Continued artificial selection for running endurance in rats is associated with improved lung function

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Kirkton SD, Howlett RA, Gonzalez NC, Giuliano PG, Britton SL, Koch LG, Wagner HE, Wagner PD. Continued artificial selection for running endurance in rats is associated with improved lung function. J Appl Physiol 106: 1810–1818, 2009. First published March 19, 2009; doi:10.1152/japplphysiol.90419.2008.—Previous studies found that selection for endurance running in untrained rats produced distinct high (HCR) and low (LCR) capacity runners. Furthermore, despite weighing 14% less, 7th generation HCR rats achieved the same absolute maximal oxygen consumption ($V_{\text{O}2_{\text{max}}}$) as LCR due to muscle adaptations that improved oxygen extraction and use. However, there were no differences in cardiopulmonary function after seven generations of selection. If selection for increased endurance capacity continued, we hypothesized that due to the serial nature of oxygen delivery enhanced cardiopulmonary function would be required. In the present study, generation 15 rats selected for high and low endurance running capacity showed differences in pulmonary function. HCR, now 25% lighter than LCR, reached a 12% higher absolute $V_{\text{O}2_{\text{max}}}$ than LCR, $P < 0.05$ (49% higher $V_{\text{O}2_{\text{max}}}$/kg). Despite the 25% difference in body size, both lung volume (at 20 cmH2O airway pressure) and exercise diffusing capacity were similar in HCR and LCR. Lung volume of LCR lay on published mammalian allometrical relationships while that of HCR lay above that line. Alveolar ventilation at $V_{\text{O}2_{\text{max}}}$ was 30% higher, $P < 0.05$ (78% higher, per kg), arterial $P_{\text{O}2}$ was 4.5 mmHg (17%) lower, $P < 0.05$, while total pulmonary vascular resistance was (insignificantly) 5% lower (30% lower, per kg) in HCR. The smaller mass of HCR animals was due mostly to a smaller body frame rather than to a lower fat mass. These findings show that by generation 15, lung size in smaller HCR rats is not reduced in concert with their smaller body size, but has remained similar to that of LCR, supporting the hypothesis that continued selection for increased endurance capacity requires relatively larger lungs, supporting greater ventilation, gas exchange, and pulmonary vascular conductance.

pulmonary O2 transport; lung diffusing capacity; lung volume; endurance capacity; genetic models

TERRESTRIAL VERTEBRATES TRANSPORT oxygen from the atmosphere to mitochondria in a series-coupled cascade via the lungs, heart, circulation, and muscles (29, 33). Overall oxygen delivery depends on the integrative matching of each step in the process; if any one part has reduced oxygen conductance, then the entire system’s oxygen delivery will be limited (30). Furthermore, not all oxygen transport components respond to increased oxygen demand equally. For example, exercise training leads to improvements in oxygen conductance due to changes in the heart, circulation, and skeletal muscles but not in the lungs (1).

Evidence from exercising humans and animals suggests that lungs may play a role in limiting maximal oxygen delivery to the muscles. In humans, ~50% of trained subjects show a decrease in arterial $P_{\text{O}2}$ ($P_{\text{O}2}$) and saturation during maximal exercise and subsequent improvements in maximal $V_{\text{O}2}$ ($V_{\text{O}2_{\text{max}}}$) while breathing gas of increased oxygen concentration (6). In thoroughbred racehorses, pulmonary limits on oxygen delivery during maximal exercise are substantial. Hypocapnia, hypoxemia, and desaturation [to 75–80% (32)] can be severe. Pulmonary artery pressures (PAP) are substantially increased, resulting in exercise-induced pulmonary hemorrhaging (35), which can be fatal.

While the training-induced responses in the cardiovascular and muscle components of the oxygen delivery pathway are well documented, the underlying genetic basis is unknown, as is the reason why the lungs fail to adapt to such stimuli. Although variation in aerobic capacity may be partly explained by genetic factors (3), separating genetic and environmental influences in oxygen transport adaptations is difficult. However, artificial selection studies using well-researched animal models that are subjected to similar environmental conditions provide one method to better understand the genetic factors responsible for aerobic capacity. For example, untrained rats selected and bred for either high or low endurance running capacity (23), show differences in mass-specific maximal $V_{\text{O}2}$ ($V_{\text{O}2_{\text{max}}}$) (20). Since these animals are subjected to the same environmental conditions, oxygen delivery adaptations seen in these selected rat lines are the result of genetic differences (4). In addition, this animal model has provided insight into the relationship between aerobic exercise capacity and both cardiovascular risks and metabolic syndrome, such as higher blood pressure (36).

We previously found that after seven generations of selection (G7), high capacity running rats (HCR) had the same $V_{\text{O}2_{\text{max}}}$ as low capacity running rats (LCR) (20), despite weighing 14% less. The higher $V_{\text{O}2_{\text{max}}}$ per kilogram in HCR was explained by peripheral improvements in the muscles of HCR rats that enhanced muscle oxygen extraction and use (21). Conversely, there were no differences in the heart, circulation, or lungs of HCR and LCR rats that would have improved central oxygen delivery in HCR animals (20).

In the present study, we examined whether continued selection through 15 generations (G15) for endurance running has lead to further divergence in $V_{\text{O}2_{\text{max}}}$ between HCR and LCR rats. If $V_{\text{O}2_{\text{max}}}$ continued to diverge with enhanced selection, we reasoned that because oxygen transport is accomplished through a serial pathway, additional physiological improvements in just one element alone—muscle oxygen extraction/use—would likely not result in increased oxygen availability to...
support additional differences in \( V'_{O2}\text{max} \). Therefore, we hypothesized that enhanced \( V'_{O2}\text{max} \) in HCR rats after 15 generations would require additional changes in the oxygen transport cascade, such as lung size or structure to enhance pulmonary function which would thereby improve systemic oxygen delivery. We recently described cardiovascular differences in these rats and showed higher heart mass and exercise cardiac output in HCR than LCR (12).

**METHODS**

**Animal model.** All procedures were carried out according to the Guide for the Care and Use of Laboratory Animals and were approved by the University of California, San Diego Institutional Animal Care and Use Committee. The founder population of 96 male and 96 female genetically heterogeneous rats (N:NIH stock) (16) were from different parentage and created a broad initial genetic variance (17). The development of the HCR and LCR lines through artificial selection for treadmill running capacity and divergent breeding has been described previously (23). Similar procedures were continued to develop G15 HCR and LCR rats used in the present study. At 11 wk of age, an endurance test was performed and the maximal distance run was over tenfold greater in HCR animals (2,349 \pm 243 m) than LCR (229 \pm 27 m; \( P < 0.001 \)) used in this study (12).

Animals were obtained from the University of Michigan, transported to the University of California, San Diego, and acclimated (but not endurance trained) for \( \approx 1 \) mo before the experiment. Animals were \( \approx 8 \) mo old at the time of the experiment. Twenty-four hours before the exercise protocol, animals were anesthetized with Nembutal (30 mg/kg ip), a polyethylene catheter (PE-50) was placed in the aortic arch via the left carotid artery, and a PE-10 catheter was advanced into the pulmonary artery via the right jugular vein with the aid of a J-shaped introducer (20). Placement of the catheters was verified by both pressure waveform and autopsies after the experiment. The heparinized catheters were tunneled subcutaneously, exteriorized at the back of the neck, cut at a length of 4 cm external to the skin, and flame sealed. The animals recovered from anesthesia and were studied on the following day. To ensure that surgery did not adversely affect the animals, we observed their spontaneous activity and behavior in their cages both before and after surgery and before the experiment began each day.

**Maximal exercise protocol.** Each animal exercised maximally twice: once in normoxia [inspired \( P_{O2} \) (\( P_{O2} \)) \( \approx 45 \) mmHg] and once in hypoxia \( (P_{O2} \approx 70 \) mmHg) with 3 h between trials. Hypoxia testing stressed the diffusion process in the lung, while at the same time minimizing the effects of ventilation/perfusion mismatching and shunting. The order of \( P_{O2} \) administered was balanced over the rats of each group. As it turned out, maximal exercise capacity during the second run was not affected by the previous run, regardless of inspired oxygen level (12).

After measurement of rectal temperature, the animals were placed on a treadmill that was enclosed in an airtight Lucite chamber adapted for open circuit measurements of \( O2 \) uptake (\( V_{O2} \)) and \( CO2 \) production (\( V'_{CO2} \)). The catheters were connected to pressure transducers (P-23-ID, Gould Instruments, Cerritos, CA) through sampling ports located on the top of the Lucite chamber. After animals rested 30 min in the Lucite chamber, arterial and mixed venous blood samples were obtained (total volume removed was 0.5 ml). The blood was replaced with an equal volume of fresh blood from donor rats. The treadmill was set at a speed of 10 m/min and inclination of 10\(^{\circ}\). After 2–3 min, the speed was increased by 5 m/min every 90–120 s, until \( V'_{O2}\text{max} \) was reached. \( V'_{O2}\text{max} \) was defined as the \( V_{O2} \) after which an increase in work rate was not associated with an additional increase (\( \pm 5\% \)) in continuously measured \( V_{O2} \).

When the \( V_{O2} \) and \( V'_{CO2} \) values were steady during the last 1–2 min of exercise, arterial and mixed venous blood samples were obtained (total volume removed was 0.5 ml). After the first run was completed, the rectal temperature was immediately measured and both fresh donor blood (1 ml) and 0.5 ml/100 g of a 0.15 mM NaHCO\(_3\) solution were administered intravenously to correct for blood loss and metabolic acidosis. After the second run, animals were humanely killed with an overdose of pentobarbital sodium, 150 mg/kg iv, and lungs, heart, and selected skeletal muscles were removed. Given the large amount of data collected in this study, the results of cardiovascular and skeletal muscle studies are reported separately to allow more detailed structural and functional analyses of each system (12, 22).

**Gas exchange and \( O2 \) transport determinations.** Inspired gas from premixed gas tanks was connected to the front of the treadmill chamber and flowed at a constant rate of 6 l/min, exiting through ports at the rear. Inflowing and outflowing \( O2 \) and \( CO2 \) concentrations were measured by mass spectrometry (Perkin Elmer MGA 1100, Pomona, CA). \( V_{O2} \) and \( V'_{CO2} \) were calculated from the inspired and expired \( O2 \) and \( CO2 \) concentrations and flow rate using standard gas exchange equations, as described previously (20).

Arterial and mixed venous blood samples were analyzed for \( P_{O2} \) and \( P_{CO2} \) on an IL 1745 electrode system at 37°C (Instrumentation Laboratories). Values were then corrected to rectal temperature using correction factors for rat blood (13). Alveolar ventilation (\( V_{A} \)) was calculated from the ratio of \( V_{O2} \) to \( P_{ACO2} \), using the alveolar ventilation equation.

Hemoglobin concentration ([Hb]) and \( O2 \) saturation (\( S_{O2} \)) were measured with an IL 482 co-oximeter (Instrumentation Laboratories). \( O2 \) contents of arterial and of mixed venous blood were calculated from measured values of [Hb], \( P_{O2} \), and \( S_{O2} \) by using an HbO\(_2\) binding factor of 1.34 ml STPD/g, as previously described (20). Maximal cardiac output (\( Q_{max} \)) was calculated as the ratio of \( V_{O2}\text{max} \) to arteriovenous \( O2 \) content difference ([a-v]\( O2 \)). Systemic oxygen delivery (\( Q_{O2} \)) was calculated from \( Q_{max} \), [Hb], arterial \( P_{O2} \), and \( S_{O2} \) (\( Q_{O2} = Q_{max} \times [Hb] \times 1.34 \times \text{arterial SO}_2 + 0.003 \times \text{arterial PO}_2 \)).

Systemic and pulmonary arterial pressures (\( P_{AP} \)) were recorded continuously, with mean pressures obtained by electronic integration. \( P_{AP}/Q_{max} \) was calculated as an estimate of pulmonary vascular resistance.

The effective lung diffusing capacity (\( D'_{LCO2} \)) was determined during maximal hypoxic exercise as the ratio of \( V_{O2}\text{max} \), to the difference between alveolar and mean pulmonary capillary \( P_{O2} \), the latter calculated by numerical analysis. We made the assumption that all of the difference between alveolar and arterial \( P_{O2} \) ([A-a]\( P_{O2} \)) in the rat is due to diffusion limitation (13). \( D'_{LCO2} \) could not be calculated in normoxia because the (A-a)\( P_{O2} \) was not different than zero in either group, indicating no diffusion limitation.

Following death, the lungs were removed and dried inflated at an airway pressure of 20 cmH\(_2\)O for 2 wk. After the trachea was clamped, lung volume was measured by water displacement.

**Statistical analysis.** The data are expressed as means \( \pm \) SE because we are interested in group mean differences, rather than individual variation among members of each group. Standard deviations can be calculated by multiplying the SE by the square root of the number of animals in each group (HCR, normoxic exercise: 10; HCR, hypoxic exercise: 10; HCR, hypoxic exercise: 7). A two-way ANOVA using post hoc t-tests with Bonferroni correction for multiple comparisons was used to evaluate the effect of exercise capacity (HCR vs. LCR) and the effect of inspired oxygen (hypoxia vs. normoxia) in G15 animals. A \( P \) value \(< 0.05 \) was considered to indicate a significant difference, unless otherwise indicated. It should also be noted that our a priori hypothesis was that HCR rats would have enhanced, and not diminished or simply different, lung size and function. Therefore, we reasoned that our data should be evaluated with a one-tailed test. That said, almost all of the outcomes remain significant had a two-tailed test been used. This can be confirmed by doubling the stated \( P \) values in each case. We made G15 comparisons with both mass-specific and mass-independent val-
uses because body mass varied significantly between HCR and LCR animals. Both sets of values are reported.

Comparisons between G7 and G15 of HCR and LCR animals were also made using a two-way ANOVA. However, for cross-generational comparisons, the P value was evaluated using a two-tailed test. All comparisons between G7 and G15 were made using mass-specific data. All statistical analyses were performed using SigmaStat (Chicago, IL).

RESULTS

In this paper we present novel findings about gas exchange, pulmonary hemodynamics, and lung volume in rats artificially selected for endurance capacity running. However, to interpret these pulmonary findings, we also include data on a few key variables already published (12). In particular, we repeat here data on body weight, V\O\textsubscript{2max} and cardiac output. Furthermore, in the DISCUSSION, we relate the findings of our previous study of G7 animals (20) to the current results at G15, also requiring the presentation of previously published data.

A complete set of blood gas data could not be obtained for all HCR and LCR animals in both normoxia and hypoxia due to technical limitations (all associated with catheter obstruction). The number of animals providing complete data within each category are HCR, normoxic exercise: 10; HCR, hypoxic exercise: 9; LCR, normoxic exercise: 10, and LCR, hypoxic exercise: 7. However, there were no noticeable affects of surgery on behavior, rectal temperature, arterial blood gases, pulmonary gas exchange, and V\O\textsubscript{2max} compared with previously published data (7, 8, 10, 11, 14, 15, 18, 19).

Body weight on the day of testing was significantly less in HCR than LCR: 194 ± 3 vs. 259 ± 9 g, respectively (P < 0.0005; n = 10 each). Thus HCR rats weighed 75% as much as LCR rats. Since it was impossible to match HCR and LCR animals for both age and body mass, we present both mass-independent and mass-specific results.

Pulmonary gas exchange values at rest. There was no significant difference in any resting pulmonary gas exchange variables between HCR and LCR rats compared within normoxia (Table 1). However, in hypoxia, the HCR rats had a 57% greater resting (A-a)PO\textsubscript{2} than the LCR (P < 0.05). All other pulmonary gas exchange variables were not significantly different at rest within hypoxia (Table 1).

Pulmonary gas exchange during maximal exercise. Except for VO\textsubscript{2}, HCR and LCR pulmonary gas exchange differences during maximal normoxic exercise were statistically significant regardless of whether the data were analyzed mass independently or mass specifically. During normoxic exercise, mass-independent V\O\textsubscript{2max} was 12% higher in HCR than in LCR (P < 0.05) but not different in hypoxia (Table 2). As reported by Gonzalez et al. (12), V\O\textsubscript{2max} normalized for body weight was 49% higher in HCR than in LCR in normoxic exercise (P < 0.0005); in hypoxia, the difference was ~30% (P < 0.0005, Table 2). When analyzed mass independently, HCR and LCR animals had similar VO\textsubscript{2} during maximal exercise in both normoxia and hypoxia (Table 2). Mass-specific V\CO\textsubscript{2} during maximal exercise was 43% higher in HCR than in LCR in normoxia (P < 0.0005) and 36% greater in hypoxia (P < 0.0005, Table 2).

Mass-independent alveolar ventilation (V\A) during maximal exercise was 30% higher in HCR than LCR in normoxia (P < 0.001), but not different in hypoxia (Table 2). Mass-specific V\A during maximal exercise was 78% higher in HCR than LCR in normoxia (P < 0.0005) and 47% greater in hypoxia (P < 0.0005, Table 2). When normalized to V\O\textsubscript{2max}, V\A in HCR was 15% higher than in LCR in normoxia (P < 0.035) and 16% greater in hypoxia (P < 0.025, Table 2).

PaO\textsubscript{2} was 4.5 mmHg lower in HCR than LCR during normoxia (P < 0.0005) and 2.4 mmHg lower in hypoxia (P < 0.04, Table 2). Alveolar PO\textsubscript{2} (PA\O\textsubscript{2}) was 2.9 mmHg higher in HCR than LCR during normoxia (P < 0.025) and 2.8 mmHg higher in hypoxia (P < 0.045, Table 2). There were no significant differences in arterial PaO\textsubscript{2} or (A-a)PO\textsubscript{2}, although arterial PO\textsubscript{2} values followed those of alveolar gas at each FiO\textsubscript{2} (Table 2).

The DLO\textsubscript{2} was not different between HCR and LCR animals (Table 2). However, when normalized for body mass, DLO\textsubscript{2} was numerically 21% higher in HCR than LCR, a difference that was on the borderline of statistical significance (P < 0.07, Table 2).

Pulmonary hemodynamics during maximal exercise. Pulmonary hemodynamic data are presented in Table 3. When analyzed independent of mass, the absolute cardiac output (Q\textsubscript{max}) was not significantly different between HCR and LCR animals during maximal exercise in either normoxia or hypoxia (Table 2).
3). However, the mass-specific $Q_{\text{max}}$ of HCR was 42% greater ($P < 0.0005$) in normoxia and 28% higher in hypoxia ($P < 0.025$) compared with LCR. Mean PAP during exercise was also not significantly different for HCR and LCR animals exercising maximally in either normoxia or hypoxia (Table 3). However, due to the higher mass-specific $Q_{\text{max}}$, the mass-specific PAP/$Q_{\text{max}}$ of HCR animals was 44% less than in LCR in normoxia ($P < 0.025$) and 33% less in hypoxia ($P < 0.025$; Table 3). The ratio of lung diffusing capacity to cardiac output ($D_{LO2}/Q_{\text{max}}$) was not significantly different between HCR and LCR animals (Table 3).

Lung volume. The lung volumes of 9 HCR and 7 LCR animals are presented in Table 4. While there were no significant differences in absolute lung volume, mass-specific lung volume was 28% greater in HCR animals ($P < 0.0005$; Table 4).

### DISCUSSION

**Summary of main findings at G15 and G7.** When evaluated after seven generations (G7) of selection for either HCR or LCR, HCR rats had a 12% greater mass-specific $V_{O2\text{max}}$. The increased $V_{O2\text{max}}$ of HCR at G7 was due entirely to greater transport and use capacity for $O_2$ in the skeletal muscle (21). In particular, there were no measurable differences between HCR and LCR in cardiopulmonary elements (20). However, in the present study of rats bred through G15, the disparity in endurance capacity was much greater. HCR mass-specific $V_{O2\text{max}}$ in G15 was 49% higher than LCR in normoxia and 30% greater than LCR in hypoxia. On the basis of the serial nature of the oxygen conductances from the mouth to the mitochondria and the associated concept of symmorphosis (33), we expected to find not only additional differences between lines in skeletal muscle structure and function between HCR and LCR at G15 but also differences in cardiopulmonary function. The disproportionate augmentation of a single element in an in-series transport system is incapable of providing substantial increases in overall system function (28). Indeed, companion studies have found increased cardiovascular function (12) and enhanced skeletal muscle structure and function (22). In addition, our study has produced multiple lines of evidence indicating differences in pulmonary function between G15 animals including alveolar ventilation, arterial $PCO_2$, alveolar $PO_2$, mass-specific $D_{LO2}$, and mass-specific PAP/$Q_{\text{max}}$.

**Body mass differences.** Although matched for age, HCR animals weighed significantly less (194 g) than LCR animals (250 g). Since some differences in pulmonary function between HCR and LCR are evident only when analyzed mass specifically (lung size, $V_{CO2\text{max}}$, $D_{LO2}$, and PAP/$Q_{\text{max}}$), it is important to understand whether the mass-specific results indicate active changes in the respiratory system or simply result from lack of changes in the lungs despite systematically smaller body size. Using mass-specific analysis is appropriate for rats, because unlike humans where there is a strong predictive relationship between human lung volume and human height, the relationship between rat lung volume and rat body length is not as strong (2). Therefore, it is more common for

### Table 2. Pulmonary gas exchange in maximal exercise

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<th>Normoxic Exercise</th>
<th>Hypoxic Exercise</th>
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<td></td>
<td>HCR $n = 10$</td>
<td>LCR $n = 10$</td>
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<td></td>
<td>HCR $n = 9$</td>
<td>LCR $n = 7$</td>
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<tr>
<td>$V_{O2\text{max}}, \text{ml STPD/min}$</td>
<td>$13.4 \pm 0.4^+$</td>
<td>$12.0 \pm 0.5^+$</td>
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<td>$12.0 \pm 0.6^+$</td>
<td>$9.7 \pm 0.4$</td>
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<tr>
<td>$V_{CO2\text{max}}, \text{ml STPD/min}$</td>
<td>$62.9 \pm 1.2^+$</td>
<td>$50.6 \pm 3.9^+$</td>
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<td>$46.3 \pm 1.1^+$</td>
<td>$38.6 \pm 2.3$</td>
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<td>$V_{DLO2\text{max}}, \text{ml STPD-min}^{-1} \cdot \text{kg}^{-1}$</td>
<td>$72.4 \pm 2.4^+$</td>
<td>$53.1 \pm 2.1^+$</td>
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<td>$50.6 \pm 1.4^+$</td>
<td>$38.9 \pm 1.5$</td>
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<tr>
<td>$V_{A}/V_{DLO2\text{max}}, \text{ml BTPS/ml STPD}$</td>
<td>$2.5 \pm 0.1^+$</td>
<td>$2.2 \pm 0.1^+$</td>
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<td></td>
<td>$1.4 \pm 0.0$</td>
<td>$1.5 \pm 0.1$</td>
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<td>$P_{AO2}, \text{mmHg}$</td>
<td>$123.1 \pm 1.0^+$</td>
<td>$120.2 \pm 0.9^+$</td>
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<td>$121.5 \pm 2.2^+$</td>
<td>$56.6 \pm 1.0^+$</td>
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<tr>
<td>$P_{AO2}, \text{mmHg}$</td>
<td>$124.0 \pm 1.1^+$</td>
<td>$120.2 \pm 0.9^+$</td>
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<td>$121.5 \pm 2.2^+$</td>
<td>$56.6 \pm 1.0^+$</td>
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<tr>
<td>$(A-a)P_{DLO2}$, mmHg</td>
<td>$-0.9 \pm 0.9^+$</td>
<td>$-1.3 \pm 2.0^+$</td>
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<td></td>
<td>$-1.3 \pm 2.0^+$</td>
<td>$-1.3 \pm 2.0^+$</td>
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<tr>
<td>$D_{LO2}$, ml STPD-min$^{-1} \cdot \text{mmHg}^{-1}$</td>
<td>$22.0 \pm 0.9^+$</td>
<td>$17.8 \pm 0.6^+$</td>
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<td>$26.5 \pm 0.6^+$</td>
<td>$20.2 \pm 0.7$</td>
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<td>$D_{LO2}$, ml STPD-min$^{-1} \cdot \text{mmHg}^{-1} \cdot \text{kg}^{-1}$</td>
<td>$3.5 \pm 0.3^+$</td>
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<td>$3.5 \pm 0.3^+$</td>
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Values are means ± SE. $D_{LO2}$, effective lung diffusing capacity. *$P < 0.05$, HCR vs. LCR; †$P < 0.05$, normoxia vs. hypoxia; ‡$P = 0.07$, HCR vs. LCR for $D_{LO2}$.

### Table 3. Pulmonary hemodynamic variables in maximal exercise

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<td>LCR $n = 10$</td>
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<td>HCR $n = 9$</td>
<td>LCR $n = 7$</td>
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<tr>
<td>$Q_{\text{max}}, \text{ml/min}$</td>
<td>$94 \pm 8$</td>
<td>$89 \pm 6$</td>
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<td></td>
<td>$90 \pm 4$</td>
<td>$94 \pm 5$</td>
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<tr>
<td>$Q_{\text{max}}, \text{ml/min} \cdot \text{kg}^{-1}$</td>
<td>$487 \pm 24^+$</td>
<td>$343 \pm 15$</td>
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<td>$465 \pm 24^+$</td>
<td>$363 \pm 20$</td>
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<td>$PAP, \text{mmHg}$</td>
<td>$25.8 \pm 0.8$</td>
<td>$25.6 \pm 1.0$</td>
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<td>$25.7 \pm 0.6$</td>
<td>$26.1 \pm 1.5$</td>
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<tr>
<td>$PAP/\text{max}, \text{mmHg} \cdot \text{min}^{-1}$</td>
<td>$284 \pm 22$</td>
<td>$298 \pm 22$</td>
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<td>$293 \pm 24$</td>
<td>$287 \pm 35$</td>
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<tr>
<td>$PAP/\text{max}, \text{mmHg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$</td>
<td>$54.0 \pm 4.0^+$</td>
<td>$77.5 \pm 4.9$</td>
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<td></td>
<td>$56.3 \pm 4.4^+$</td>
<td>$75.1 \pm 9.1$</td>
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<tr>
<td>$D_{LO2}/Q_{\text{max}}, \text{ml STPD} / \text{mmHg}^{-1}$</td>
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<td>$0.007 \pm 0.001$</td>
<td>$0.007 \pm 0.001$</td>
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Values are means ± SE. $Q_{\text{max}}$, maximal cardiac output; PAP, mean pulmonary arterial pressure. *$P < 0.05$, HCR vs. LCR.
studies to compare rat lung volume using a mass-specific analysis rather than lung volume per body length (or frame size). However, due to the significant differences in body mass and frame size in our rats, we have chosen to use both methods to compare lung volume in HCR and LCR rats.

The lower mass of HCR rats is attributable mainly to a smaller body frame size and not to lower body fat content. While we did not measure lean body mass and fat content in our particular rats, others have in these populations. Thus Noland et al. (24) showed that HCR rats were smaller in body length and femur size, and R. T. Hepple (personal communication, with permission) found HCR to have 9% body fat while LCR had 13% body fat. The body fat data applied to our rats indicate lean body mass of HCR was 79% of that in LCR. Furthermore, Fig. 1 shows lung volume in our rats plotted on a background of published allometric data for lung size as a function of body mass (lung volume = 45.994-body mass^{1.059}) (9). It is evident that in our rats, LCR animals had lung volumes consistent with the data of Gehr et al. (9) while HCR rats had lung volumes greater than predicted for body mass (P < 0.0001). Although our measurements of lung volume are in vitro and may not reflect in vivo differences, the findings that all G15 lungs were the same size (at the same inflation pressure) and that HCR rats were significantly smaller and lighter suggest a difference in relative lung volume that was not seen in the relative proportions of heart and skeletal muscles of HCR and LCR G15 rats (12, 22).

We draw two conclusions from these findings. First, it is HCR and not LCR rats that differ from expected in terms of lung size in relation to body mass, and second, HCR rats have high lung volumes for their body mass (even if absolute lung volumes are similar in HCR and LCR).

We cannot resolve from the current study which of two possibilities explain our findings. 1) Whether, as HCR rats evolved toward a smaller body frame size, all organs and tissues underwent active downsizing, with, however, a specific restorative pulmonary genetic program activated to maintain lung size as an important attribute needed to support greater aerobic capacity, or 2) whether, as HCR rats evolved toward a smaller body frame size, all organs and tissues except the lungs developed genetic programs that led to smaller size. However, the recognition that, of all components of the O2 transport system ventilation, diffusion, and perfusion, this makes sense because the lungs are themselves a “mini” in series oxygen transport system such that enhancing just one step—ventilation, or diffusion, or perfusion—would not greatly augment overall gas transport. A coordinated increase in all three elements is consistent with the concept of symmorphosis (33).

Ventilation. Enhanced pulmonary gas exchange (substantially higher VO_{2max} without hypoxemia, hypercapnia, or an increase in (A-a)PO_{2} in either normoxia or hypoxia) could have occurred without structural changes or maintenance of lung size, provided there was sufficient ventilatory functional reserve. Indeed, rats are known to exchange gas very efficiently (13). Unlike larger mammals, rats do not exhibit any significant (A-a)PO_{2} at VO_{2max} in either normoxia or hypoxia (13). Furthermore, rats consistently hyperventilate, reaching a considerably higher alveolar and arterial PO_{2} and lower arterial PCO_{2} than humans (13). Both HCR and LCR lines behaved in a similar manner in the present study in normoxia, with the PaO_{2} at ~120 mmHg, PaCO_{2} at ~25 mmHg, and (A-a)PO_{2} near zero. The rise in PaO_{2} from rest to exercise in both the HCR and LCR are similar to previously published data (7, 8, 10, 11, 14, 15, 18, 19).

A simple calculation using the alveolar ventilation equation shows that to produce the higher VO_{2max} in HCR without increasing alveolar ventilation, alveolar PO_{2} would have had to fall from the measured value of 123 mmHg to ~113 mmHg. Given the efficiency of gas exchange in the rat, arterial PO_{2} in species with increased activity (34). Similarly, developing marsupials increase their rate of lung growth at the transition from ectothermy to endothermy (26).

The greater relative lung volume of HCR animals promotes improved exercise capacity and allowed maintenance of gas exchange (and even a small increase in alveolar PO_{2} and fall in alveolar PCO_{2}) along with an absence of an increase in PAP. These physiological changes appear to reflect differences in all three main functional components of the pulmonary gas exchange system ventilation, diffusion, and perfusion. This makes sense because the lungs are themselves a “mini” in series oxygen transport system such that enhancing just one step—ventilation, or diffusion, or perfusion—would not greatly augment overall gas transport. A coordinated increase in all three elements is consistent with the concept of symmorphosis (33).
would have likely also been ~113 mmHg. Even with the high standard P50 of 30–35 mmHg, this would provide essentially full saturation. The same calculation for CO2 reveals that alveolar (and thus arterial) PCO2 would have had to be ~29 mmHg to support the higher V̇CO2 without changes in alveolar ventilation. This is a value regarded as below normal for a human. Thus, while the HCR lung allowed alveolar gas tensions to be maintained through greater ventilation, the absence of any increase in alveolar ventilation would not have produced hypoxemia or hypercapnia by human standards despite the higher metabolic rate. Indeed, in athletic humans, the tendency is to let arterial PCO2 rise at V̇O2max and at the same time accept some degree of hypoxemia (25).

It is therefore all the more interesting to note that HCR rats, in the face of substantially higher V̇O2max, displayed clearly lower arterial PCO2 values than LCR rats due to relative hyperventilation. A clue as to why this took place is in the arterial lactate values, which were considerably higher in HCR than LCR (22) and likely provided additional stimulus to breathe that resulted in a lower arterial PCO2. Indeed arterial pH values at V̇O2max were lower in HCR (7.34) than in LCR (7.45) as a result of higher lactate values (22).

**Blood flow and PAP/Q̇max.** A larger lung may be advantageous because it would buffer possible increases in pulmonary artery pressure during exercise. However, in the present study, absolute cardiac output and PAP were not significantly different between HCR and LCR rats during exercise. Had lung size in HCR decreased in proportion to body mass, HCR would presumably have had a higher PAP and vascular resistance. The potential effect is substantial and can be estimated as follows.

The mass-specific cardiac output (at normoxic V̇O2max) in HCR was 42% greater than in LCR. Neglecting left atrial pressure, and for the same PAP/Q̇max, Ohm’s law predicts that mean pulmonary artery pressure would have had to be 42% higher as well. This would have caused mean PAP to be nearly 40 mmHg. Figure 2 shows this: when PAP is plotted as a function of mass-specific cardiac output, HCR have a consistently lower pressure when related to cardiac output. This difference would be expected to disappear had lung size not been maintained.

While 40 mmHg is nowhere near as great as the 100 mmHg pulmonary pressures seen in racehorses (35), such a level would be considered high in humans, and if the right ventricle were often subjected to such a pressure, it is possible that pathological changes might develop over time. Therefore, unlike the above calculations for alveolar PO2 and PCO2 (which suggested that gas exchange would suffer minimally even in the face of unchanged alveolar ventilation), failure to lower mass-specific PAP/Q̇max would be predicted to have significance for right heart function. We suggest that there may be value in maintaining lung size to prevent undue pulmonary hypertension during activity.

**Blood flow and alveolar-capillary diffusion.** Similar logic can be applied to the diffusing capacity. It is therefore likely that maintenance of lung size in HCR was associated with a maintained capillary blood volume that sustained diffusing capacity and buffered the red cell transit time in the face of a higher mass-specific cardiac output, leading to maintained arterial oxygenation and no increase in (A-a)PO2 compared with LCR rats. While we did not directly measure capillary blood volume, diffusing capacity should track with it (27).

Using numerical analysis techniques, we calculated that had mass-specific diffusing capacity not been 21% higher in HCR (than in LCR) in the face of the 28% higher mass-specific cardiac output at V̇O2max in hypoxia, arterial PO2 would have been 2.6 mmHg lower than measured, and (A-a)PO2 would have been 2.6 mmHg higher. All other factors unchanged, this would have caused systemic O2 delivery to fall by 4.9%, which at a constant peripheral O2 extraction would have reduced V̇O2max by the same amount. Thus a relatively larger lung with an attendant increase in pulmonary vasculature not only helps to prevent significant increases in PAP, but also increases diffusing capacity and helps to maintain red cell transit time to enhance diffusional exchange of O2. This is consistent with findings in human populations as well (27).

**G7 vs. G15.** While the preceding sections dealt with differences between LCR and HCR rats at G15, it is of interest to examine the basis for the continued divergence across lines between our original study (G7) and the current effort (G15). The general question is whether the continued divergence in exercise capacity occurs because 1) the HCR rats are improving; 2) the LCR rats are worsening, or 3) both. A priori, one would expect c) “both,” since the breeding selection process identified both better and poorer runners. The G7 vs. G15 analysis was limited to data collected during maximal exercise in normoxia (except for DLVO2, determined in all rats as mentioned only in hypoxia). At G7, a complete data set during normoxic exercise was reported for six HCR rats (215 ± 9 g body wt) and five LCR, rats (250 ± 4 g body wt) (20).

Figure 3 shows mean data central to lung function at V̇O2max in normoxia for both lines at G7 and G15. Alveolar ventilation (Fig. 3A), while similar at G7, was different by G15 due to downward trends in LCR combined with upward trends in HCR, although neither trend alone reached statistical significance. These results are therefore in accordance with the general hypothesis: HCR continuing to improve and LCR continuing to deteriorate. Figure 3B shows that arterial po2 increased in both lines from G7 to G15, with very little difference between lines. For the most part, this suggests that
between lines, ventilation tracked metabolic rate. Why arterial PO2 appears to be slightly higher at G15 compared with G7 in both groups is not clear; however, since the values are so high in both generations (116 mmHg at G7 and 122 mmHg at G15), and the differences so small (~6 mmHg), the effects on arterial O2 saturation and content are negligible. Arterial PCO2 (Fig. 3C) did not change between G7 and G15 for either HCR or LCR. A small difference of 3 mmHg between HCR and LCR persists, although due to high variance at G7 the difference was not statistically significant. The systematically lower PCO2 in HCR likely reflects the added stimulus of higher lactate values (22). (A-a)PO2 (Fig. 3D) is approximately zero in either group at either generation. Thus the lungs are in essence completely efficient in normoxia at both times in both lines.

Similar to ventilation, total systemic O2 delivery [the product of cardiac output and arterial O2 content; Fig. 3E; adapted from Gonzalez et al. (12)] improved by 27% in HCR rats from G7 to G15 (P < 0.05) and deteriorated in LCR between G7 and
There were no differences at G7 but total systemic oxygen delivery was 48% higher in HCR than LCR at G15 (P < 0.0005). As with ventilation, the movement of the two lines in opposite directions conforms to the nature of the breeding process.

On the basis of arterial PO2 and Hb concentration not being different between the lines, it is clear that the divergence in oxygen delivery must reflect corresponding differences in cardiac output (confirmed in Fig. 4A). Also in Fig. 4B, mean pulmonary artery pressure showed no differences between lines at either generation. The changes in mass-specific Qmax resulted in a 44% higher PAP/Qmax in LCR compared with HCR at G15, which was due mostly to a substantial 55% increase in PAP/Qmax from G7 to G15 in LCR (P < 0.001; Fig. 4).

The effective lung diffusing capacity (Fig. 5), similar at G7, was interpreted as likely being statistically different between HCR and LCR at G15, although the P value did not quite reach 0.05 (P = 0.07). This argument is strengthened by finding that the diffusing capacity of HCR rats significantly increased (by 68%) between G7 and G15. The higher HCR DlO2 at G15 explains why (A-a)PO2 did not change even with increasing cardiac output from G7 to G15.

For G7 vs. G15, two primary variables—alveolar ventilation (Fig. 3) and cardiac output (Fig. 4)—diverged further between G7 and G15 with both increases in HCR and decreases in LCR. The remaining variables, because they depend on these primary factors, changed as expected when differences in relative lung size were taken into account.

In summary, we showed that continued selective breeding of rats (over 15 generations) for endurance running ability caused further divergence in parameters of O2 transport. Although HCR animals were 25% smaller than LCR, lung size was similar in both groups. Absolute VO2max was 12% higher (49% higher, per kg). Our data suggest that this was due in part to the maintenance of lung size, which permitted hyperventilation, and kept pulmonary vascular resistance and the (A-a)PO2 from rising. It is not known whether the maintenance of lung volume was the result of an active genetic program that intervenes to maintain lung size when other organs and tissues are downsizing, or whether downsizing simply does not involve the lungs. However, taken together with prior observations that in the racehorse relatively small lungs lead to hypercapnia, hypoxemia, and severe pulmonary hypertension, the present findings support the importance of genetic control of lung structure and function in attainability of high levels of exercise.
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