Electrocardiograms of Mice Selectively Bred for High Levels of Voluntary Exercise: Effects of Short-term Exercise Training and the Mini-muscle Phenotype

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Abstract

Changes in cardiac function that occur with exercise training have been studied in detail, but those accompanying evolved increases in the duration or intensity of physical activity are poorly understood. To address this gap, we studied electrocardiograms (ECGs) of mice from an artificial selection experiment in which four replicate lines are bred for high voluntary wheel running (HR) while four non-selected lines are maintained as controls (C). ECGs were recorded using an ECGenie (Mouse Specifics, Inc.) both before and after six days of wheel access (as used in the standard protocol to select breeders). We hypothesized that HR mice would show innate differences in ECG characteristics and that the response to training would be greater in HR mice relative to C mice because the former run more. After wheel access, in statistical analyses controlling for variation in body mass, all mice had lower heart rates, and mice from HR lines had longer PR intervals than C lines. Also after wheel access, male mice had increased heart rate variability, whereas females had decreased heart rate variability. With body mass as a covariate, six days of wheel access significantly increased ventricle mass in both HR and C males. Within the HR lines, a subset of mice known as mini-muscle individuals have a 50% reduction in hindlimb muscle mass and generally larger internal organs, including the heart ventricles. As compared with normal-muscled individuals, mini-muscle individuals had a longer QRS complex, both before and after wheel access. Some studies in other species of mammals have shown correlations between athletic performance and QRS duration. Correlations between wheel running and either heart rate or QRS duration (before wheel running) among the eight individual lines of the HR selection experiment or among 17 inbred mouse strains taken from the literature were not statistically significant. However, total revolutions and average speed were negatively correlated with PR duration among lines of the HR selection experiment for males, and duration of running

was negatively correlated with PR duration among 17 inbred strains for females. We conclude that HR mice have enhanced trainability of cardiac function as compared with C mice (as indicated by their longer PR duration after wheel access), and that the mini-muscle phenotype causes cardiac changes that have been associated with increased athletic performance in previous studies of mammals.

Keywords: Artificial Selection, Cardiac Hypertrophy, Electrocardiogram, Exercise, Heart, Wheel Running

1. Introduction

The effects of endurance-exercise training (physical conditioning) on the heart are well documented in both humans and other mammals [1–3]. Effects include hypertrophy (increased size of cardiomyocytes), increased stroke volume and cardiac output, reduced resting heart rate, and changes in the durations of various aspects of the electrocardiogram (ECG), such as the PR interval and the QRS complex. In contrast, changes in cardiac function that might accompany the evolution of endurance-exercise behavior have been studied in only a few mammals [i.e. greyhounds, sled dogs, thoroughbred horses, and some rodents; ,4–6,see 7 for review]. Some of the evolutionary responses to increased endurance exercise are similar to the effects of endurance training (see below).

Many studies determine cardiac response to training by examining the hypertrophic response (usually determined by ventricular mass). Swim training, treadmill training, and voluntary wheel running are all common experimental protocols with rats and mice, and studies have shown mixed results for cardiac hypertrophic responses [8–12,see 13,and 3 for reviews]. For example, male rats swim-trained for at least 8 weeks, 5 days/week, 75 minutes/day, showed no statistical differences in heart mass (dry heart mass/body mass) between control and trained groups [14], but adult male rats swim-trained for 1 hour daily for 10 weeks showed a significant increase in ventricular mass [expressed as dry mass; ,15]. Also, increases in the duration or intensity of swim training do not necessarily increase the hypertrophic response of rodents [16,17]. In swim-trained rats and voluntary wheel-running mice, females have a significantly greater hypertrophic response than males [18,17,19,20]. These sex differences have not been reported in all rodent models. In laboratory mice, Swallow et al. [21] found that log ventricle mass (adjusted for log body mass by analysis of covariance) increased in both males and females when given

access to wheels for 8 weeks [see also 22 on females]. Heart mass has been shown to increase in mice with as little as 4 weeks of wheel access [8]. In a follow-up study, these researchers found that resistance loading of wheels reduced the magnitude of the response in heart mass, while also leading to a significant increase in the relative mass of the soleus, but not of the plantaris, gastrocnemius, or tibialis anterior muscles [9].

Horses have been bred for various types of locomotor behavior or performance. As a result of selective breeding, thoroughbred horses have numerous cardiovascular traits that increase locomotor performance, including larger hearts [and 7,based on echocardiography or gross anatomy after death; see 23,24], larger spleens [25], and increases in maximal aerobic capacity [VO_2max], stroke volume, and cardiac output [26–30]. However, some studies have not included rigorous control groups, such as untrained thoroughbred horses or trained horses that are not thoroughbreds, which makes it difficult to disentangle the effects of training from those of selective breeding.

The domestic dog is also a good model of the possible changes that occur during selective breeding for increased exercise behavior or performance [31–33]. Sled dogs and greyhounds have been bred for either endurance or speed, respectively, and this selection has resulted in a suite of changes to the cardiovascular system. For instance, greyhounds have increased heart mass to body mass ratios (greater than 1% of body mass) compared to other breeds [~0.8% of body mass; ,7,34–36]. Greyhounds also have increased red blood cell counts, higher hemoglobin levels, and increased packed cell volume as compared with mongrel dogs [37]. Other differences in greyhounds, relative to other breeds or mongrels, include increases in maximal heart rate, cardiac index, left ventricular wall and septal thickness, ventricular cavity dimensions, mean arterial pressure, and increased capillarity in the semitendinosus [38–41].

106 Several experiments have bred rodents for various aspects of locomotor behavior (see
107 Feder et al. [42] for review), but the two most widely studied are the Koch-Britton rats, bred for
108 high or low treadmill endurance capacity [43] and the Garland "High Runner" (HR) mice, bred for
109 high voluntary wheel running [44]. In the Koch-Britton rats, selective breeding has caused a suite
110 of cardiac changes, including higher stroke volume and cardiac output in high-capacity rats versus
111 low-capacity rats, differences in cardiac gene expression, differences in aortic and coronary flow,
112 altered risk of tachyarrhythmias, and altered expression of antioxidant genes involved in cardiac
113 stress signaling [5,45–47].
114 As compared with their four non-selected control (C) line counterparts, mice from the four
115 replicate High Runner lines have higher endurance capacity, higher VO_2max during forced
116 treadmill exercise [44,48–50], and in some studies, increased ventricular mass [21,50,51,22].
117 Additionally, a subset of the HR mice, known as the mini-muscle mice, have a 50% reduction in
118 hindlimb muscle mass as a result of a mutation in an intron of the myosin heavy chain IIb (*Myh4*)
119 gene [52]. The mini-muscle phenotype also has associated traits that may be adaptive for
120 endurance activity, including reduced type IIb muscle fibers, increased capillarity in the
121 gastrocnemius, higher VO_2max (especially during hypoxia), and increased ventricular mass
122 [22,50,51,53–56].
123 In addition to heart size and contractile properties, cardiac changes that occur during
124 endurance training or as a result of artificial selection include changes in the electrocardiogram
125 (ECG) of humans and dogs [57,36,58]. Changes in the ECG that occur after training include
126 bradycardia (reduced heart rate), increased PR (or PQ) duration, and increased QRS amplitude
127 and/or duration. Reduced resting heart rate in response to endurance training is common in
128 mongrel and sled dogs [59,60], as well as humans [61–63]. However, previous studies using the

129 Garland mice have not shown differences in resting heart rate (before or after 6 days of wheel
130 access) between HR and C lines [51]. Elongation of the PR interval is typically seen in resting
131 human athletes [62,64], as compared with non-athletes, and elongation of the QRS complex in
132 response to training has been documented in both sled dogs [58] and human athletes [65]. Similar
133 changes in the ECG are not necessarily seen due to selective breeding. For example, greyhounds
134 do not have lower resting heart rates than mongrels [35], and the longer QRS duration seen in
135 some thoroughbred horses is not always reflective of their higher athletic abilities [7 and
136 references therein]. Moreover, for some of these studies it is not possible to tease apart the role
137 that genetics plays. For example, studies attempting to determine whether human athletes are
138 "born or bred" with respect to athletic ability have not always included all of the necessary control
139 groups: untrained individuals chosen randomly from a comparable population (e.g., males of a
140 given age), trained individuals chosen from that same population, and athletes that have not
141 recently been in training.
142 In the present study, non-invasive, high-throughput phenotyping of electrocardiograms
143 [66,67] was applied to mice before and after 6 days of wheel access [as used routinely to pick
144 breeders in the ongoing selection experiment; ,44] to test for both genetic and environmental
145 (training) influences on cardiac properties. The use of a non-invasive technique allowed the
146 subject mice to continue on as potential or actual breeders in the selection experiment. Based on
147 the studies outlined above, and the increased ability for aerobic exercise in the HR mice, we
148 hypothesized that HR and C mice would differ in various aspects of the ECG even prior to wheel
149 access, and also that 6 days of wheel access would cause changes in the ECG. Because HR mice
150 run ~3-fold more revolutions per day than C mice, we expected that training effects might be
151 greater in the former [e.g., see 21,68,69]. In addition, because of the occasional correlation

between performance and QRS duration, we attempted to correlate aspects of the ECG (prior to wheel access) with wheel running metrics in the HR and C mice, as well as in various inbred mouse strains.

2. Materials and Methods

2.1. Animals

Male and female mice were sampled from the 68th and 74th generation of an ongoing artificial selection experiment in which mice have been selectively bred for voluntary wheel running [44,70]. The founding population was 224 outbred Hsd:ICR mice (*Mus domesticus*). Four selected High Runner (HR) lines are bred based on wheel running, while four control (C) lines are bred without regard to wheel running. No sibling mating is allowed. At ~6-8 weeks of age, mice are given access to wheels for six days, during which time they are housed individually and given *ad libitum* food and water. The cages are attached to Wahman-type activity wheels (1.12 m circumference, 10 cm wide, 35.7 cm diameter) interfaced to a computer that records the number of revolutions in one-minute intervals. Mice from selected lines are then bred based on the mean number of revolutions from days 5 plus 6. For the present study, 50 male and 50 female mice (split equally among the 8 replicate lines with an additional 4 mice from laboratory designation line 6 which is polymorphic for the mini-muscle phenotype) from generation 68 were used [these are the same mice used in 71]. Mice were weaned at 21 days of age and housed individually with food (Harlan Teklad Laboratory Rodent Diet (W)-8604, Los Angeles, CA, USA) and water provided *ad libitum* and a 12:12 photoperiod. All experimental conditions and protocols were approved by the UCR IACUC.

175 *2.2. ECG Recordings*

176 Mice from generation 68 were tested on a motorized transparent treadmill for gait
177 kinematics [71] one day before the first electrocardiogram (ECG) recordings. These mice were
178 treadmill tested again after 6 days of wheel running and then placed back in cages attached to
179 wheels overnight to ensure that no detraining occurred before the second ECG recordings were
180 made. ECG recordings were made using an ECGenie (Mouse Specifics, Framingham, MA USA)
181 before and after 6 full days of wheel access. This method takes ECG recordings passively through
182 the feet of the mouse, and hence requires them to stand with 2–3 feet touching the recording pad to
183 obtain adequate signal, and is comparable to published telemetry measurements [66,72,73]. The
184 pad is raised approximately 30.5 cm from the bench top. The recording chamber is enclosed on
185 three sides with the opening facing the experimenter. Platforms on each side allow additional
186 mice to acclimate while recording is occurring. Mice were placed on one acclimation stage and
187 then allowed to recover from handling and to acclimate to the height of the stage. To minimize
188 further handling, sliding doors on either side of the recording chamber were opened to allow
189 experimental mice to voluntarily enter the recording area, and then closed once the mouse was
190 fully inside. The electrical signals run through a bio-amplifier into an analog/digital converter and
191 are displayed in Chart 7 software (AD Instruments, Colorado Springs, CO USA). Sampling rate
192 was 2,000 samples/second with a high-pass filter set at 100 Hz and a low-pass filter set at 0.3 Hz.
193 Chart files were analyzed using eMOUSE^{TM} software (Mouse Specifics, Framingham, MA USA).
194 One of the authors (TGH) visually examined each trace for clear P, Q, R, and S morphology
195 before accepting the automatic calculations. From the raw ECG recordings (Fig. 1 left) we
196 recorded heart rate, heart rate variability (variability in R-R duration), and the durations of the PR
197 and QRS intervals (Fig. 1 right).

198

199 *2.3. Organ Masses*

200 Male mice were allowed to breed as part of the ongoing selection experiment and were
201 then euthanized, whereas female mice were allowed to wean pups and then euthanized via CO_2
202 inhalation ~21 days after weaning. Triceps surae calf muscles were dissected, blotted, and
203 weighed. Plots of muscle mass versus body mass were used to determine which individuals
204 expressed the mini-muscle phenotype [74].

205 Some of the mice used for ECGs (above) were required as breeders for the ongoing
206 artificial selection experiment, and therefore ventricle mass was not recorded for any individual
207 from generation 68. To determine changes in ventricle mass that occurred after 6 days of wheel
208 access, male mice from generation 74 were housed individually and half were allowed to run on
209 wheels for 6 days while the other half had no wheel access. After 6 days, mice were euthanized
210 via decapitation, their hearts were dissected, the atria removed, the ventricles were blotted to
211 remove excess blood, and then weighed.

212

213 *2.4. Statistical Analysis*

214 Following numerous previous studies of this selection experiment [e.g., 51,71,75], we used
215 the Mixed Procedure in SAS 9.1.3 (SAS Institute, Cary, NC, USA) to apply a nested analysis of
216 covariance (ANCOVA) with replicate lines nested within linetype (HR vs. Control). These
217 models were conducted as repeated-measures analyses, including values measured both before and
218 after the 6-day period of wheel access [see also 71]. In all cases, body mass was used as a
219 covariate, and presence or absence of the mini-muscle phenotype was an additional main effect.
220 Degrees of freedom for testing the effects of sex, selective breeding, and training were 1 and 6.

Main effects were considered statistically significant at $p < 0.05$; interactions were considered significant at $p < 0.10$ because the power to detect interactions is generally lower than for detecting main effects [e.g., see 76–78]. SAS Procedure Mixed was used to calculate least squares means and associated standard errors for main effects and interactions.

To determine whether HR mice had a more plastic response to training, or if their increased response was attributable to their increased wheel running, average wheel-running metrics across all 6 days were added as covariates (one at a time) to the statistical model [22]. Measurements of wheel running on the day after the second treadmill test (Day 7) were not included in the analysis because mice were removed from wheels earlier than on other days. In some cases, dependent variables were transformed to normalize the distribution of residuals from the statistical models.

For strain comparisons, we used Pearson correlations in SPSS to test for relationships between ECG characteristics and wheel-running traits [79,80]. The data for these correlations comes from the Mouse Phenome Database (The Jackson Laboratory). Specifically, the values for wheel running traits come from Lightfoot et al. [81], and the values for ECG characteristics come from Hampton (https://phenome.jax.org/projects/Hampton1).

3. Results

3.1. Wheel Running

As in numerous previous studies [e.g., see 78], HR mice ran significantly more revolutions across 6 days than their C counterparts ($p < 0.0001$), with a strong linetype*day interaction ($p < 0.0001$). Examination of Figure 2A shows that HR mice drastically increased their wheel running during the 6-day trial from ~7,000 revolutions on day 1 to ~15,000 revolutions on day 6

(+ ~114%). In contrast, C mice ran ~3,500 revolutions on day 1 and increased to ~4,500 revolutions on day 6 (+ ~29%). Figure 2A also shows that female mice ran more than males regardless of linetype ($p = 0.0094$).

Similar linetype effects (e.g. HR>C) occurred for the average speed and the maximum revolutions in any one-minute interval across the 6 days ($p = 0.0002$ and $p < 0.0001$, respectively; Fig. 2C and 2D). Mice with the mini-muscle phenotype had increased one-minute maximum revolutions compared to normal-muscle individuals ($p = 0.0207$). Linetype*day and sex*day interactions were observed for one-minute maximum revolutions ($p < 0.0001$ and $p = 0.0004$, respectively). The amount of time spent running showed a significant sex*linetype interaction ($p = 0.0328$). As shown in Figure 2B, males from C lines spent less time running than the other three groups.

3.2. Heart Rate

Heart rate was significantly lower after the 6-day period of wheel access for all groups of mice (Table 1: $p = 0.0338$; Fig. 3A and 3B). Further examination of Figure 3A and B shows that the decrease in heart rate after exercise tended to be greater in HR mice than C, although the linetype*training interaction was not statistically significant ($p = 0.1129$). The heart rate of mini-muscle mice did not significantly differ from normal-muscled mice ($p = 0.1762$; Fig. 3A and 3B). Heart rate was not significantly correlated with body mass (mean of values before and after wheel access) in the ANCOVA model ($p = 0.4833$). When body mass was removed as a covariate, all p-values were similar (results not shown). Adding the mean amount of running on days 1 through 6 as a covariate in the analysis had little effect on the p-values shown in Table 1 (results not shown), and running accounted for little of the variance in heart rate ($F = 0.18$, $p = 0.6745$). Similar results

were found when using either the average duration of running or the average speed of running on days 1 through 6 ($p = 0.8512$ and $p = 0.4746$, respectively).

3.3 Heart Rate Variability

The variability in heart rate (variability in R-R duration) tended to be higher in HR mice than in C ($p = 0.0584$; Table 1). Training differentially affected the heart rate variability in the two sexes, increasing variability in males while decreasing variability in females (sex*training $p = 0.0452$). Heart rate variability was not significantly different between mini- and normal-muscled mice ($F = 0.680$, $p = 0.4111$) and was not significantly correlated with body mass ($F = 0.010$, $p = 0.9139$). Generally, adding either the amount of wheel running or the amount of time spent running as a covariate had little effect of the p-values (results not shown). However, when average running speed was added to the model as a covariate ($p = 0.2742$, negative effect), the effect of linetype became statistically significant ($p = 0.0353$), with HR mice having higher variability than C mice.

3.4. PR Duration

Six days of wheel access differentially affected HR and C mice, tending to increase PR duration in HR mice but decrease it in C mice (linetype*training interaction $p = 0.0931$; Fig. 3C and 3D). Mini-muscle mice tended ($p = 0.0517$) to have longer PR durations than wild-type individuals (Fig. 3C and 3D; Table 1). PR duration was not significantly correlated with body mass. When body mass was removed as a covariate, all p-values were similar. Adding the amount of running as a covariate had little effect on the p-values shown in Table 1, and amount of running accounted for little of the variance in PR duration ($F = 1.07$, $p = 0.3015$). Similar effects

were seen when adding the average speed of running during days 1 through 6; however, adding the average running duration on days 1 through 6 increased the significance of the sex*linetype interaction ($F = 4.14$, $p = 0.0882$) and the mini-muscle effect ($F = 4.67$, $p = 0.0323$).

3.5. QRS Duration

Mini-muscle mice had longer QRS durations than wild-type mice, both before and after wheel access (Fig. 3E and 3F; Table 1; $p = 0.0068$). In general, male mice tended to have longer QRS durations than females (Fig. 3E and 3F; $p = 0.0975$). Body mass was not a predictor of QRS duration ($p = 0.7394$). When body mass was removed as a covariate, the effect of sex became significant ($p = 0.0306$), with males (LSMean ± SE = 10.9095 ± 0.1325ms) having longer values than females (10.4601 ± 0.1485ms). Adding the amount of running as a covariate had little effect on p-values (results not shown), and running did not account for an appreciable amount of the variance in QRS duration ($F = 2.25$, $p = 0.1361$). However, the average duration of wheel running across all 6 days of the trial tended to be a positive predictor of QRS duration ($F = 3.08$, $p = 0.0813$).

3.6. Ventricle Mass

With body mass as a covariate, six days of wheel access significantly increased ventricle mass in both HR and C males (Fig. 4, $p = 0.0060$; see Table 3). Additionally, Fig. 4 shows that mice from HR lines tended to have larger ventricles than those from C lines ($p = 0.0842$) and mini-muscle mice had larger ventricles than normal-muscled mice regardless of wheel access ($p = 0.0008$). In a separate analysis, we found no significant interaction between the mini-muscle phenotype and wheel access (results not shown).

313

314 *3.7 Strain Comparisons*

315 Using data from the Mouse Phenome Database for 17 inbred strains of mice, the amount of
316 time spent running was negatively correlated with the duration of the PR interval in females ($p =$
317 0.022; Table 4). Male mice showed no significant correlations between any of the wheel running
318 metrics and ECG characteristics tested (Table 4). In the 4 lines of HR mice and the 4 lines of C
319 mice, PR duration was significantly negatively correlated with the total number of revolutions and
320 the speed of running ($p = 0.0420$ and $p = 0.0210$, respectively; Table 5). Female HR and C mice
321 showed no significant correlations (Table 5).

322

323 # *4. Discussion*

324 *4.1. Heart Rate*

325 Six days of wheel access caused lowered resting heart rates for all mice, and this effect was
326 marginally greater in the HR lines (Table 1; Figs. 3A and 3B), supporting our hypotheses that 6
327 days of wheel access would cause changes in ECG characteristics and that these changes would be
328 greater in HR mice (however, this was not true of all ECG characteristics). A slower heart rate
329 (bradycardia) is a common response to endurance training in humans [see 62,82,83], dogs [59,60],
330 and sometimes in horses [84]; however, this is not common in rodents in such a short time [i.e.
331 less than 2 weeks; ,16,85,86]. For example, when given wheel access for 2 weeks, adult female
332 C57/BL6 mice showed a decrease in heart rate when compared to sedentary controls, but did not
333 exhibit this difference after only 1 week of wheel access [86]. Also, heart rates are similar
334 between HR and C mice before training (Table 2), which is similar to results in rats that have been
335 selectively bred for high and low treadmill endurance capacity [5].

Adding the amount of wheel running to the statistical model as a covariate did not account for a significant amount of the variation between HR and C lines, suggesting that the greater reduction in heart rate for HR mice does not appear to be caused by their increased running alone. Thus, the cardiac response to training in HR mice is more plastic (or occurs more rapidly) than in C mice, which can be interpreted as an example of the evolution of increased adaptive plasticity [22,87], given that a lower resting heart rate is generally associated with better cardiovascular health [88,89]. As the training effect is caused by voluntary behavior, this can be termed self-induced adaptive plasticity [21]. In future studies, it would be of considerable interest to identify the genetic basis of this physiological plasticity in HR mice [e.g., see 90–92].

Enhanced plasticity in muscular and cardiovascular traits has been shown before in HR mice [21,22,87,93], but previous studies used longer durations of wheel access. However, Gomes et al. [69] showed that 5 days of wheel access is able to increase the concentration of glucose transporters type 4 (GLUT4) in the gastrocnemius in all mice, and the increase was greater in HR lines than C lines. The present is the first study of HR and C mice to show that the plastic response of cardiac muscle to exercise is greater in HR mice than in C mice in such a short time span [for longer time spans, see 22,94].

4.2. Heart Rate Variability

Heart rate variability tended to be higher in HR mice than in C, and training caused differential responses between the sexes (Table 1). Low heart rate variability is known to be an indicator of such pathologies as coronary heart disease [95], whereas increased heart rate variability is seen in endurance athletes, or in individuals following endurance training [96–98]. The increased variability in HR mice suggests that they have more "athletic hearts." In humans,

359 higher resting-state heart rate variability correlates with better task-switching, which relies in part
360 on the prefrontal cortex [99]. Previously, we found that in mice prevented from running after they
361 had 6 days of wheel access, c-Fos (an immediate early gene used as an indicator of neuronal
362 activity) was higher in HR than in C in the medial frontal cortex [100], a brain region involved in
363 motivation, among other functions.

364 Human females tend to have less variation in heart rate than males [101], but this does not
365 seem to be the case with the female mice used in this study. However, some studies suggest that
366 the reduction in heart rate variability seen in females may be protective against cardiac
367 arrhythmias [101,102]. The increase in heart rate variability seen in male mice after training
368 suggests that their hearts are benefiting from the training. Over-trained individuals can also have
369 decreased heart rate variability similar to sedentary individuals when compared to trained humans
370 [103], and the differences seen between male and female mice after training could be related to the
371 higher amount of wheel running done by females.

372

373 *4.3. PR Duration*

374 As with heart rate, we observed no innate (untrained) differences between HR and C lines,
375 but HR mice had an increased plastic response with regards to their PR duration. Mice from the
376 HR lines had an increased PR duration after wheel access, whereas the duration of the PR interval
377 decreased in male C mice and showed little change in female C mice (Table 1, Figs. 3C and 3D).
378 These results are similar to those for heart rate.

379 An increased resting PR duration in response to training in humans is seen more often in
380 human athletes than in the general population [see 62]. During exercise, the PR interval shortens
381 with increasing heart rate [65,104–108]. Therefore, we have no reason to believe that this effect is

382 pathological in nature, but instead probably represents the evolution of adaptive plasticity within
383 the HR lines. Interestingly, we do not see the same differences in PR interval between trained and
384 untrained individuals in general as we do for heart rate. Longer access to running wheels may be
385 necessary for C lines to show a training response in this particular ECG characteristic.
386 Alternatively, C mice may not run enough to elicit training responses [see Discussion in 94,109].
387 Additionally, mice with the mini-muscle phenotype tended to have a longer PR duration
388 than non-mini-muscle individuals, regardless of wheel access. This finding is similar to ventricle
389 mass (see section 3.6). As the PR duration reflects the time of the electrical signal to travel
390 through the AV node (from the atria into the ventricles), the increased PR duration of mini-muscle
391 mice may reflect their increased ventricular (and probably atrial) mass because the increased mass
392 could slow the signal.
393
394 *4.4. QRS Duration*
395 Neither a history of artificial selection for increased voluntary wheel running (linetype) nor
396 access to wheels for 6 days (training) had a significant effect on QRS duration. In sled dogs and
397 humans, endurance training alone (for weeks or months) causes an increase in QRS duration
398 [58,65,110–112]. The results of our study show that QRS duration is not significantly different
399 before or after 6 days of wheel running, suggesting that there is not an innate difference in the HR
400 mice. However, this time course (or exercise type) may not be sufficient to elicit a plastic
401 response to exercise in either the C or HR lines.
402 Mice with the mini-muscle phenotype had a longer QRS duration than normal-muscled
403 individuals (Figs. 3E and 3F). QRS duration generally correlates positively with left ventricular
404 size among individuals within greyhounds, sled dogs, and humans [36,58,113,114]. Given that

405 QRS duration is, in part, dependent on the Purkinje system, the longer QRS duration seen in mini-
406 muscle mice may be caused by longer depolarization times related to the increased ventricular size
407 (see Section 3.5) [21,48,74,115,50,22]. However, it is possible that elongation of the QRS interval
408 is attributable to slower conduction in the Purkinje fibers in general.
409
410 *4.5. Ventricle Mass*
411 In a separate sample of male mice (Table 3), we found that wheel access for 6 days
412 increased the mass of the ventricles and that mini-muscle individuals had larger ventricles than
413 wild-type mice, regardless of wheel access. Also, male HR mice tended to have larger ventricles
414 than male C mice ($p = 0.0842$). In a previous study with no wheel access versus 8 weeks of wheel
415 access beginning at weaning, the training effect on ventricle mass was greater in HR mice,
416 indicating increased plasticity [see Fig. 4.2 in 94]. In the present study, increased plasticity of
417 ventricle mass in HR (i.e., a wheel access*linetype interaction) was not found (Table 3, $p =$
418 0.5427), perhaps due to the much shorter duration of wheel access. In the present study, increased
419 ventricle mass is likely one cause of the lower heart rates seen after 6 days of wheel access in both
420 HR and C lines (Table 1, training $p = 0.0338$), as larger ventricles lead to stronger contractions
421 and increased stroke volume. Normal responses to increased stroke volume include lowering of
422 heart rate to maintain normal cardiac output while at rest.
423 Mini-muscle mice, which are found in two of the four replicate HR lines, also had larger
424 relative ventricular mass, regardless of training, which corroborates several other studies [e.g.,
425 22,51,53]. Increased ventricular mass related to selective breeding is commonly seen in dogs and
426 horses [see 7]. However, these evolutionary changes in ventricular mass are coupled with other
427 modifications to the cardiovascular system that are not seen in mini-muscle mice. For example,

greyhounds and deerhounds have higher blood pressure and mean arterial pressure (MAP) at rest than other dog breeds [38,116]. In contrast, mini-muscle mice do not have some of the other phenotypic differences that are typically associated with selective breeding for aerobic exercise capacity, such as lower resting heart rates than normal-muscled individuals [Table 2; ,51], nor do they have increased endurance capacity when treadmill tested [49]. Also, the larger ventricular mass of mini-muscle individuals is not associated with increased resting blood pressure measured by tail cuff [51].

4.6. Strain Comparisons

Some previous studies have correlated a longer QRS duration with increased athletic performance in human athletes and for selectively bred animals [57,117,118,36]. However, the correlation between QRS duration and athletic performance is not always present [119–122]. Using the Mouse Phenome Database (The Jackson Laboratory), we correlated wheel-running traits with ECG characteristics across 17 strains of inbred mice, and also calculated correlations using mean values for our 8 lines of mice. Among the 17 inbred strains, only one correlation was found between any of the available metrics of wheel running (distance, duration, average speed: see Table 4). Across the four HR and four C lines, males showed negative correlations between running speed and PR interval before training and between total revolutions and PR interval before training (Table 5). No significant correlations were found between the duration of the QRS complex before training and any of the wheel traits considered. This finding indicates that, at least among the inbred strains tested and the HR and C lines, QRS duration does not predict athletic performance as has been found occasionally in horses, dogs, and humans.

451 *4.7. Conclusions and Future Studies*

452 Here we have demonstrated that cardiac responses to short-term endurance training (6 days
453 of voluntary exercise) occur in both selectively bred HR lines of mice and their non-selected
454 control lines, for both heart rate and ventricle mass. However, the response in heart rate and PR
455 interval by the HR mice is more plastic than in C mice, indicating the evolution of increased
456 adaptive phenotypic plasticity in HR mice [see also 21,22,68,69,87]. Also, we have shown that
457 mice with the mini-muscle phenotype show innate differences in cardiac mass and QRS duration
458 compared to normal-muscled individuals. Mini-muscle mice do not necessarily run more or less
459 on wheels as compared with normal-muscled mice in the HR lines, and the lines with and without
460 the mini-muscle allele may have evolved somewhat differently in response to the same selection,
461 which is an example of multiple solutions [123]. Additionally, this study has shown that heart rate
462 variability is affected by wheel running, but that effect differs between the sexes. This may
463 indicate a pathological response in females to exercise, potential overtraining, or a mechanism to
464 reduce cardiac arrhythmias. Future studies should examine biochemical and histological markers
465 for physiological and pathological hypertrophy [see 124 for review]. Histological analyses of the
466 ventricular wall should be able to determine if the longer QRS duration seen in mini-muscle mice
467 is attributable to slower conduction time in the Purkinje fiber network or if the longer duration is
468 pathological in nature. Furthermore, histological analysis could be used to determine whether the
469 decreased heart rate variability seen in females after exercise is associated with any pathological
470 traits. Studies of cardiac output should be performed to determine if six days of wheel access also
471 increases stroke volume.

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Table 1. Significance levels (p-values) from repeated-measures ANCOVA analyzing ECG characteristics

		Heart Rate		Heart Rate Variability		PR		QRS	
	df	F	p	F	p	F	p	F	p
Factor									
Sex	1, 6	0.00	0.9861+	0.36	0.5710-	0.05	0.8339+	3.85	0.0975+
Linetype	1, 6	3.11	0.1284-	5.44	0.0584+	0.05	0.8305-	0.36	0.5685+
Training	1, 6	7.50	**0.0338-**	0.55	0.4864+	0.17	0.6908+	0.42	0.5423+
Mini-muscle	1, ~150	1.85	0.1762-	0.68	0.4111+	3.85	0.0517+	7.53	**0.0068+**
Sex * Linetype	1, 6	1.61	0.2513	0.02	0.9019	3.15	0.1262	0.15	0.7132
Sex * Training	1, 6	2.06	0.2014	6.36	**0.0452**	0.45	0.5261	0.27	0.6190
Linetype * Training	1, 6	3.44	0.1129	0.00	0.9771	3.98	**0.0931**	0.28	0.6160
Sex * Linetype * Training	1, 6	0.00	0.9466	2.00	0.2071	0.36	0.5693	0.36	0.5683
Mini * Training	1, ~150	0.91	0.3414	0.32	0.5710	0.12	0.7286	0.29	0.5891
Body mass	1, ~150	0.49	0.4833-	0.01	0.9139+	1.30	0.2568-	0.11	0.7394-

Bold values indicate significant differences ($p < 0.05$ or $p < 0.10$ for interactions). Positive (+) indicates direction HR > C, Male > Female, Mini > Normal, and After Training > Before Training (negative signs indicate the opposite). Training refers to 6 days of wheel access.

Table 2. Significance levels (p-values) from ANCOVA analyzing ECG characteristics before training, split by sex

		Heart Rate		Heart Rate Variability		PR		QRS	
Females	df	F	p	F	p	F	p	F	p
Factor									
Linetype	1, 5	1.83	0.2335-	7.52	**0.0407+**	0.02	0.8978-	0.52	0.5024+
Mini-muscle	1, 5	0.03	0.8717+	0.31	0.5788-	0.69	0.4126+	0.81	0.3729+
Body Mass	1, ~35	0.03	0.8605+	0.17	0.6801+	0.76	0.3899-	0.11	0.7408-

		Heart Rate		Heart Rate Variability		PR		QRS	
Males	df	F	p	F	p	F	p	F	p
Factor									
Linetype	1, 6	0.14	0.7254+	1.16	0.3237+	8.60	**0.0262-**	0.93	0.3711-
Mini-muscle	1, 6	0.97	0.3309-	0.31	0.5788+	0.64	0.4292+	5.43	**0.0251+**
Body Mass	1, ~35	1.56	0.2193+	0.02	0.9014-	9.08	**0.0046-**	1.14	0.2920-

Bold values indicate significant differences ($p < 0.05$ or $p < 0.10$ for interactions). Positive (+) indicates direction HR > C and Mini > Normal (negative signs indicate the opposite).

Table 3. Significance levels (p-values) from ANCOVA analyzing ventricle mass of male mice

All Mice
N = 105

Effect	df	F	P
Training	1, 6	17.24	**0.0060+**
Linetype	1, 6	4.27	0.0842+
Training*Linetype	1, 6	0.42	0.5427
Mini-muscle	1, 87	12.06	**0.0008+**
Body Mass	1, 87	249.13	**<0.0001+**

Bold values indicate significant differences ($p < 0.05$ or $p < 0.10$ for interactions).
Positive (+) indicates direction HR > C, Trained > Untrained, and Mini > Normal.

Table 4. Correlations between strain means for wheel-running traits and ECG characteristics in 17 inbred mouse strains, split by sex

		Females			Males			
		Dist	**Dur**	**RPM**		**Dist**	**Dur**	**RPM**
Heart Rate	Pearson Correlation	0.269	0.190	0.286	**Heart Rate** Pearson Correlation	0.099	-0.045	0.151
	Sig. (2-tailed)	0.296	0.465	0.266	Sig. (2-tailed)	0.705	0.863	0.562
PR	Pearson Correlation	-0.465	**-0.552**	-0.422	**PR** Pearson Correlation	-0.066	-0.177	0.100
	Sig. (2-tailed)	0.060	**0.022**	0.091	Sig. (2-tailed)	0.801	0.497	0.704
QRS	Pearson Correlation	-0.437	-0.349	-0.426	**QRS** Pearson Correlation	-0.176	-0.037	-0.182
	Sig. (2-tailed)	0.080	0.169	0.088	Sig. (2-tailed)	0.499	0.889	0.484

Run = daily running distance, Int = daily running duration, RPM = revolutions per minute (average running speed). Wheel running data are from Lightfoot and ECG data are from Hampton, both accessed from https://phenome.jax.org (see Methods). P-values considered statistically significant are in bold, along with their correlation.

Table 5. Correlations between LS means of wheel running traits and ECG characteristics measured before wheel access in C and HR lines, split by sex

		Females			Males			
		Dist	**Dur**	**RPM**		**Dist**	**Dur**	**RPM**
Heart Rate	Pearson Correlation	-0.350	-0.603	-0.235	**Heart Rate**	Pearson Correlation	-0.073	-0.493	0.159
	P value	0.396	0.113	0.575		P value	0.864	0.214	0.707
PR	Pearson Correlation	-0.084	-0.086	-0.010	**PR**	Pearson Correlation	**-0.724**	-0.358	**-0.785**
	P value	0.843	0.839	0.982		P value	**0.042**	0.384	**0.021**
QRS	Pearson Correlation	0.107	0.525	-0.027	**QRS**	Pearson Correlation	-0.262	0.157	-0.496
	P value	0.801	0.182	0.950		P value	0.532	0.710	0.212

P-values considered statistically significant are in bold, along with their correlation.

831 *Figure Legends*

832

833 Figure 1. From the raw ECG recordings, graphed as milliseconds versus millivolts (left: compare
834 with samples shown in Figure 1 of Chu et al. 2001 and Figure 1 of Xing et al. 2009) we recorded
835 heart rate, duration of the PR interval, and duration of the QRS complex (right).

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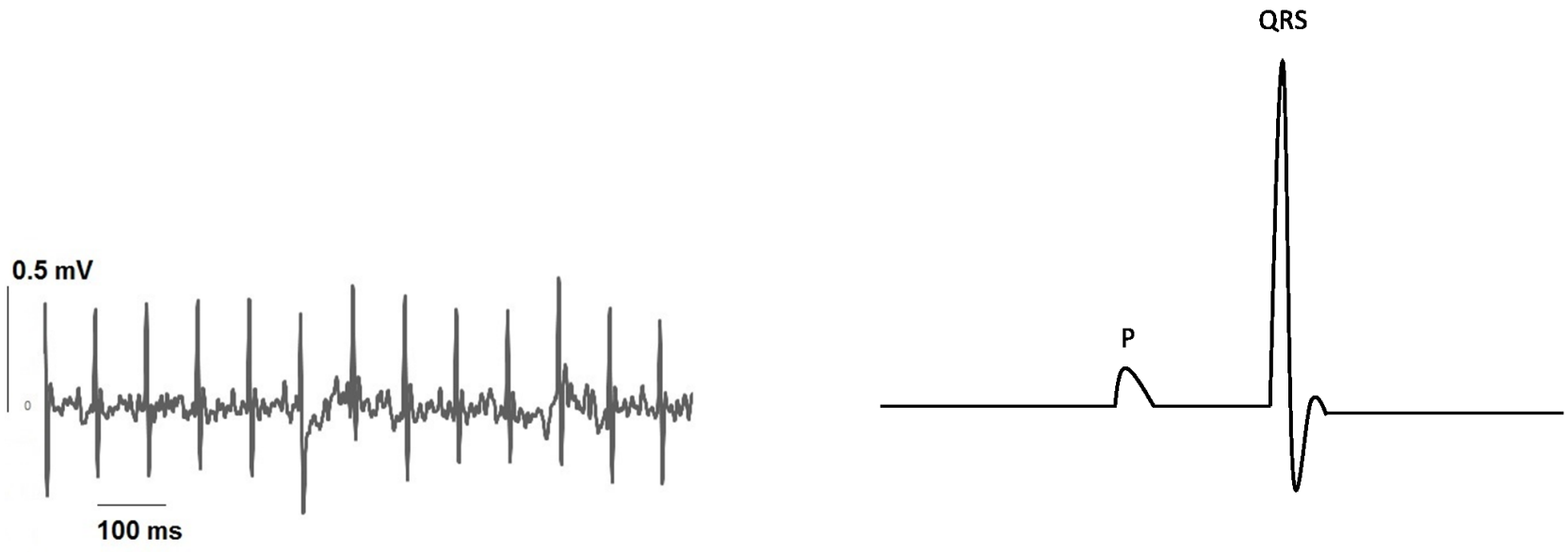
837

838 Figure 2. Average daily wheel running metrics across six days. (A) Total revolutions. (B)
839 Number of one-minute intervals with at least one revolution (running duration). (C) Revolutions
840 per minute (average speed). (D) Maximum number of revolutions in any one-minute interval.
841 Values are LS means ± standard errors from SAS Procedure Mixed repeated-measures ANCOVA.
842 N = ~95. Mice from HR lines ran more revolutions, at a faster average speed, and had higher
843 maximum speeds than C mice ($p < 0.0001$, $p = 0.0002$, and $p < 0.0001$, respectively). Female mice
844 ran more revolutions than males ($p = 0.0094$). C male mice ran for less time than other groups
845 (sex*linetype $p = 0.0328$).
846 Figure 3. Effects of selective breeding (HR vs. C lines), training (before 6 days of wheel access
847 vs. after 6 days of wheel access), and the mini-muscle phenotype on the ECG characteristics of
848 mice. (A) Heart rate in beats per minute. (B) PR duration in milliseconds. (C) QRS duration in
849 milliseconds. See Table 1 for statistical analyses. Values are LS means ± standard errors from
850 SAS Procedure Mixed ANCOVA with repeated measures, done separately for females and males.
851 N = ~93. Heart rate was significantly lower in all groups after 6 days of wheel access ($p =$
852 0.0338). Wheel access differentially affected the PR duration in C and HR mice, increasing

853 duration in HR, while decreasing it in C mice (linetype*training interaction p = 0.0931). QRS
854 duration was longer in mini-muscle mice regardless of wheel access (p = 0.0068).
855 Figure 4. Effects of selective breeding (HR vs. C lines), training (6 days of wheel access), and the
856 mini-muscle phenotype on ventricle mass, in grams, of male mice. See Table 2 for statistical
857 analyses. Values are LS means ± standard errors from SAS Procedure Mixed ANCOVA. N =
858 105. Ventricle mass increased after 6 days of wheel access in all mice (p = 0.0060). Mini-muscle
859 individuals had larger ventricles than normal-muscled mice regardless of wheel access (p =
860 0.0008).
861

862 Figure 1.



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865 Figure 2.
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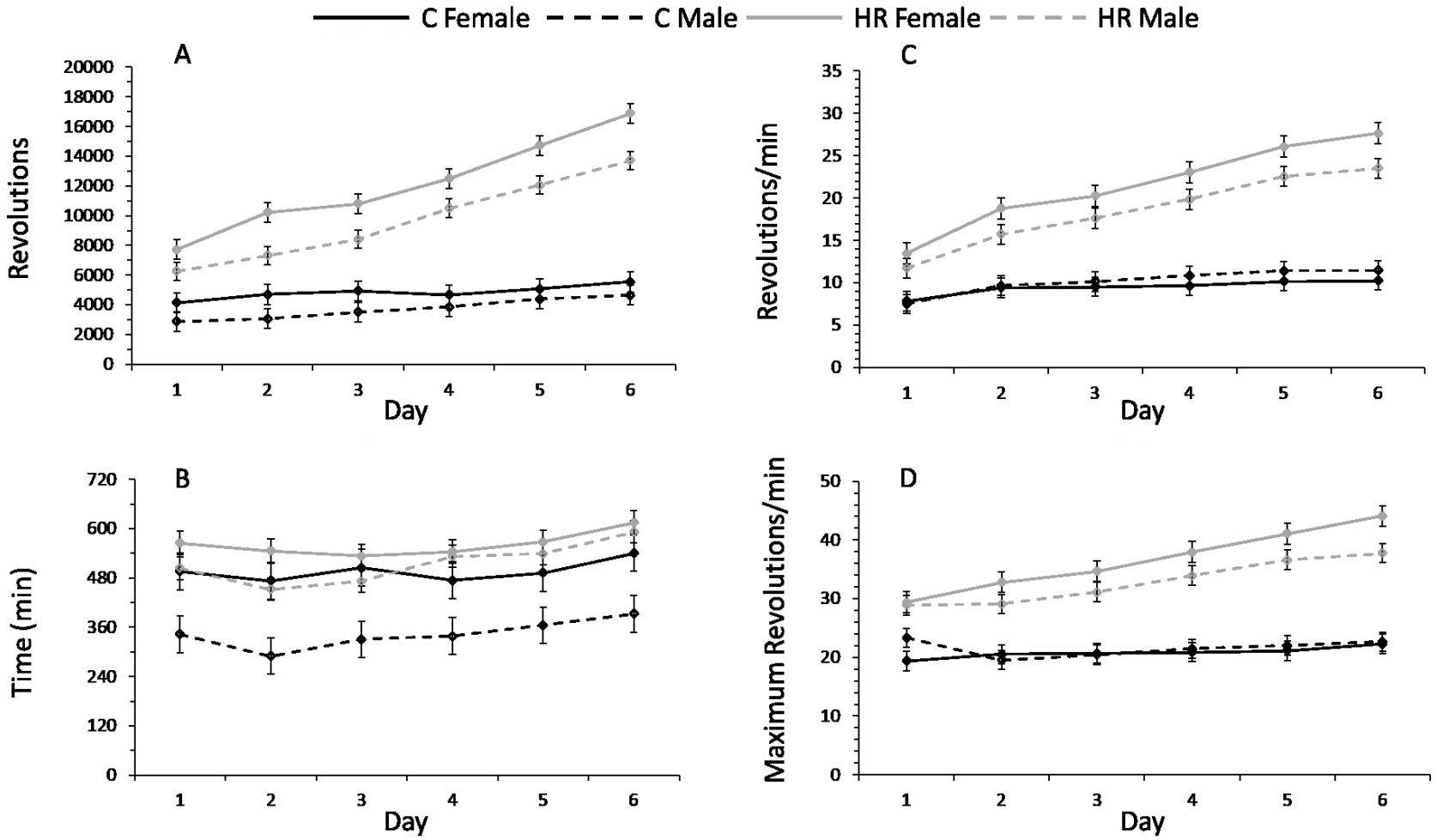
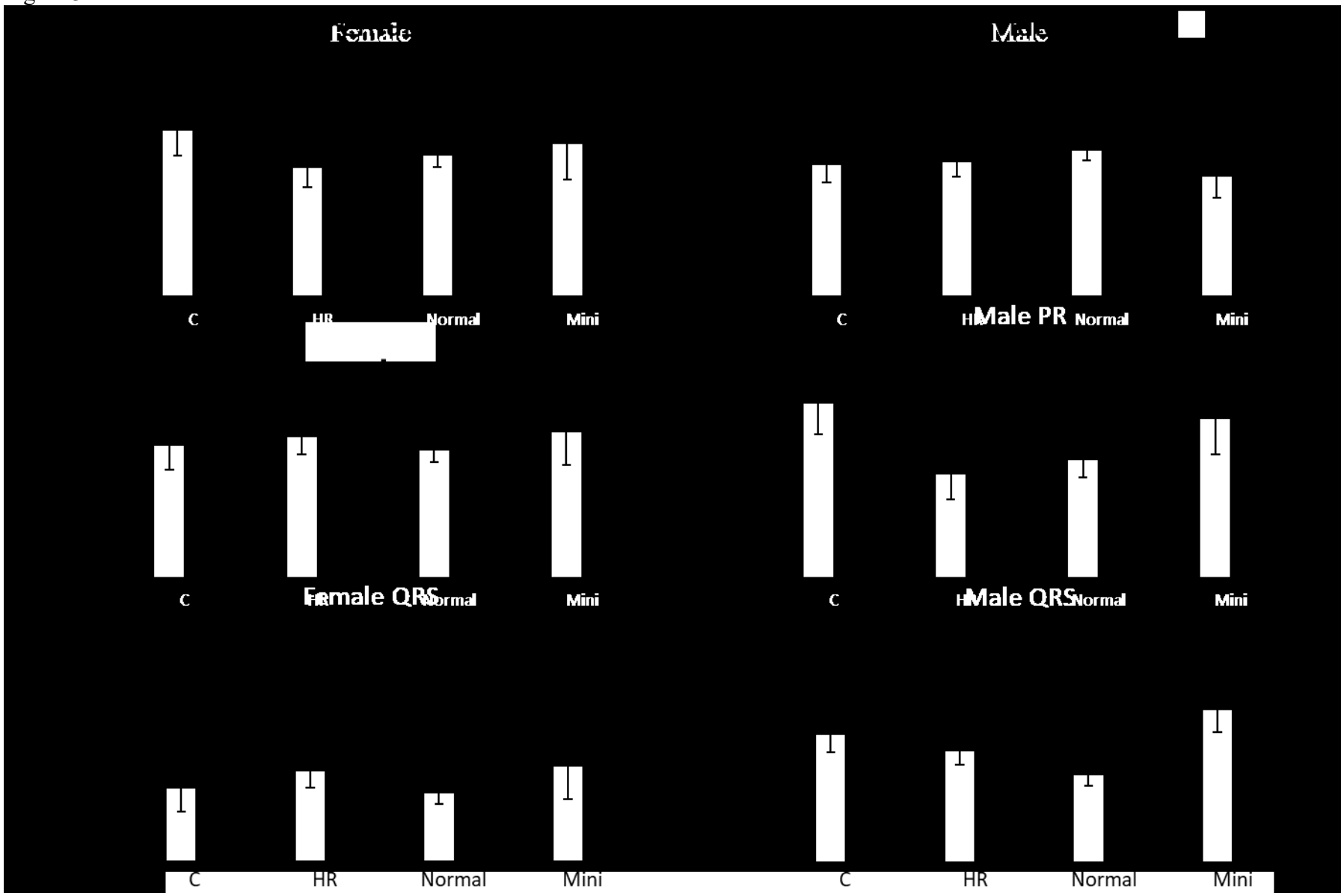


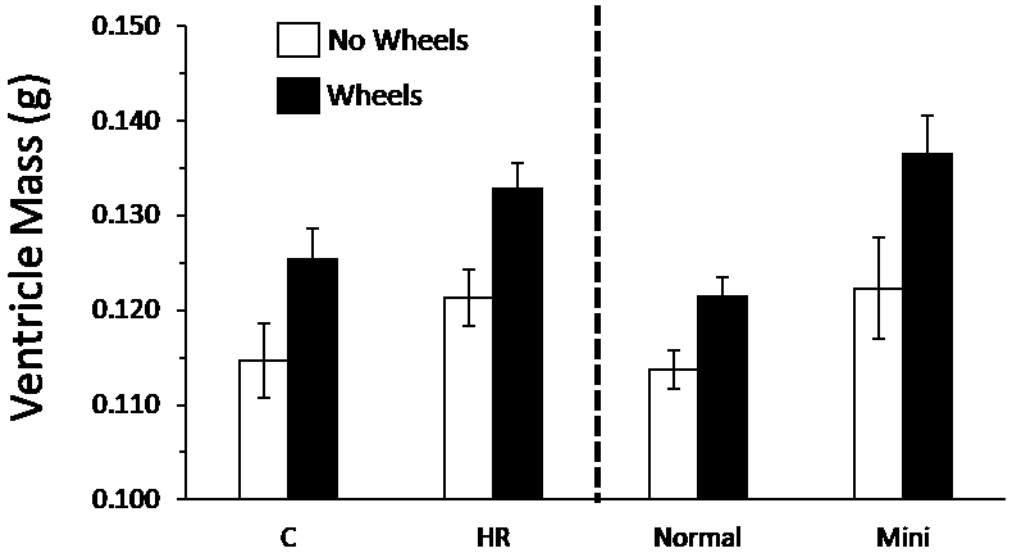
867

868 Figure 3.



869

870 Figure 4.



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