muscle mice. Mice from replicate lines bred for high voluntary exercise did not all have inherent positive cardiac functional or structural characteristics, although a genetically unique subset of mini-muscle individuals did have greater functional cardiac characteristics, which in conjunction with their previously described peripheral aerobic enhancements (e.g., increased capillarity) would partially account for their increased $\dot{V}O_{2max}$.

Introduction

Routinely engaging in moderate to vigorous physical activity is associated with reduced risk for non-communicable diseases,[1–3] including the incidence of cardiovascular disease, hypertension, type II diabetes, and some types of cancer.[1,4] In humans, the regulation of physical activity is under complex control by both environmental (access to sidewalks, availability of public transportation, perceived safety of the environment, and high socioeconomic status)[5] and biological factors (ability of skeletal muscle to perform contractions, ability of the cardiovascular system to delivery oxygen to muscle, and neurobiological factors related to motivation, reward, and fatigue).[4,6]

Given that genetic and genomic variants must underlie variation in some of the biological factors that regulate physical activity, the Garland Laboratory has used selective breeding for voluntary exercise to develop a mouse model for the control of physical activity.[7] Specifically, four replicate lines of High Runner (HR) mice have been bred for voluntary wheel-running behavior as young adults, while four non-selected control (C) lines are bred without regard to wheel running.[8] Since reaching selection limits around generations 17–27 (depending on replicate line and sex), mice from the HR lines run ~2.5-3-fold more revolutions/day.[9] Even without access to exercise wheels (i.e., in the absence of exercise training), the HR lines have a variety of co-segregating traits that are generally viewed as being associated with positive health outcomes and longevity, including greater activity when housed without wheels,[10] reduced body fat,[11,12] higher endurance on a motorized treadmill,[13] and higher maximum aerobic capacity [i.e., maximal oxygen consumption, ($\dot{V}O_{2max}$)],[14–17] as compared with the control lines.

One remarkable discovery in the HR mouse selection experiment is a muscle-mass polymorphism, in which some individuals have triceps surae and whole hindlimb muscles ~50% as heavy as in normal

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List of abbreviations:

C	Control mouse
HR	High Runner mouse
$\dot{V}O_{2max}$	Maximal Oxygen Consumption
C-to-T	Cytosine to Thymine
Hsd	**ICR** Harlon-Sprague-Dawley
N	Sample size
CO_2	Carbon Dioxide
°C	Degrees Celsius
M-mode	Motion Mode
B-mode	Brightness Mode
REML	Restricted Maximum Likelihood
EKG	Electrocardiogram
DF	Degrees of Freedom
LSM	Least Squares Mean
SE	Standard Error
g	Grams
mg	Milligrams
mL	Milliliters
μL	Microliters
mm	Millimeters

individuals.[18] This "mini-muscle" phenotype is caused by a single C-to-T base pair change (*Myh4*^{Minimsc}) between exon 11 and 12 of the *Myh4* skeletal muscle gene[19] that behaves as a Mendelian recessive (i.e., heterozygotes have the normal phenotype). The reduced muscle mass is attributable to a strong reduction, and sometimes absence, of the fast glycolytic (type 2B) fiber type in locomotor muscles that normally contain this fiber type.[20–22] Mini-muscle mice have a variety of correlated phenotypes (i.e., pleiotropic effects of the mini-muscle allele), including increased capillarity in medial gastrocnemius, increased mass-specific citrate synthase and myoglobin concentration in gastrocnemius, larger soleus muscles, larger hearts and other internal organs, a longer QRS complex in electrocardiograms, and a higher $\dot{V}O_{2max}$ even than other HR, non-mini mice.[15,16,23–27] However, it is unknown if mini-muscle individuals have functional cardiovascular traits that would facilitate their high voluntary running speeds on wheels and their high $\dot{V}O_{2max}$. Along with the muscle phenotype displayed in the mini-muscle mice there could be inherent/genetic adaptations to the heart. In particular, eccentric remodeling of the heart is associated with increased left ventricle chamber size and modest increases in myocardial wall thickness, which increases cardiac health and reduces mortality from cardiovascular disease.[28]

The purpose of the present investigation was to determine if selective breeding for high physical activity also results in an altered cardiac phenotype resembling cardiac eccentric remodeling when measured by echocardiography. If mice from the HR lines, or perhaps just the subset of mini-muscle HR mice, have inherent cardiac differences (i.e., in the absence of chronic exercise) that resemble eccentric remodeling, then further investigations could reveal potential genetically based cardioprotective mechanisms. Cardiac differences observed between mini-muscle and non-mini muscle mice would, specifically, suggest peripheral adaptations may assist with cardiac remodeling to facilitate greater oxygen delivery. However, if such phenotypes do not exist in untrained HR or mini-muscle mice, then the importance of regular engagement in physical activity to reduce the risk of cardiovascular disease would be reinforced. All mice included in this study were female, which we hypothesized would highlight inherent cardiac differences due to higher levels of wheel running in female mice.[18]

Methods

Ethical approval

All animals were housed in a room temperature controlled at ~22 °C under a 12-h dark/light cycle. Food and water were provided *ad libitum* throughout the experimental procedures, and all procedures were approved by the University of California, Riverside Institutional Animal Care and Use Committee.

Selection experiment

The ongoing selection experiment was initiated in 1993 with 224 outbred Hsd:ICR (Harlan-Sprague-Dawley) mice. Mice were bred randomly for two generations and then separated into eight lines, with four to be bred for high activity (HR, n = 31) and four non-selected control (C, n = 32) lines.[8] Briefly, at ~6–8 weeks of age, mice are housed individually in cages with attached wheels for 6 days. In the HR lines, breeders are chosen based on revolutions run on days 5 and 6; in the C lines, breeders are chosen without regard to running (for further details, see[8,9]).

Originally present at low frequency in the base population (~7%), one of the C lines and two of the HR lines had individuals with hindlimb muscles that were ~50% smaller than normal-muscled individuals.[18,24] This "mini-muscle" phenotype is no longer present in the C line, is fixed in HR line 3, and is polymorphic in HR line 6.[15,29,30] Population-genetic modeling indicate that the mini-muscle trait was either neutral or under negative selection in the C lines, but favored in the HR lines.[18] Of the 63 mice included in this study, all 7 in HR line 3 had the mini-muscle phenotype and 1 of the 8 mice in HR line 6 had it (n = 55 normal, n = 8 mini-muscle).

Experimental subjects and timeline

From generation 88, we sampled a total of 63 females (eight from each line, except seven from HR line 3). Each had experienced six days of wheel access as part of the routine testing protocol at an average starting age of 55 days (range = 48–65). Subsequently, each female had been a breeder, and all had given birth, with 60 of 63 successfully rearing their litter until weaning at 21 days of age. Echocardiography (see next section) was done 10–14 days after weaning (mean age = 137 days, range = 129–141), which was an average of 76 days (range = 68–84) after the end of the six-day period of wheel access. Given the amount of time that elapsed between wheel testing and echocardiography, any differences between the HR and C mice, or between mini- and normal-muscled individuals, should reflect inherent genetic differences, rather than possible effects of differential wheel running.

The following morning, mice were weighed, and body composition was measured by non-invasive quantitative magnetic resonance (EchoMRI-100; Echo Medical Systems LLC, Houston, Texas, USA), which independently calculated fat and lean mass. Mice were then euthanized via CO_2, and the heart ventricles were dissected free, blotted to remove blood, and weighed. As line HR line 6 is polymorphic for the mini-muscle phenotype (see above), we also collected and weighed the triceps surae muscle group for this line to identify mini-muscle individuals.[18]

Echocardiography

Investigators from Michigan State University traveled to the University of California, Riverside with a portable echocardiograph (Vivid IQ equipped with Hockey Stick probe and rodent analysis software, GE Healthcare, Chicago, IL, USA). As in previous studies,[31] mice were anaesthetized using 2% isoflurane and placed in a supine position on a heated handling table. Limbs were secured to the table, and the hair over the trunk area was removed with clippers followed by application of hair removal gel (Nair: Church & Dwight Co. Ewing, NJ, USA). Isoflurane concentration was then reduced to 1% and maintained throughout the rest of the measurement period. Body temperature was maintained at 37 °C via a supplemental heating lamp. Two dimensional echocardiographic images (M-mode short axis views at mid-papillary level and B-mode parasternal long axis images) were collected for measures of cardiac structure and function in both short (left ventricular volume) and long axis (end diastolic volume) when applicable. The anesthesia process took less than 10 min, including hair removal and induction. Image analysis was conducted by a single researcher blinded to the mouse treatment

Table 1
Body mass, composition, and cardiovascular echocardiography results.

Variable Name, Units	*N*	C *LSM*	C *SE*	HR *LSM*	HR *SE*	Normal *LSM*	Normal *SE*	Mini-Muscle *LSM*	Mini-Muscle *SE*	Line Type *DF*	Line Type *F*	Line Type *p*	Mini-muscle *DF*	Mini-muscle F	Mini-Muscle *p*
Body Mass at Dissection (g)	62	34.66	1.78	31.07	1.57	34.71	1.10	31.02	1.93	6	2.64	0.155 1	53	3.89	**0.053 9**
Fat Mass (g)	59	0.67	0.03	0.62	0.03	0.53	0.02	0.76	0.04	6	1.89	0.218 1	49	22.81	**< 0.000 1**
Lean Mass (g)	59	1.45	0.02	1.41	0.02	1.45	0.01	1.41	0.02	6	3.16	0.125 6	50	3.81	**0.056 4**
Ejection Fraction (%)	36	1.87	0.01	1.82	0.01	1.81	0.01	1.89	0.02	6	9.09	**0.023 6**	24	15.72	**0.000 6**
Fractional Shortening (%)	35	36.64	1.78	32.71	1.28	29.98	0.96	39.37	2.29	6	4.10	0.09	25	13.56	**< 0.000 1**
Ventricle Mass at Dissection (mg)	60	152.80	5.06	158.30	4.30	145.30	2.90	165.70	6.20	6	0.83	0.396 1	50	9.94	**0.002 7**
Left Ventricle Mass (mg)	54	127.24	15.34	114.03	12.16	108.84	8.10	132.42	19.49	6	0.54	0.488 5	44	1.25	0.269 7
End Systolic Volume (μL)	37	−1.44	0.08	−1.36	0.06	−1.32	0.04	−1.49	0.10	6	0.71	0.431 2	26	2.36	0.136 6
End Diastolic Volume (μL)	37	−0.88	0.05	−0.88	0.04	−0.87	0.03	−0.89	0.06	6	0.00	0.999 7	26	0.13	0.722 9
Left Ventricle Volume Diastolic (μL)	54	142.93	22.85	97.97	18.10	105.17	12.01	135.73	29.06	6	2.83	0.143 5	43	0.94	0.337 4
Left Ventricle Volume Systolic (μL)	53	51.78	8.52	32.88	6.78	31.79	4.48	52.87	10.91	6	3.57	0.107 9	42	3.14	0.083 5
Anterior Wall Thickness (Diastole, mm)	53	0.86	0.05	0.99	0.04	0.88	0.03	0.97	0.06	6	5.09	**0.065 0**	43	1.80	0.186 4
Anterior Wall Thickness (Systole, mm)	54	1.32	0.08	1.45	0.06	1.37	0.04	1.41	0.11	6	1.71	0.239 1	44	0.12	0.728 3
Posterior Wall Thickness (Systole, mm)	53	1.21	0.08	1.25	0.06	1.18	0.04	1.27	0.09	6	0.18	0.688 8	43	0.68	0.414
Posterior Wall Thickness (Diastole, mm)	53	0.87	0.06	0.90	0.05	0.86	0.03	0.90	0.08	6	0.14	0.716 6	43	0.30	0.586 1
Relative Wall Thickness (mm)	37	−0.36	0.04	−0.34	0.03	−0.34	0.02	−0.37	0.06	6	0.15	0.710 2	28	0.28	0.599 6
Stroke Volume (μL)	53	91.69	15.07	63.88	12.21	68.88	8.20	86.69	18.98	6	2.48	0.166 3	42	0.77	0.386 5
Cardiac Output (mL/min)	53	54.52	9.12	38.65	7.40	41.55	4.98	51.63	11.46	6	2.21	0.188 0	42	0.67	0.416 1
Heart Rate (beats/min)	61	620.01	19.92	604.36	14.88	600.04	10.05	624.32	27.53	6	0.61	0.465 7	52	0.67	0.418 2

All data presented as Least Squares Mean and Standard Errors. Bold and underline *p* values denote significance ($p < 0.05$). *N* = Sample size, *DF* = degrees of freedom, *LSM* = least squares means, *SE* = standard error, C= Control mice, HR = high runner mice, g = grams, mg = milligrams, mL = milliliters, μL = microliters, mm = millimeters.

groups using dedicated software (EchoPAC; GE Healthcare, Horten, Norway). Standard measures of left ventricular structure and function were determined from the average of three cardiac cycles through EchoPAC software calculations.[32]

Statistics

Images were evaluated for quality resulting in an *N* of 62 for M-mode images and *N* of 37 for B mode. Data were tested for normality using the Shapiro-Wilk test, and most residuals were found to be normally distributed, but the following variables were log-transformed: relative wall thickness, end systolic volume, left ventricle mass, posterior wall thickness (during diastole), left ventricle volume (during diastole), left ventricle internal diameter (during systole), left ventricle volume (during systole), ejection fraction, stroke volume, and cardiac output.

As in numerous previous studies of these lines of mice,[10,13,15,17] data were analyzed with mixed models in SAS Procedure Mixed (SAS 9.4, SAS Institute, Inc., Cary, NC), using REML estimation and Type III tests of fixed effects. Line type (HR vs. C) and mini-muscle status were the main effects, and replicate line was nested within line type as a random effect. Degrees of freedom for testing the line type effect were always 1 and 6, whereas those for testing the mini-muscle effect varied with sample size. As another way to approach the data, we also used SAS Procedure Mixed with REML estimation and Type III tests of fixed effects with *a priori* contrasts to compare three groups: mice from the non-selected Control lines (1,2,4,5), those from the High Runner lines that had the mini-muscle (3, some of line 6), and those from High Runner lines that had normal muscles (some of line 6,7,8). Unlike the primary analyses presented in the text, these analyses did not use line as nested random effect.

Body mass and/or heart rate were used as covariates. Specifically, analyses of structural variables included body mass, whereas both body mass and heart rate were included for functional variables. Ejection fraction and fractional shortening percentage used only heart rate.

In all analyses, residuals were checked for skew and dependent variables were transformed as needed to improve normality (see Table 1). Outliers were removed based on criteria established *a priori*: when standardized values exceeded approximately 3 and/or were ≥1 unit from the next value. Significance was set at $p \leq 0.05$, and trends were considered at $0.05 < p \leq 0.10$.

Results

Table 1 presents significance levels as well as Least Squares Means and Standard Errors for both line type and mini-muscle status for all traits. HR and C mice did not differ statistically for body mass (Fig. 1A), fat mass (Fig. 1B), or lean mass (Fig. 1C). When comparing the data separated into three groups [mice from the non-selected Control lines (1,2,4,5), those from the High Runner lines that had the mini-muscle (3, some of line 6), and those from High Runner lines that had normal muscles (some of line 6,7,8)] (Supplemental Table 1A), C mice had significantly greater body mass ($p < 0.000\,1$, Supplemental Fig. 1A) and lean mass ($p < 0.000\,1$, Supplemental Fig. 1C) compared to normal-muscle HR mice. No statistical differences in fat mass were observed between normal-muscle HR and C mice ($p = 0.174\,5$, Supplemental Fig. 1B).

HR mice had significantly lower ejection fractions ($p = 0.023\,6$, Fig. 2A) and tended to have a lower fractional shortening percentage ($p = 0.090\,0$, Fig. 2B) compared to the C lines. Furthermore, HR mice tended to have thicker anterior walls than C mice during diastole ($p = 0.065\,0$, Fig. 3A). When separating mice into the three groups, the ejection fraction difference ($p = 0.005\,2$, Supplemental Fig. 2A) and fractional shortening percentage trend ($p = 0.051\,5$, Supplemental Fig. 2B) remained. However, normal-muscle HR mice had significantly thicker anterior walls during diastole compared to C mice ($p = 0.028\,6$, Supplemental Fig. 3A).

As compared with normal-muscle mice, the mini-muscle phenotype was associated with reduced total body ($p = 0.053\,9$, Fig. 1A) and lean mass ($p = 0.056\,4$, Fig. 1C), but significantly higher fat mass ($p < 0.000\,1$ with lean mass as a covariate, Fig. 1B). Furthermore, mini-muscle mice had significantly greater ejection fraction ($p = 0.000\,6$, Fig. 2A) and fractional shortening percentage ($p < 0.000\,1$, Fig. 2B) compared to non-mini muscle mice.

When separating the normal-muscled and mini-muscle HR mice, the latter had significantly lower body mass ($p = 0.002$) and lean mass ($p < 0.000\,1$) compared to C mice. Mini-muscle mice also had significantly greater fat mass (with lean as a covariate) compared to both C mice ($p = 0.001\,7$) and normal-muscle HR mice ($p < 0.000\,1$, Supplemental Fig. 1B). Mini-muscle mice had greater ejection fraction ($p = 0.000\,4$) than normal-muscle HR mice, and greater fractional shortening percentage than both C ($p = 0.031\,6$) and normal-muscle HR mice ($p = 0.000\,9$). Mini-muscle mice had significantly thicker anterior walls ($p = 0.002\,6$, Supplemental Fig. 3A) compared to C mice.

Finally, mini-muscle mice had significantly greater ventricular mass weighed at dissection ($p = 0.002\,7$ with body mass as a covariate, Fig. 4A) and tended to have greater left ventricular volumes during systole as estimated by echocardiography ($p = 0.083\,5$). We observed no statistical differences in the other cardiac traits between HR and C mice or between mini- and normal-muscle mice. Differences in ventricular mass at dissection remained after separating the normal-muscle HR mice, with mini-muscle mice having greater ventricular mass than both C ($p < 0.000\,1$) and normal-muscle HR mice ($p < 0.000\,1$, Supplemental Fig. 4A) at dissection.

Discussion

Work with rodent models demonstrates that physical activity engagement is regulated by both environmental[33–36] and biological[4,7,37–44] factors, as well as epigenetic mechanisms.[45,46] Over the past 30 years, the Garland laboratory has bred mice for high physical

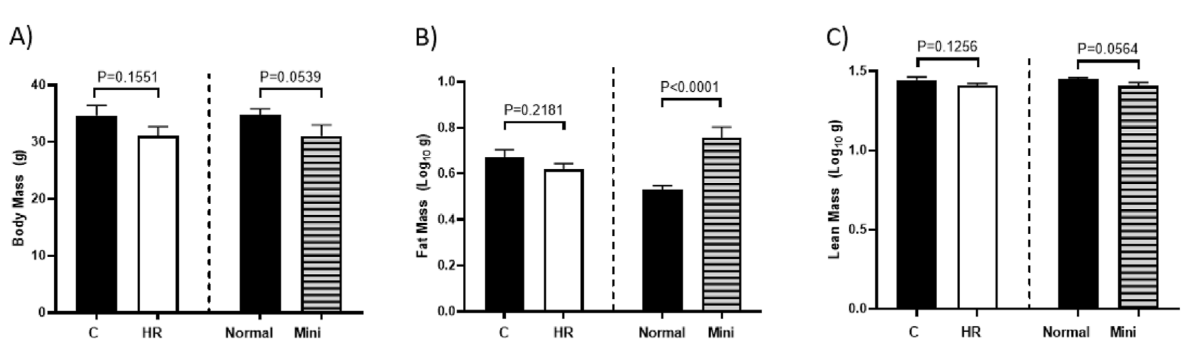

Fig. 1. 1A) Body mass, 1B) fat (lean mass as covariate), and 1C) lean mass of control (C), high runner (HR), normal, and mini muscle mice. LS Means and associated standard errors from SAS Procedure Mixed (see text). g = Grams.

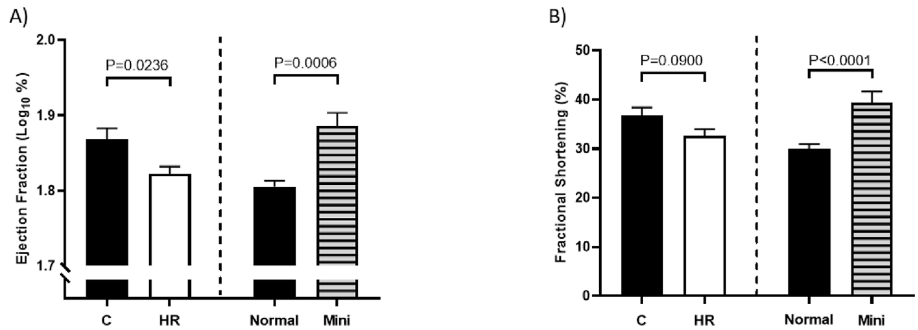

Fig. 2. 2A) Ejection fraction and 2B) fractional shortening percentage of control (C), high runner (HR), normal, and mini muscle mice (heart rate as a covariate). LS Means and associated standard errors from SAS Procedure Mixed (see text).

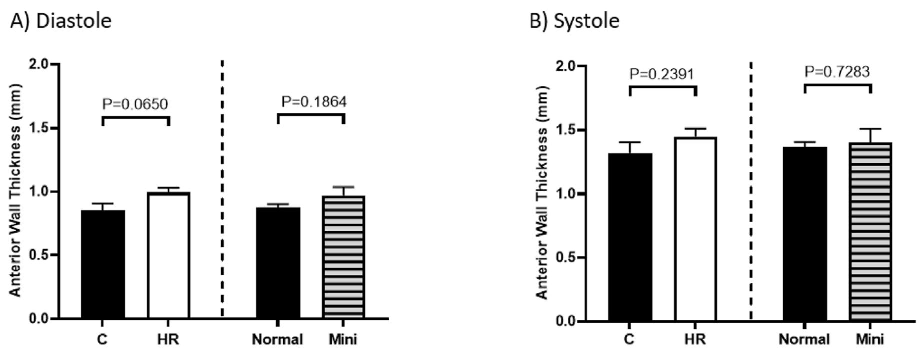

Fig. 3. Anterior wall thickness during 3A) Diastole and 3B) Systole of control (C), high runner (HR), normal, and mini muscle mice (body mass as a covariate). LS Means and associated standard errors from SAS Procedure Mixed (see text). mm = millimeters.

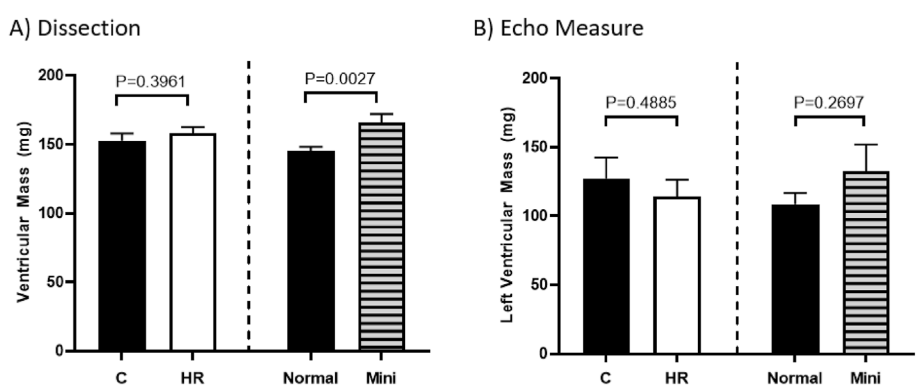

Fig. 4. Left ventricular mass measured via 4A) Dissection and 4B) Echocardiography of control (C), high runner (HR), normal, and mini muscle mice (body mass as a covariate). LS Means and associated standard errors from SAS Procedure Mixed (see text). mg = milligrams.

activity (as measured by voluntary wheel running).[7,47,48] Over the course of almost 100 generations, various neural, anatomical, and functional adaptations that promote or support high levels of endurance running have evolved in the HR lines,[12,13,17,18,20,24,25] and some of the underlying genetic and genomic changes have been identified.[49–51]

Regular engagement in physical activity is viewed as cardioprotective and is known to elicit favorable changes in cardiac structure and function, including left ventricular eccentric remodeling. Whether individuals, genetic strains or even species that regularly engage in physical activity have innate (i.e., genetically "programmed") cardiac features that might resemble those of eccentric remodeling is unknown (see also Kay et al., 2018 [29]). We tested this hypothesis by use of the unique, selectively bred High Runner (HR) lines of mice. We observed no statistically significant differences in any cardiovascular structures between HR mice and those from the non-selected control lines; rather, HR mice had reduced ejection fraction and tended to have thicker anterior walls, contrary to our initial hypotheses. These results are counterintuitive and may indicate that even though HR mice are bred for high activity during a 6-day period of wheel access, they need to engage in endurance activity for some length of time to exhibit characteristics of cardiac remodeling. Although eccentric remodeling has not previously been examined in the HR model, a previous study[25] reported that chronic wheel access (13–14 weeks) led to a greater degree of ventricular hypertrophy in HR than in C mice, which could be explained statistically by the greater amount of running by the former (i.e., "more pain, more gain"). Similarly, HR mice have enhanced trainability of cardiac function as compared with C mice over six days of wheel access, as indicated by their longer PR duration (measured via electrocardiogram) afterwards.[23] Indeed, an initial investigation into cardiac gene expression in the HR and control lines indicated that chronic exercise (20–33 months) offset many age-related gene expression changes observed in the ventricles of sedentary animals.[52]

A primary limitation to aerobic exercise capacity is an adequate cardiovascular system to provide necessary oxygen to the skeletal muscle mitochondria for sustained contraction (e.g., see[53–56]). Among various changes, consistent engagement in endurance activity elicits eccentric remodeling of the heart that primarily consists of increased ventricular volume, modest increases in wall thickness, and a reduction in relative wall thickness.[28] Previous research has observed minimal differences between the HR lines bred for high activity and the non-selected control lines, despite HR lines previously demonstrating greater $\dot{V}O_{2max}$, greater lipid utilization during exercise, increased myoglobin concentration in their ventricles, and faster speeds throughout wheel running sessions compared to non-HR mice.[25,26,57,58] Although the reduction in ejection fraction of HR mice, in the present investigation, was surprising, cardiovascular measurements in elite cyclists showed similar reductions when compared to sedentary volunteers,[59] and professional basketball players had lower ejection fraction than the general population while maintaining normal systolic function.[60] Therefore, despite the traditional association between lower ejection fraction and systolic dysfunction, we propose the HR mice rather have evolved cardiac traits (lower left ventricular ejection fraction, thicker anterior walls) similar to those observed in the "athlete's heart",[61–64] see also Kay et al., 2019.[23]

Although we did not observe many universal differences between the HR and control-line mice, the subset of HR individuals with the mini-muscle phenotype had cardiac differences (when compared to non-mini muscle mice) indicative of health-positive adaptations, including significantly greater ejection fraction, fractional shortening percentage, and left ventricular mass (the last also shown in previous studies, e.g.,[13,15,17,18,65]). The elevated ejection fraction and fractional shortening percentage may be explained by a longer duration QRS complex (measured via live EKG analysis:[23]), which is positively correlated with left ventricular size in humans[23,66] but does not predict athletic performance.[23,67,68] Previous work shows that mini-muscle mice have greater $\dot{V}O_{2max}$ (greater than other HR mice in some studies:[15,26,58]) and peripheral adaptations in greater capillary density and capillary-to-fiber ratio of their gastrocnemius.[15,27,58] Mini-muscle mice also have greatly reduced hindlimb muscle masses in conjunction with alterations in skeletal muscle enzyme concentrations and fiber types that appear beneficial for prolonged aerobic activity.[23,25,27,36] The greater capillary density, in addition to smaller skeletal muscle (and more resistance to flow through them), may require mini-muscle mice to generate a higher blood pressure to facilitate blood flow through these tissues and ensure perfusion of the working tissue, although higher blood pressures were not observed in tail-cuff blood pressure measurements.[69] The greater activity of skeletal muscle enzymes in the mini-muscle mice may also necessitate greater blood delivery, and result in further cardiac remodeling to facilitate the increased need. Therefore, in addition to the peripheral alterations previously observed, the mini-muscle mice also have altered cardiovascular characteristics to facilitate blood flow to smaller tissues that are ultimately conducive to prolonged, aerobically supported physical activity.

The inherent cardiac functional and structural differences between the mini-muscle and non-mini muscle HR mice demonstrate the idea of multiple solutions for high voluntary activity from a given starting point[57]; however, it is important to note we investigated adult mice that were not exposed to more than 6 days of running, and even that short exposure occurred an average of 76 days prior (see Methods). Therefore, although the HR mice were genetically bred to be highly active, they were not regularly participating in physical activity at the time of investigation, so the cardiac phenotype observed in the HR mice should be representative of their untrained or baseline state. Specifically, the HR mice may have a reduced ejection fraction at baseline, but an increased capacity to adapt, which may result in an improved cardiac phenotype later in life if trained. Evidence of increased plasticity to training has been demonstrated in the HR mice previously,[23,25] and thus structural changes in response to training should be investigated in future studies.

Although the present results are interesting, we note several limitations. First, data collection was conducted by investigators traveling with a portable echocardiography. The machine was not equipped with the stationary mounting system common on most mouse echocardiographs, which reduced quality of the images and consequently sample size for some measures. Second, measurements were conducted after anesthetization, so more differences could emerge during a pharmacological stress test on the mice to examine maximal cardiac function. Thirdly, this study included only females, and as such, inherent genetic differences could be present in males that are absent in females. Finally, cardiac differences might be observed at the single myocyte level, as both cardiac calcium transit kinetics and contractile functioning differences were observed between rats bred for high- and low endurance capacity during forced treadmill exercise[70] and which also differ in voluntary wheel running.[71]

Conclusions

In summary, even though mice were bred for high activity, it may be necessary for mice to engage in moderate to vigorous physical activity to observe positive eccentric remodeling of the heart. Additional studies would be needed to test this hypothesis. Although mice from the HR lines bred for high activity showed limited structural or functional differences from the non-selected control lines, the subset of HR mini-muscle mice have high ejection fraction and fractional shortening percentage than non-mini muscle mice, which could aid $\dot{V}O_{2max}$ and endurance activity engagement.[31]

Submission statement

All authors have read and agree with the contents of the manuscript. While this manuscript is being reviewed for this journal, the manuscript will not be submitted elsewhere for review and publication.

Authors' contribution

TG and DF devised the study. TG and DF led data collection, assisted by NS and EL. EL led analysis of data, assisted by AM, KD, and DF. TG ran statistical analysis with assistance from DF and NS. EL, TG, and DF drafted the manuscript. All authors read and approved the manuscript.

Ethical approval statement

All animals were housed in a room temperature controlled at 22 °C under a 12-h dark/light cycle. All food and water was provided ad libitum throughout the experimental procedures, and all experimental procedures were approved by the University of California, Riverside Institutional Animal Care and Use Committee.

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Data availability

Data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.smhs.2023.07.003.

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