Research Paper

Exercise training effects on hypoxic and hypercapnic ventilatory responses in mice selected for increased voluntary wheel running

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New Findings

- **What is the central question of this study?**
  We used experimental evolution to determine how selective breeding for high voluntary wheel running and exercise training (7–11 weeks) affect ventilatory chemoreflexes of laboratory mice at rest.
- **What is the main finding and its importance?**
  Selective breeding, although significantly affecting some traits, did not systematically alter ventilation across gas concentrations. As with most human studies, our findings support the idea that endurance training attenuates resting ventilation. However, little evidence was found for a correlation between ventilatory chemoreflexes and the amount of individual voluntary wheel running. We conclude that exercise 'training' alters respiratory behaviours, but these changes may not be necessary to achieve high levels of wheel running.

Ventilatory control is affected by genetics, the environment and gene–environment and gene–gene interactions. Here, we used an experimental evolution approach to test whether 37 generations of selective breeding for high voluntary wheel running (genetic effects) and/or long-term (7–11 weeks) wheel access (training effects) alter acute respiratory behaviour of mice resting in normoxic, hypoxic and hypercapnic conditions. As the four replicate high-runner (HR) lines run much more than the four non-selected control (C) lines, we also examined whether the amount of exercise among individual mice was a quantitative predictor of ventilatory chemoreflexes at rest. Selective breeding and/or wheel access significantly affected several traits. In normoxia, HR mice tended to have lower mass-adjusted rates of oxygen consumption and carbon dioxide production. Chronic wheel access increased oxygen consumption and carbon dioxide production in both HR and C mice during hypercapnia. Breathing frequency and minute ventilation were significantly reduced by chronic wheel access in both HR and C mice during hypoxia. Selection history, while significantly affecting some traits, did not systematically alter ventilation across all gas concentrations. As with most human studies, our findings support the idea that endurance training (access to wheel running) attenuates resting ventilation. However, little evidence was found for a correlation at the level of the individual variation between ventilatory chemoreflexes and performance (amount of individual voluntary wheel running).

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We tentatively conclude that exercise 'training' alters respiratory behaviours, but these changes may not be necessary to achieve high levels of wheel running.

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Introduction

Complex traits, such as ventilatory behaviour, are regulated by multiple genetic and environmental influences. For example, genetic influences on ventilatory control have been reported in both humans (Hirshman *et al.* 1975; Sahn *et al.* 1977; Collins *et al.* 1978) and rodents (Strohl *et al.* 1997; Han & Strohl, 2000; Tankersley, 2001; Strohl, 2003; Yamaguchi *et al.* 2003; Tankersley & Broman, 2004; Balbir *et al.* 2007). Respiratory control is also affected by experience (i.e. phenotypic plasticity; Mitchell & Johnson, 2003; Kelly *et al.* 2012). Endurance-trained human athletes exhibit attenuated hypoxic and hypercapnic ventilatory responses at rest, demonstrating diminished chemoreflex responses (Byrne-Quinn *et al.* 1971; Mirayama *et al.* 1976; Scoggin *et al.* 1978; for review see Dempsey *et al.* 1984, 1985). However, it is unclear whether these correlations result from a causal relationship (i.e. exercise training reduces resting chemoresponsiveness) or if they are both connected to a common factor (i.e. the same genetic or experience-related factors affect both the exercise ventilatory response and resting chemoresponsiveness). A major goal of the present study was to distinguish between genetic and exercise-training (access to wheel running) effects on resting ventilatory responses to hypoxia and hypercapnia by taking advantage of a rodent model selectively bred for high levels of voluntary wheel running (Swallow *et al.* 1998*a*,*b*; Rhodes *et al.* 2005; Careau *et al.* 2013). We also investigated possible non-additive effects of selective breeding and exercise, meaning genotype-by-environment interactions.

Although exercise training is associated with alterations in ventilatory control at rest, it is unknown whether these effects enhance exercise performance [here, defined as the amount of wheel running; see Rezende *et al.* 2005, 2006*a*,*b*; Meek *et al.* 2009 for associations between amount of wheel running, maximal rates of oxygen consumption ($\dot{V}_{O_2\,max}$) and treadmill endurance]. There are conflicting reports concerning relationships between the resting acute hypoxic ventilatory response (AHVR) and $\dot{V}_{O_2\,max}$ in humans; while Byrne-Quinn *et al.* (1971) observed a positive correlation between the resting AHVR and $\dot{V}_{O_2\,max}$, Sheel *et al.* (2006) could not confirm this relationship in human subjects with variable aerobic capacities [highly trained ($\geq$60 ml O_2 kg^{-1} min^{-1}), moderately trained (50–60 ml O_2 kg^{-1} min^{-1}), and untrained ($<$50 ml O_2 kg^{-1} min^{-1})]. Thus, another goal of the present study was to test the hypothesis that diminished chemoreflex responses at rest are associated with increased exercise performance.

We used an experimental evolution approach (Garland & Rose, 2009) to determine whether ventilatory chemoreflexes at rest are affected by selective breeding for high voluntary wheel running *versus* exercise training *per se* (wheel availability). The replicated selection experiment began in 1993 from an outbred, genetically variable base population of mice (Hsd:ICR; Swallow *et al.* 1998*a*). By generation 16, high-running (HR) lines showed an increase of ~170% in total revolutions per day compared with control (C lines), with little further change in subsequent generations (Careau *et al.* 2013). Increased running was primarily due to faster, more intermittent running in HR mice, rather than a greater amount of time spent running (Girard *et al.* 2001). However, the relative importance of the two components differs somewhat between the sexes, with HR males showing more of an increase in running duration than HR females (Swallow *et al.* 1998*a*; Garland *et al.* 2011*a*). Even when denied access to running wheels, the HR lines differ from their control lines in a number of morphological, physiological and behavioural traits, including increased $\dot{V}_{O_2\,max}$ (Kolb *et al.* 2010 and references therein), increased open-field turning behaviour (Bronikowski *et al.* 2001) and home-cage activity (Malisch *et al.* 2009), and altered brain dopaminergic function (Rhodes *et al.* 2001, 2005; Rhodes & Garland, 2003).

Methods

Animals

All procedures in this study were approved by and are in accordance with guidelines set forth by the Institutional Animal Care and Use Committee at The University of California, Riverside. Mice used in this study were from generation 37 of the artificial selection experiment for high voluntary wheel-running activity (for full details, see Swallow *et al.* 1998*a*; Careau *et al.* 2013). Twelve females from each of the eight lines (four HR and four C; $n = 96$) were randomly chosen at weaning (21 days of age) and were not administered the usual 6 day wheel test. All individuals from each line came from a different family, with the exception of one selected line that was represented by only nine families. Mice were maintained in randomly assigned, same-sex groups of four in standard

cages. Water and food [Harlan Teklad Laboratory Rodent diet (W) 8604, Placentia, CA, USA] were provided *ad libitum*, photoperiod was a constant 12 h–12 h light–dark (lights on at 07.00 h), and rooms were controlled for temperature (∼22°C).

At 74–81 days of age (mean, 79 days), mice were weighed, placed into individual cages, and either granted (active) or denied (sedentary) access to a wheel (1.12 m circumference, Wahman-type; Lafayette Instruments, Lafayette, IN, USA). Thus, the following four groups of adult mice were compared: C lines housed without wheels (Sedentary C, $n = 24$); C lines housed with wheels (Active C, $n = 24$); HR lines housed without wheels (Sedentary HR, $n = 24$); and HR lines housed with wheels (Active HR, $n = 24$). The treatment was maintained for 13–14 weeks (7–11 weeks prior to ventilation assessment). Wheel running was recorded daily, in 1 min bins for ∼23 h, for the duration of the experiment using photocell counters interfaced with computers. Wheels were checked daily to remove food pellets and wood shavings and to ensure freedom of rotation.

Ventilation measurements

Seven to eleven weeks after the start of the experiment, ventilatory responses to acute hypoxia (10% O_2 and balance N_2) and hypercapnia (21% O_2, 5% CO_2 and balance N_2) were measured using a flow-through, whole-body plethysmograph (e.g. Drorbaugh & Fenn, 1955; Chappell, 1985; Szewczak & Powell, 2003). Due to logistical constraints and to minimize any time-of-day effects, mice were processed in batches ($n = 8$). Mice ($n = 12$) were randomly assigned to each batch, with the constraint that each line was represented at least once and half of the mice were assigned to the wheel access group (not all from one linetype). Thus, any effects potentially introduced by the variation in the length of wheel access (7–11 weeks) were equally distributed across all experimental groups.

When assessing ventilation in unrestrained and unanaesthetized mice, behaviour is critically important to the accuracy of the resting measurements. To minimize confounding effects of exposure to a novel environment (e.g. Hennessy, 1991), each mouse had constant access to an open metabolic chamber (500 ml) placed in its cage 1 week before trials began. This chamber remained in the cage for the duration of the experiment and was used during all ventilation trials for that animal. Each mouse was also acclimated to the chamber with normoxic conditions (flow 1 l min^{-1} ± 1%) for 1 h, 1 day prior to measurements.

On each measurement day, prior to placement in a metabolic chamber, mice were weighed, and body temperature (±0.1°C) was measured using a rectal thermocouple connected to a Baily BAT-12 thermometer (Sensortek Inc., Fort Wayne, IN, USA). Animals were then allowed to acclimate to the plethysmograph for at least 60 min (flow 1 l min^{-1} ± 1%) and to become quiescent before data collection began. A differential pressure transducer (PT-100; Sable Systems International, Inc., Las Vegas, NV, USA) was connected to the chamber, and baseline measurements of ventilation (normoxia) were performed for 6 min. Baseline measurements were repeated prior to exposure to each gas exposure, and all responses were assessed relative to corresponding baseline values. Following baseline measurements, the chamber was flushed for 3 min with hypoxic or hypercapnic gas, and ventilation was assessed for an additional 6 min. A 'washout' period of 3 min was allowed between normoxia and experimental gas concentrations. Flow rate (1 l min^{-1} ± 1%) was maintained with a Tylan mass flow controller (Mykrolis Corporation, Billerica, MA, USA), relative chamber relative humidity and temperature were constantly monitored with a dew point meter (RH-100; Sable Systems), and temperature was continuously monitored (TC-1000; Sable Systems). Gas concentrations were continuously monitored with O_2 and CO_2 analysers (S-3A from AEI Technologies, Pittsburgh, PA, USA; and CA-2A from Sable Systems, respectively) to ensure stable gas concentrations during measurements. The system was calibrated for tidal volume measurements immediately after each trial by repeatedly injecting a known volume of air (0.5 ml) into the chamber at rates approximately matching inhalation cycles. Animals were then removed from the chamber, and final body temperature was assessed.

For each mouse, exposure to the two experimental gas concentrations occurred on two consecutive days to minimize carry-over effects and, to reduce stress, the order of gas exposure was always hypoxia followed by hypercapnia. To ameliorate possible detraining effects, mice were returned to their original conditions (wheel or no wheel) following each measurement day. Given that the total blood volume for a mouse (weighing 25 g) is ∼2 ml (Wish *et al.* 1950), arterial blood gases were not assessed (see also Tankersley *et al.* 1994).

Metabolic measurements

Oxygen consumption ($\dot{V}_{O_2}$; in millilitres per minute) and carbon dioxide production ($\dot{V}_{CO_2}$; in millilitres per minute) were monitored while measuring ventilation. Excurrent air from the chamber was sampled at ∼100 ml min^{-1}, dried with magnesium perchlorate, passed through a CO_2 analyser (CA-2a; Sable Systems), scrubbed of CO_2 (soda lime), redried and, finally, passed through an O_2 analyser (S-3A; AEI Technologies, Pittsburgh, PA, USA).

Data acquisition

All data (ventilation and metabolic measurements) were collected automatically (125 Hz) using 'Labhelper' software (www.warthog.ucr.edu) and analysed with 'LabAnalyst' software (www.warthog.ucr.edu). Breathing frequency (f; in hertz), tidal volume (V_T; in millilitres) and minute ventilation ($\dot{V}_E$; in millilitres per minute) were calculated according to Malan (1973); we assumed that lung temperature was 37°C and that gas in the respiratory tract was 100% saturated with water vapour. Although data collection was continuous through the 15 min trial, data were analysed only when animals were quiescent and exhibited stable breathing patterns. Within the first 6 min (baseline conditions), a 15 s representative sample was chosen to determine f, V_T, $\dot{V}_E$, $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$. During the final 6 min of exposure to experimental gases, two 15 s periods were chosen for analysis, one during the initial 2 min and one during the final 4 min. This was done because of the known biphasic hypoxic ventilatory response in mice, which consists of an initial hyperventilatory response to acute hypoxic stimulus followed by a reduction in ventilation within 2–3 min below the peak level (roll-off) (e.g. see Hoop *et al.* 1999). For a graphical representation of the procedure described above, see Fig. 1.

Statistical analysis

Changes in ventilatory variables were assessed during gas exposures (normoxia, hypoxia and hypercapnia) and were related to selection history or activity condition by use of a repeated-measures analysis of covariance (ANCOVA) in SAS (SAS Institute, Cary, NC, USA). Here, we are addressing whether ventilatory variables within a given gas exposure are altered by effects of genetics (HR *versus* C, in the absence of wheels), the environment (wheels *versus* no wheels) and/or the amount of exposure time (sample). Using the present approach, we can also examine all potential interactions between the main grouping factors. The three main grouping factors linetype (HR *versus* C), activity (wheel access *versus* no wheel access) and sample (the phase of the exposure to test gases) were considered fixed effects. Trials consisted of three samples (normoxia, initial 2 min of gas exposure and final 4 min of gas exposure; Fig. 1), taken during two separate gas concentrations, resulting in a total of six samples. Sample-by-activity, sample-by-linetype, activity-by-linetype and sample-by-activity-by-linetype interactions were also examined. The variance in the ventilatory traits was assumed to be constant, and the multiple measurements were autocorrelated. Therefore, an autoregressive covariance structure was used, because it assumes homogeneous variances that decline exponentially with distance. Body mass ($\log_{10}$ transformed), $\dot{V}_{O_2}$, age, time and batch (never statistically significant) were included as additional covariates, where applicable. These analyses revealed significant highest-order interactions (selection-by-sample-by-activity) for some traits (e.g. breathing frequency, $P = 0.0337$); thus, for simplicity, each gas concentration trial was considered separately. Repeated-measures analyses assessed the effects of activity (wheels *versus* no wheels) and selection history (HR *versus* C) on varying ventilatory patterns within a gas trial (normoxia *versus* experimental gas). Significance was judged at the level of $P = 0.05$. In accordance with previous studies of these lines of mice (e.g. Houle-Leory *et al.* 2000), interaction terms were deemed significant at or below $P = 0.1$, because ANOVAs typically have relatively low power to detect interactions (Wahlsten, 1990, 1991).

We also used one-way and two-way analysis of variance (ANOVA) to test for effects of other factors (e.g. wheel running, body mass and body temperature) on ventilatory patterns. For mice housed with wheel access, we tested whether the individual variation in running during the final week of wheel access (performance) was associated with ventilatory patterns. To accomplish this, we used methods previously outlined by Garland & Kelly (2006; see their Table 2 and accompanying text). Briefly, we used SAS Procedure Mixed to implement a mixed-model, nested ANOVA (or ANCOVA if such covariates as age or body mass are included in the model), in which replicate line is a random effect nested within line type (HR or C). Within the active group (with wheel access), we tested whether a model that does or does not include the amount of wheel running as an additional covariate fits the data better.

Results

Wheel running

The HR lines ran more than C on the wheels (Table 1), and their increased running was primarily attributable to increased running speed rather than running time, as reported previously (e.g. Girard *et al.* 2001; Koteja & Garland 2001; Kelly *et al.* 2006; Malisch *et al.* 2009; Garland *et al.* 2011*a*). Both HR and C mice increased total revolutions run from day 1 to 20, which then declined as the study continued (Fig. 1), similar to previous studies on these lines of mice (e.g. Koteja *et al.* 1999; Dumke *et al.* 2001; Morgan *et al.* 2003).

Body temperature and mass

Body temperature (both pre- and postventilation measurements) was unaffected by any factor in this study ($P > 0.05$; results not shown). Mice from HR lines were lighter than C mice, as has been reported previously (e.g. Swallow *et al.* 1999). For body mass, two-way nested ANCOVAs indicated a significant interaction

Table 1. Analysis of wheel-running traits during first 6 days, middle 6 days and last 6 days of wheel access

Trait	*n*	High runner	Control	High runner/control	$P_{selection}$
Days 1–6					
Revolutions	47	10,805 ± 406	4081 ± 400	2.65	<0.0001
Minutes	47	515.8 ± 37.4	446.0 ± 37.1	1.16	0.2347
Mean r.p.m.	47	20.2 ± 1.0	8.8 ± 1.0	2.30	0.0002*
Maximal r.p.m.	47	33.6 ± 1.1	18.9 ± 1.1	1.77	<0.0001*
Days 36–41					
Revolutions	47	11,407 ± 565	4652 ± 574	2.45	0.0002*
Minutes	47	447.4 ± 20.0	387.1 ± 20.3	1.16	0.0791
Mean r.p.m.	47	25.3 ± 1.6	11.8 ± 1.6	2.14	0.0009*
Maximal r.p.m.	46[a]	43.3 ± 2.3	22.6 ± 2.3	1.92	0.0007*
Days 88–93					
Revolutions	44	11,533 ± 668	3684 ± 701	3.13	0.0002*
Minutes	44	407.8 ± 22.1	302.8 ± 22.8	1.35	0.0176*
Mean r.p.m.	43[a]	26.9 ± 1.1	11.7 ± 1.2	2.30	<0.0001*
Maximal r.p.m.	43[a]	46.9 ± 2.0	22.1 ± 2.0	2.12	0.0001*

Significance levels (*P* values; $^*P < 0.05$, two-tailed, unadjusted for multiple comparisons), as well as least-squares adjusted means and standard errors from nested analysis of covariance models implemented in SAS Procedure Mixed. [a]Indicates removal of one outlier based on formal statistical test (see Cook & Weisburg, 1999). Age and wheel freeness were included as covariates (results not shown)

between linetype and activity prior to hypoxia (two-tailed, $P = 0.0642$), which also approached significance prior to exposure to hypercapnia (two-tailed, $P = 0.1769$). Least-squares adjusted means and standard errors corresponding to the masses prior to the two trials indicated that wheel access reduced body mass in C lines but not in HR lines (see Fig. 2 for example). When examining only mice with wheel access, HR lines tended to be smaller than C lines, but the difference was not simply a function of greater wheel running in HR mice,

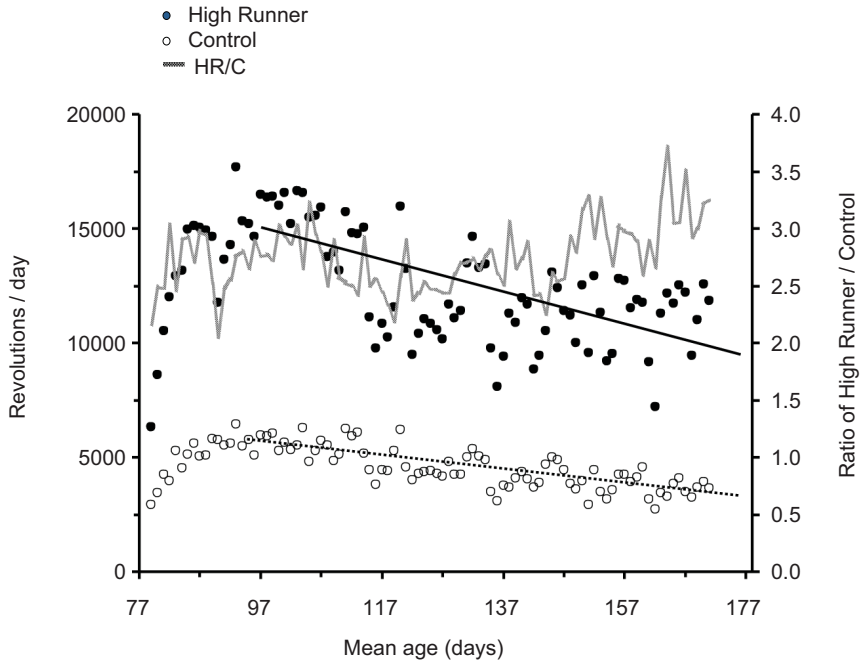

Figure 1. Simple means for wheel running in female mice from high-runner (HR; filled symbols) and unselected control lines (C; open symbols)
Following an initial ∼15 days of increasing activity, wheel running declined in both HR (continuous line) and C lines (dashed line). In C lines, the decrease was approximately linear, as indicated by the least-squares linear regression (dotted line) fitted to days 94–171. In HR lines, the decrease appeared non-linear, but a linear regression (continuous line) is shown for comparison with the C lines. The HR mice were significantly different from the C mice in the amount of running during early, middle and late stages of wheel exposure (see Table 1).

given that HR lines were significantly smaller ($P < 0.05$) for sedentary mice. Therefore, body mass was used as a covariate for all ventilation and metabolic analyses.

Hypoxic responses

Ventilatory responses. Controlling statistically for body mass and $\dot{V}_{O_2}$ (Fig. 3*A*), hypoxia significantly increased ventilation, as expected ($P \leq 0.004$; see Table S1). There were significant interactions between sample (the phase of the exposure to test gases) and activity (wheel access *versus* no wheel access) for f and $\dot{V}_E$ ($P = 0.0709$ and $P = 0.0077$, respectively). A Tukey *post hoc* test indicated that wheel access significantly reduced both f ($P = 0.0216$) and $\dot{V}_E$ ($P = 0.0023$; Fig. 3*B*) during hypoxia, but not during normoxia. Tidal volume increased significantly during hypoxia, but was not affected by selection history or wheel access (Table S1). A one-way ANCOVA examining mice with wheel access revealed that no ventilatory trait correlated significantly with individual variation in the amount of running over the final week (see Tables S2 and S3).

Metabolic rate responses. Repeated-measures analyses revealed statistically significant interactions between linetype (HR *versus* C) and sample (the phase of the exposure to test gases) for $\dot{V}_{O_2}$ ($P = 0.0014$) and $\dot{V}_{CO_2}$ ($P = 0.0046$; Fig. 3*A*; Table S1). *Post hoc* analyses (Tukey) revealed that samples measured in hypoxia were significantly different from normoxia, and this effect was dependent on linetype ($\dot{V}_{O_2}$, $P = 0.0004$; and $\dot{V}_{CO_2}$, $P = 0.0013$). A two-way ANCOVA of only normoxic values indicated that HR mice had significantly lower $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ compared with C mice ($P < 0.05$); however, during hypoxia there was no difference in $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$ between HR and C mice, because C mice exhibited lowered $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ whereas these parameters remained stable in HR mice (Fig. 3*A*).

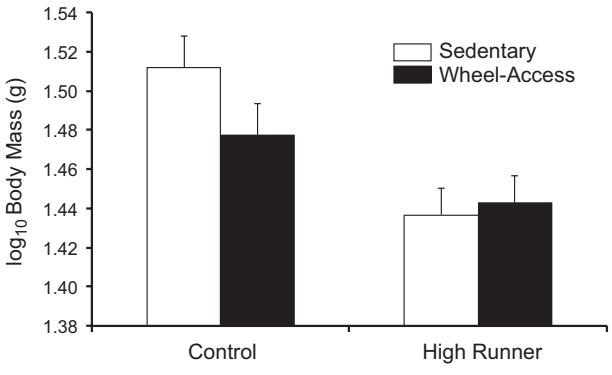

Figure 2. Log_{10} body mass, prior to hypoxia trial, for HR and C lines housed with (filled bars) and without wheel access (open bars)
Two-way nested ANCOVA revealed that HR mice were lighter than C mice ($P = 0.0214$) and that wheel access significantly reduced body mass in C lines but not in HR lines (linetype by activity interaction term, two-tailed $P = 0.0642$). Values are least-squares means ± SEM from SAS Procedure Mixed (covariates included age and time of day).

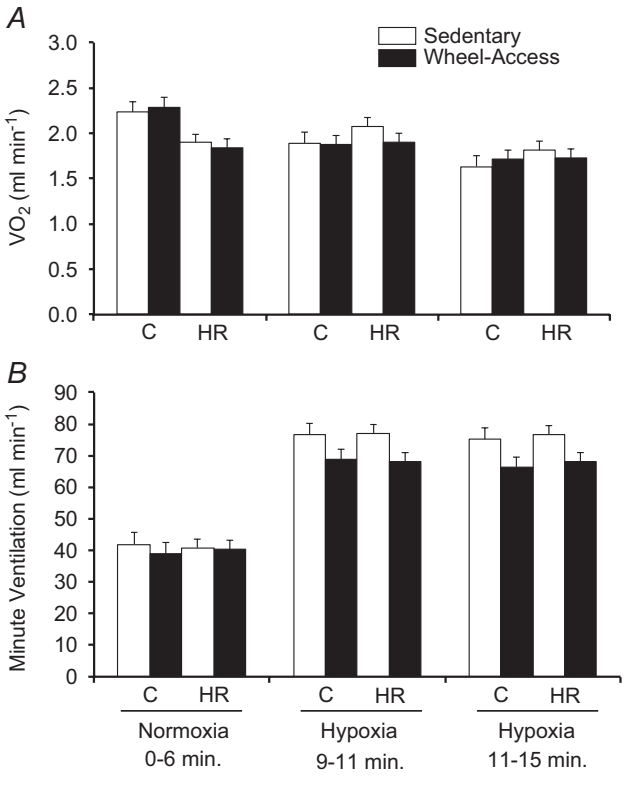

Figure 3. Oxygen consumption ($\dot{V}_{O_2}$; in millilitres per minute) and minute ventilation ($\dot{V}_E$; in millilitres per minute) in response to normoxia (21% O_2) and hypoxia (10% O_2, balance N_2), for HR and C lines housed with (filled bars) and without wheel access (open bars)
A, repeated-measures analyses revealed that $\dot{V}_{O_2}$ in hypoxia was significantly different from normoxia, and this effect was dependent on linetype (HR *versus* C; $F_{1,12} = 23.41$; $P = 0.0004$). During normoxic exposure, HR mice had significantly lower $\dot{V}_{O_2}$ compared with C mice (two-way ANCOVA, $P < 0.05$). However, in hypoxia there was no difference in $\dot{V}_{O_2}$ between HR and C mice, primarily due to C mice lowering $\dot{V}_{O_2}$ while HR values remained stable. *B*, $\dot{V}_E$ was significantly increased during exposure to hypoxia, and a repeated-measures ANCOVA revealed a statistically significant interaction with activity ($P = 0.0077$, Table S1). A Tukey *post hoc* test indicated that wheel access significantly reduced $\dot{V}_E$ during hypoxia, but not during exposure to normoxic conditions ($F_{1,201} = 9.58$; $P = 0.0023$). The *post hoc* analysis simultaneously tested for the effects of normoxia *versus* hypoxia by wheel access, not discriminating between the two time points during hypoxia. For both *A* and *B*, values are least-squares means ± SEM from SAS Procedure Mixed. Covariates included body mass, age, time of day and the squared term for *z*-transformed time of day. Similar results were observed for $\dot{V}_{CO_2}$. Results of full analyses can be found in Table S1.

Hypercapnic responses

Ventilatory responses. Exposure to hypercapnia significantly increased ventilatory traits (after accounting for body mass and $\dot{V}_{O_2}$; Fig. 4*A*) *versus* normoxic/normocapnia ($P \leq 0.008$; see Table S4). Breathing frequency was significantly altered by selection history and activity condition, but these effects were due to a significant interaction between linetype and activity ($P = 0.0588$; Fig. 4*B*). Separate two-way ANCOVAs of each sample (1, 2 and 3) revealed that wheel access significantly reduced *f* during hypercapnia for C mice only. Tidal volume and $\dot{V}_E$ increased significantly during hypercapnia, but were unaffected by selection history or wheel access (Table S4). Finally, a one-way ANCOVA examining mice with wheel access revealed that no ventilatory trait correlated significantly with individual variation in the amount of running over the final week (see Tables S2 and S3).

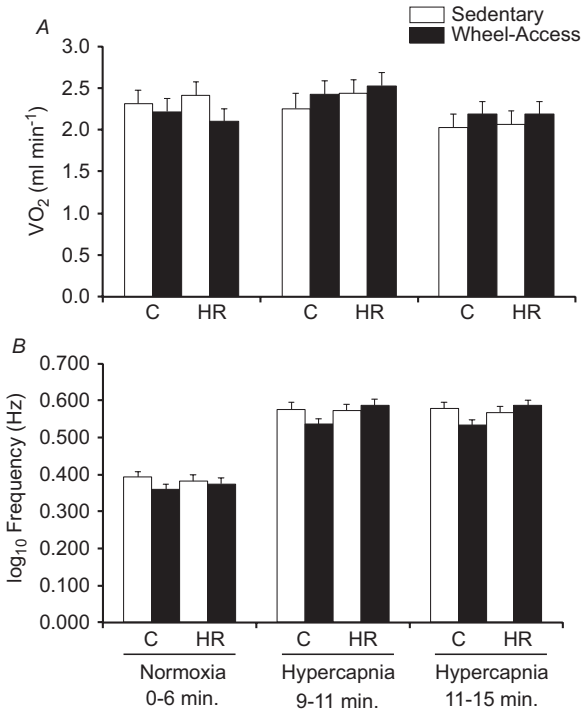

Figure 4. Oxygen consumption (in millilitres per minute) and breathing frequency (*f*; in herz) in response to normoxia (21% O_2) and hypercapnia (21% O_2, 5% CO_2 and balance N_2), for HR and C lines housed with (filled bars) and without wheel access (open bars)
A, repeated-measures analyses revealed that $\dot{V}_{O_2}$ in hypercapnia was significantly different from normoxia, and this effect was dependent on activity (wheels *versus* no wheels; $F_{1,194} = 5.30$; $P = 0.0224$). During normoxia, wheel access caused attenuation of $\dot{V}_{O_2}$, but during exposure to hypercapnia wheel access caused increases relative to sedentary mice. *B*, wheel access significantly reduced *f*, but this effect was dependent upon sample (wheel access-by-sample interaction term, $P = 0.0588$). Further inspection of the least-squares means revealed that this reduction was apparent in control lines only. For both *A* and *B*, values are least squares means ± SEM from SAS Procedure Mixed. Covariates included body mass, age, time of day and the squared term for *z*-transformed time of day. Similar results were observed for $\dot{V}_{CO_2}$. Results of full analyses can be found in Table S4.

Metabolic rate responses. The interaction between activity and sample was significant for $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ ($P < 0.07$). *Post hoc* tests (Tukey) revealed that samples obtained during hypercapnia were significantly different from baseline, and this effect was dependent on wheel access for $\dot{V}_{O_2}$ ($P = 0.0224$; Fig. 4*A*) and $\dot{V}_{CO_2}$ ($P = 0.0083$). Separate two-way ANCOVAs of individual samples indicated that wheel access tended to reduce $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ during normoxia, but increase $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ during hypercapnia.

Discussion

We examined the relationships between ventilatory chemoreflexes, artificial selection for high voluntary wheel running and long-term voluntary exercise (7–11 weeks of wheel access). We acknowledge that the use of variable types (e.g. forced treadmill exercise) and intensities of exercise training may have yielded different results. We chose the present system to reflect the conditions in which the HR mice were selectively bred (voluntary wheel running), thereby minimizing any interpretational issues associated with altering the type of exercise for training. It should be noted that we were not attempting to mirror the exercise regimen of highly trained human endurance athletes. Rather, we have attempted to examine the effects of genetics and exercise training on a relative basis, and in a way directly relevant to the present model (HR *versus* C). In addition, wheel running by rodents is viewed as a model for human voluntary exercise (Garland *et al.* 2011*b*). Our results support the idea that endurance training decreases ventilatory drive and ventilatory chemoreflexes at rest. However, we did not find a statistically significant relationship between ventilatory traits and a primary measure of exercise performance (the amount of wheel running) among individuals. Although wheel access decreased ventilatory drive and ventilatory chemoreflexes at rest, among the individuals that had wheel access the amount of running and resting ventilatory behaviour were unrelated. Evidence of a genetic association between selective breeding for high voluntary wheel running and some aspects of respiratory control were observed, and this genetic association was independent of recent wheel-running activity.

Effects of selective breeding

Inbred mouse strains vary in ventilatory responsiveness to hypoxia and hypercapnia, primarily because V_T varies

in some strains and f varies in others (Tankersley *et al.* 1994). The present study suggests that selective breeding for high voluntary exercise has altered at least some aspects of respiratory control, as indicated by linetype-by-activity and sample-by-linetype interactions for f in hypercapnic conditions (Figs 3 and 4 and Tables S1 and S4). Selection history also altered $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ during normoxia and hypoxia.

The small respiratory differences between sedentary HR and C mice observed in this study may potentially be explainable by known neurobiological differences between HR and C mice. For example, increased running by HR mice is related to altered dopaminergic function (Rhodes *et al.* 2001, 2005; Rhodes & Garland, 2003). Dopaminergic function in the peripheral chemoreceptors and central nervous system plays a key role in ventilatory control (Schlenker, 2007; Gargaglioni *et al.* 2008), particularly during hypoxia (Huey *et al.* 2003; Schlenker, 2008). However, we acknowledge that this hypothesis may be premature given the number of neuromodulators involved in ventilatory control. Additional investigations are needed to test causality between dopaminergic function and alterations in ventilatory control in HR mice. Also, as pointed out in the Introduction, even without access to running wheels, HR lines are more active than C lines in their home cages (Malisch *et al.* 2009). Thus, at this point, we are not able to rule out differences in home-cage activity in accounting for the differences between sedentary HR and C mice.

Although we have highlighted several results suggesting a genetic association between voluntary wheel running and respiratory control, it is important to note that most of the measured ventilatory traits were not statistically affected by selection history, either alone or with wheel access. Thus, we conclude that a reduction in chemoresponsiveness is not necessary for high levels of voluntary wheel running in the context of this artificial selection experiment. What limits voluntary wheel running in the HR lines is a subject of active research. For example, significantly increasing blood haemoglobin concentration and $\dot{V}_{O_2\,max}$ via administration of an erythropoietin analogue had little effect on wheel running in either HR or C females (Kolb *et al.* 2010). In contrast, administration of a 'Western' diet (high in fat and with added sucrose) had a remarkable stimulatory effect on male HR mice, with little effect on C mice (Meek *et al.* 2010).

Effects of wheel access

In humans, endurance-trained athletes have attenuated acute hypoxic and hypercapnic ventilatory responses at rest (AHVR and AHCVR, respectively; Byrne-Quinn *et al.* 1971; Mirayama *et al.* 1976; Scoggin *et al.* 1978; for review see Dempsey *et al.* 1984, 1985). A decrease in the AHVR has also been observed after endurance training in previously untrained individuals (Katayama *et al.* 1999). However, other comparisons of the AHVR and/or AHCVR between endurance athletes and control subjects found no difference in ventilatory drive (Mahler *et al.* 1982). The present study partly supports the idea that endurance training by long-term wheel running reduces at least some aspects of the hypoxic and hypercapnic ventilatory responses in mice, although these effects were largely dependent on selection history.

In hypoxia, our results agree with some human investigations (Byrne-Quinn *et al.* 1971; Mirayama *et al.* 1976; Scoggin *et al.* 1978; Adamczyk *et al.* 2006), indicating that endurance training (wheel running) reduces the AHVR (breathing frequency and minute ventilation). These effects in hypoxia are independent of selection history (i.e. wheel access reduced values in both HR and C lines), which is somewhat unexpected given known differences in the capacity for plasticity between HR and C lines in a number of physiologically relevant traits (Johnson *et al.* 2003; Rhodes *et al.* 2003; Garland & Kelly, 2006; Gomes *et al.* 2009). During hypercapnia, wheel access reduced f only in C lines. This differential effect of selection history may be associated with altered neural mechanisms or increases in home-cage activity in the absence of wheels, as discussed above. Thus, long-term wheel running and chemosensitivity in some ventilatory traits appear to be related, especially with regard to f and $\dot{V}_E$ during hypoxia. Mechanistically, the relationship between long-term voluntary wheel running and chemosensitivity may be facilitated via augmented carotid body chemoreceptor activity. In rabbits with chronic heart failure, exercise inhibited increases in afferent carotid body chemoreceptor activity (Li *et al.* 2008). Additional studies are needed in order to investigate this mechanistic hypothesis.

Our experimental design yielded a wide range of voluntary wheel-running values, but generally failed to show a relationship between ventilatory responses to hypoxia or hypercapnia and the amount of wheel running among individual mice. These findings lend support to human studies (e.g. Levine *et al.* 1992; Li *et al.* 2005; Sheel *et al.* 2006) indicating that high or low ventilatory responses to experimental gas concentrations may not have a functional relationship with exercise performance. Maximal oxygen consumption is a frequently used indicator of aerobic performance and has been used to examine the relationship between alterations in resting ventilation and exercise performance (e.g. see Sheel *et al.* 2006). We do acknowledge that when the range of $\dot{V}_{O_2\,max}$ is small, any variation in $\dot{V}_{O_2\,max}$ may be less predictive of overall aerobic exercise performance. Although we did not measure $\dot{V}_{O_2\,max}$ in the present experiment, previous studies assessing performance in HR mice demonstrate increases of as much as 33% (on a mass-specific basis)

in aerobic capacity with selective breeding for voluntary wheel running (Swallow *et al.* 1998*b*; Rezende *et al.* 2005, 2006*a*,*b*; Kolb *et al.* 2010). Additionally, Meek *et al.* (2009) reported elevated treadmill endurance in HR males (mean, 32.6 min) and females (mean, 35.0 min) *versus* C mice (males, mean, 26.3 min; and females, mean, 28.1 min). Thus, the elevated wheel running of HR mice appears to be associated with their increased $\dot{V}_{O_2\,max}$ in comparison to C mice. Therefore, the lack of any association between chemosensitivity at rest and exercise performance is similar to earlier reports in humans (e.g. Sheel *et al.* 2006).

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Additional Information

Competing interests

None declared.

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Supplemental material

The following supporting information is available in the online version of this article.

Table S1. Repeated-measures analysis of ventilatory traits comparing the effects of sample (within the hypoxic trial only: normoxia, initial 2 minutes, final 4 minutes), linetype (high runner *vs.* control), and activity (wheel access *vs.* no wheel access).

Table S2. One-way ANOVA to determine if including the amount of wheel running (full model) fits the data significantly better than a model that does not include this covariate (reduced model). The following ventilatory traits are examined within the within the hypoxic trial only.

Table S3. One-way ANOVA to determine if including the amount of wheel running (full model) fits the data significantly better than a model that does not include this covariate (reduced model). The following ventilatory traits are examined within the within the hypercapic trial only.

Table S4. Repeated-measures analysis of ventilatory traits comparing the effects of sample (within the hypercapnic trial only: normoxia, initial 2 minutes, final 4 minutes), linetype (high runner *vs.* control), and activity (wheel access *vs.* no wheel access).