Continued divergence in $\text{VO}_2\text{max}$ of rats artificially selected for running endurance is mediated by greater convective blood O$_2$ delivery

Norberto C. Gonzalez,¹ Scott D. Kirkton,² Richard A. Howlett,² Steven L. Britton,³ Lauren G. Koch,³ Harrieth E. Wagner, and Peter D. Wagner²

¹University of Kansas Medical Center, Kansas City, Kansas; ²University of California San Diego, La Jolla, California; and ³University of Michigan, Ann Arbor, Michigan

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Gonzalez, Norberto C., Scott D. Kirkton, Richard A. Howlett, Steven L. Britton, Lauren G. Koch, Harrieth E. Wagner, and Peter D. Wagner. Continued divergence in $\text{VO}_2\text{max}$ of rats artificially selected for running endurance is mediated by greater convective blood O$_2$ delivery. J Appl Physiol 101: 1288–1296, 2006. First published June 15, 2006; doi:10.1152/japplphysiol.01527.2005.—We previously showed that after seven generations of artificial selection of rats for running capacity, maximal O$_2$ uptake ($\text{VO}_2\text{max}$) was 12% greater in high-capacity (HCR) than in low-capacity runners (LCR). This difference was due exclusively to a greater O$_2$ uptake and utilization by skeletal muscle of HCR, without differences between lines in convective O$_2$ delivery to muscle by the cardiopulmonary system ($\text{QO}_2\text{max}$). The present study in generation 15 (G15) female rats tested the hypothesis that continuing improvement in skeletal muscle O$_2$ transfer must be accompanied by augmentation in $\text{QO}_2\text{max}$ to support $\text{VO}_2\text{max}$ of HCR. Systemic O$_2$ transport was studied during maximal normoxic and hypoxic exercise (inspired PO$_2$ 8750–7587 Torr). $\text{VO}_2\text{max}$ divergence between lines increased because of both improvement in HCR and deterioration in LCR: normoxic $\text{VO}_2\text{max}$ was 50% higher in HCR than LCR. The greater $\text{VO}_2\text{max}$ in HCR was accompanied by a 41% increase in $\text{QO}_2\text{max}$; 96.1 ± 4.0 in HCR vs. 68.1 ± 2.5 ml STPD O$_2$·min$^{-1}$·kg$^{-1}$ in LCR ($P < 0.01$) during normoxia. The greater G15 $\text{QO}_2\text{max}$ of HCR was due to a 48% greater stroke volume than LCR. Although tissue O$_2$ diffusive conductance continued to increase in HCR, tissue O$_2$ extraction was not significantly different from LCR at G15, because of the offsetting effect of greater HCR blood flow on tissue O$_2$ extraction. These results indicate that continuing divergence in $\text{QO}_2\text{max}$ between lines occurs largely as a consequence of change in the capacity to deliver O$_2$ to the exercising muscle.

intrinsic exercise capacity; genetic determinants; tissue O$_2$ diffusive conductance; maximal cardiac output

AEROBIC EXERCISE CAPACITY is a complex individual trait that is influenced by both genetic and environmental factors such as lifestyle, diet, and training and that has been proposed as predictor of mortality (35, 36). Recent research suggests that the genetic substrate includes genes that determine the intrinsic exercise capacity of the untrained individual and genes that are responsible for regulating the adaptive responses to exercise training (2, 4, 5). The exact nature of these genes has not been established; however, it is estimated that the genetic component may account for up to 50% of the variations in individual exercise capacity (3). A major obstacle for better understanding of this subject is the difficulty in estimating the relative contribution of genetic and environmental influences determining the exercise capacity in a given individual. Development of animal models in which these influences can be controlled would be of major value (6).

In 1996, large scale, two-way artificial selection for low and high treadmill running capacity in rats was initiated (29). With the exception of 1 wk to test for running capacity, the rats remain sedentary throughout their lifetime; accordingly, differences between lines reflect genetically segregating differences in intrinsic exercise capacity. After seven generations, maximal distance run to exhaustion in an incremental treadmill running protocol was approximately threefold higher in high-capacity (HCR) than in low-capacity runners (LCR) (20). Maximal O$_2$ uptake ($\text{VO}_2\text{max}$) of a group of generation 7 females was 12% higher in HCR than in LCR (20). The higher $\text{VO}_2\text{max}$ of HCR was entirely due to a greater ability to transport O$_2$ from the tissue capillaries to the cells (20), accompanied by improved utilization of O$_2$ by skeletal muscle cells (25). The greater tissue O$_2$ extraction and utilization in HCR was the result of increased tissue O$_2$ diffusive conductance (20); this was paralleled by greater capillary density and higher oxidative enzyme activity in skeletal muscle (25). There were no differences in pulmonary or cardiovascular function between the lines that would support an enhanced rate of O$_2$ delivery from the tissue capillaries in HCR (20).

Because the higher $\text{VO}_2\text{max}$ of HCR of generation 7 was exclusively due to a greater capacity of skeletal muscle to extract and utilize O$_2$, the question arises whether it would be possible to maintain a continually expanding capacity for O$_2$ uptake and utilization at the skeletal muscle level without an accompanying increase in the ability to deliver O$_2$ to the muscle. The studies presented here were designed to test the hypothesis that an increased capacity for tissue O$_2$ transfer would have to be accompanied by a parallel augmentation in the capacity to transport O$_2$ to the tissue capillaries to maintain $\text{VO}_2\text{max}$. To test this hypothesis, systemic O$_2$ transport was analyzed during maximal exercise in HCR and LCR rats of generation 15. Our results demonstrate that at generation 15 a widening difference in tissue O$_2$ diffusive conductance between HCR and LCR is accompanied by significant differences between lines in the capacity to deliver O$_2$ to the exercising muscles.

METHODS

Animal model. All procedures were carried out according to the Guide for the Care and Use of Laboratory Animals. The protocol was

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Address for reprint requests and other correspondence: N. C. Gonzalez, Dept. of Molecular and Integrative Physiology, Univ. of Kansas Medical Center, 3901 Rainbow Blvd., Kansas City, KS 66160 (e-mail: ngonzalez@kumc.edu).
approved by the Institutional Animal Care and Use Committee of the University of California San Diego Medical School.

The development of HCR and LCR rats was described in detail previously (29). Briefly, artificial, divergent, selective breeding was used to create low and high lines for treadmill running capacity. The founder population was 96 male and 96 female genetically heterogeneous rats (N:NIH stock) obtained from a colony maintained at the National Institutes of Health (17). Each rat in the founder population was of different parentage so that selection was not among brothers and sisters, which produced a broader initial genetic variance (9, 18).

In each generation, running capacity of every rat was assessed by using an incremental velocity treadmill running protocol when the animals were 11 wk of age (29). After a week of adaptation and learning to run on a treadmill, the animals were tested for maximal endurance running capacity on the following week. Each animal underwent an exercise test every day for 5 consecutive days. Starting at a speed of 10 m/min and 15° angle, speed was increased by 1 m/min every 2 min until exhaustion. For each of the five runs, total distance run was used as an estimate of endurance capacity. The single best daily run of the five trials for each rat was considered the trial most closely associated with the heritable component of endurance running capacity (29).

Systemic O₂ transport studies. The experiments reported here were carried out on female rats of generation 15 when the animals were ~8 mo old. One day before the maximal exercise test, the animals were anesthetized with pentobarbital sodium (30 mg/kg ip). A polyethylene catheter (PE50) was placed in the aortic arch via the left carotid artery, and a PE10 catheter was advanced into the pulmonary artery via the right jugular vein with the aid of a J-shaped introducer. Adequate placement of the catheters was established by the pressure waveform and verified at autopsy. The catheters were tunneled subcutaneously, exteriorized at the back of the neck, cut at a length of 4 cm of their emergence from the skin, and flame sealed. The animals recovered from anesthesia and were studied on the following day. Each animal exercised maximally twice: once in normoxia (inspired PO₂ ~150 Torr) and once in hypoxia (inspired PO₂ ~70 Torr). Both runs were carried out on the same day, with ~3 h between runs. The order of the hypoxic and normoxic runs was balanced within both HCR and LCR groups. An equal number of HCR and LCR were tested on each day.

Maximal exercise protocol. After measurement of rectal temperature, the animals were placed on a treadmill enclosed in a Lucite chamber adapted for the determination of O₂ uptake (V˙O₂) and CO₂ production (V˙CO₂) by the open-circuit method. The catheters were connected, through sampling ports located on the top of the box enclosing the treadmill, to pressure transducers. After 30 min at rest on the treadmill, arterial and mixed venous blood samples were obtained via stopcocks, the blood was replaced with fresh blood from donor rats, and the treadmill was set at a speed of 10 m/min and an angle of 10°. This work rate was maintained for 2–3 min, after which speed was increased by 5 m/min every 90–120 s, until V˙O₂ max was reached. V˙O₂ max was defined as the V˙O₂ after which an increase in work rate was not associated with a further increase (±5%) in continuously measured O₂ uptake.

Arterial and mixed venous (pulmonary artery) blood samples were obtained during the last 60–120 s of exercise, while V˙O₂ and V˙CO₂ showed steady values. After blood sampling, the box enclosing the treadmill was opened and the rectal temperature was determined within 30 s of termination of exercise. After the first run, the blood withdrawn in the exercise sample was replaced as described above, and 0.5 ml/100 g of a solution of 0.15 mM NaHCO₃ was administered intravenously to correct the metabolic acidosis of maximal exercise. After the last run, the animals were killed with an overdose of pentobarbital sodium, 60 mg/kg iv, and the heart, lungs, and selected skeletal muscles were removed for morphological analysis and measurement of metabolic markers. The results of the studies in lungs and skeletal muscle will be reported separately.

Gas exchange and O₂ transport determinations. The box enclosing the treadmill was airtight except for the front inflow and rear outflow ports. Appropriate inspired PO₂ was delivered from gas tanks with known concentrations. Inflow was maintained constant at ~6 l/min. Infloowing and outflowing O₂ concentrations and outflowing CO₂ concentration were measured continuously and simultaneously via a Perkin-Elmer model 1100 mass spectrometer (Pomona, CA). V˙O₂ and V˙CO₂ were calculated from the inflowing and outflowing O₂ concentration difference, the outflowing CO₂ concentration, and the gas flow, by standard gas exchange equations (expressed in ml STPD·min⁻¹·kg⁻¹). Maximal work rate (kg·min⁻¹·kg⁻¹) was calculated from the maximal speed, treadmill angle, and body weight.

Arterial and mixed venous blood samples were analyzed for pH, P O₂, and P CO₂ in an Instrumentation Laboratory Synthesis 45 blood-gas analyzer (Lexington, MA) with use of appropriate electrodes at 37°C. Hb concentration and O₂ saturation were measured with an Instrumentation Laboratory model 682 CO-oximeter. Values were corrected to the rectal temperature using temperature correction factors for rat blood (14) and reported at blood temperature herein.

Systemic (MABP) and pulmonary arterial pressures were measured using Gould Instruments pressure transducers (Cerritos, CA) and recorded continuously in a Clevite chart recorder (Cleveland, OH). Mean pressures were obtained by electronic integration using Gould Instruments preamplifiers. Heart rate (HR) was obtained directly from the systemic arterial blood pressure tracing. O₂ contents (ml/dl) of arterial (CaO₂) and of mixed venous blood were calculated from measured values of Hb concentration, P O₂, and O₂ saturation using an Hb-O₂ binding factor of 1.34 ml STPD/g. This constant was obtained from direct measurement of total blood O₂ content (Oxycon, Cameron Instruments, Port Aransas, TX) and of blood Hb concentration by a spectrophotometric method (Sigma Chemical, St. Louis, MO) (20). In each animal, measured O₂ saturation and the corresponding P O₂ from all samples were used to estimate standard hemoglobin P50. Cardiac output (Q; in ml·min⁻¹·kg⁻¹) was calculated as the ratio V˙O₂/C(a-v)O₂, where C (a-v)O₂ is the arterio-venous O₂ content difference.

Stroke volume (SV) was calculated as Q/HR and expressed both as ml/kg and ml/m². Mean thoracic aortic pressure (P AER) was calculated as C(a-v)O₂/CaO₂. Mean tissue capillary P O₂ (Porr) and the corresponding value for tissue O₂ conductance (D O₂, ml·min⁻¹·Torr⁻¹·kg⁻¹) were calculated by a numerical integration procedure (39, 41).

Eleven HCR and 12 LCR rats were studied. However, in a few animals V˙O₂ max, as defined above, was not reached, and/or a complete set of blood samples was not obtained. As a result data are presented on the following number of animals that did achieve V˙O₂ max and provided complete sets of blood samples: HCR, normoxic exercise: 10; hypoxic exercise 9; LCR, normoxic exercise: 10; hypoxic exercise: 7.

Data are presented as means ± SE. A two-way ANOVA with repeated measures was conducted to evaluate the effect of exercise capacity (HCR vs. LCR) and the effect of inspired oxygen (hypoxia vs. normoxia). Significance was established by using t-tests with the Bonferroni correction for multiple comparisons. A P value smaller than 0.05 was considered to indicate a significant difference. All statistical analyses were performed by using SPSS (SPSS, Version 14; Chicago IL).

RESULTS

Maximal distance run in an endurance test carried out when these rats were 11 wk of age was 2,349 ± 243 m in HCR and 229 ± 27 m in LCR (P < 0.001). Body weight on the day of maximal exercise, when the animals were ~8 mo old, was significantly different in both groups: 194 ± 3 in HCR vs. 259 ± 9 g in LCR (P < 0.05). Gastrocnemius muscle weight
was also significantly lower in HCR: 0.91 ± 0.04 g vs. 1.18 ± 0.02 (P < 0.01). However, there were no significant differences between lines in gastrocnemius muscle normalized for body weight: 4.69 ± 0.012 in HCR vs. 4.58 ± 0.009 mg/g in LCR [not significant (NS)]. Maximal work rate achieved was almost twice as high in HCR as in LCR during normoxic exercise (Table 1); in hypoxia, maximal work rate was ~65% higher in HCR. Vo2max normalized for body weight was almost 50% higher in HCR than in LCR in normoxic exercise; in hypoxia, the difference was close to 30% (Table 1). The energy cost of maximal work rate was not significantly different between lines: the relationship between V˙O2 max and max- 
imal work rate was similar in HCR and LCR, both in normoxic and in hypoxic exercise (Fig. 1). Hypoxia did not influence the energy cost of exercise; this was true for both HCR and LCR (Fig. 1).

The greater Vo2max in HCR was accompanied by a larger rate of convective blood O2 delivery to the tissues: QO2 max, the product of maximal (Qmax) and arterial blood O2 content, was 41% higher in HCR than in LCR in normoxia and 25% higher in hypoxia (Table 1); accordingly, the higher QO2 max of HCR was entirely the result of greater Qmax (Table 2). This, in turn, was mediated by a higher SV because there were no significant differences in maximal HR between lines (Table 2). The larger maximal SV (SVmax) of HCR was associated with a greater heart weight in proportion to body weight (Table 2). Although both heart and body weights were higher in LCR, the heart-to-body weight ratio was larger in HCR (Table 2). SV normalized to heart weight was also significantly higher in HCR, both in normoxic and as well as in hypoxic exercise (Table 2). There were no significant differences in mean arterial blood pressure between groups in either hypoxia or normoxia (Table 1); accordingly, the higher QO2 max of HCR was entirely the result of greater Qmax (Table 2). This, in turn, was mediated by a higher SV because there were no significant differences in maximal HR between lines (Table 2). The larger maximal SV (SVmax) of HCR was associated with a greater heart weight in proportion to body weight (Table 2). Although both heart and body weights were higher in LCR, the heart-to-body weight ratio was larger in HCR (Table 2). SV normalized to heart weight was also significantly higher in HCR, both in normoxic and as well as in hypoxic exercise (Table 2). There were no significant differences in mean arterial blood pressure between groups in either hypoxia or hypoxic exercise; however, hypoxia resulted in similar reduction of blood pressure with respect to normoxia in both lines (Table 2). The ratio MABP/Qmax, an index of systemic vascular resistance, was significantly lower in HCR than LCR only during normoxia. There was no significant effect of hypoxia on MABP/Qmax although the values tended to be lower in both lines during hypoxic exercise (Table 2).

Table 3 shows the values of variables related to the transport of O2 at the tissue level. The expected decreases in arterial, mixed venous, and mean capillary blood Po2 were observed during hypoxia; there were no significant differences between lines in any of these values. Tissue O2 diffusing capacity was significantly higher in HCR than in LCR. Hypoxic exercise was associated with an increase in DTo2 in both groups; however, the increases were similar, such that DTo2 was ~50% higher in HCR than LCR in both hypoxia and normoxia. Although there was a tendency for a larger O2ER in HCR, the differences with LCR did not reach statistical significance in either normoxic or hypoxic exercise. There was no significant effect of hypoxia per se in O2ER in either group. There was no significant difference in standard P50 between HCR and LCR (Table 3).

DISCUSSION

The central finding of the experiments presented here is that the greater Vo2max of HCR is due to both a larger rate of convective blood O2 delivery to the tissues and a greater tissue O2 diffusing capacity. However, although tissue O2 diffusive conductance is greater in HCR, its effect on the tissue O2 extraction ratio V˙O2 max/QO2max is offset by the larger tissue O2 perfusive conductance such that O2 extraction by the tissues is similar in both lines. This combination of greater rate of O2 delivery to the tissues in HCR and similar tissue O2 extraction in both lines supports a larger V˙O2 max in HCR.

### Table 1. Vo2max and convective O2 delivery variables during maximal exercise

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<th>Normoxic Exercise</th>
<th>Hypoxic Exercise</th>
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<tbody>
<tr>
<td></td>
<td>HCR (n = 10)</td>
<td>LCR (n = 10)</td>
</tr>
<tr>
<td>Maximal work rate, kg.m⁻¹.min⁻¹</td>
<td>6.94±0.25†</td>
<td>3.60±0.35</td>
</tr>
<tr>
<td>V˙O2 max, ml.¹.min⁻¹.kg⁻¹</td>
<td>69.2±2.0*</td>
<td>46.3±1.1*</td>
</tr>
<tr>
<td>QO2 max, ml.¹.min⁻¹.kg⁻¹</td>
<td>96.1±4.0*</td>
<td>68.1±2.5*</td>
</tr>
<tr>
<td>CaO2 ml/dl</td>
<td>19.9±0.5*</td>
<td>19.9±0.4*</td>
</tr>
<tr>
<td>CVO2, ml/dl</td>
<td>5.4±0.7*</td>
<td>6.3±0.4*</td>
</tr>
<tr>
<td>Blood [Hb], g/dl</td>
<td>15.3±0.4</td>
<td>15.1±0.3</td>
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</tbody>
</table>

Values are means ± SE. Maximal work rate was calculated from maximal speed, treadmill angle, and body weight. HCR and LCR, rats with high and low running capacity, respectively. Vo2max, maximal rate of O2 consumption; QO2 max, maximal rate of convective O2 delivery = cardiac output × arterial blood O2 content; CaO2, arterial blood O2 content; CVO2, mixed venous (pulmonary arterial) blood O2 content; blood [Hb], hemoglobin concentration in blood. *P < 0.05 normoxic exercise vs. hypoxic exercise; †P < 0.05 HCR vs. LCR.
**Experimental design.** Maximal exercise was studied in these experiments because it provides a measure of the capacity of the pulmonary, cardiovascular, and skeletal muscle system to transport and utilize O₂. The experimental model used here allowed us to obtain an accurate measure of the variables involved in the transport of O₂ from the atmosphere to the tissue capillaries and therefore to determine which of the linked conductances that compose the O₂ transport system contribute to the differences between HCR and LCR. Hypoxic and normoxic exercise was studied in each animal to determine whether there is a difference between the lines in their response to O₂ limitation and to establish the O₂ dependence of pulmonary and tissue diffusive O₂ conductances.

Vascular catheter implantation is necessary to measure systemic O₂ transport variables. The protocol involving maximal exercise on the day after vascular catheterization has been employed extensively in our laboratories (10, 13, 14, 19, 20, 34). VO₂ max values obtained in the HCR rats in this study are within the range of values obtained in rats without prior surgery (1, 15, 28). Values of Qmax, maximal HR, and SVmax obtained in HCR in the present studies, as well as in experiments in normoxic, untrained Sprague-Dawley rats studied before by us 1 day after surgery (10, 14, 19, 34) are within the range of values observed in rats with vascular catheters in other laboratories (22). In addition, it appears that a prior maximal exercise bout in the same day does not influence maximal exercise capacity: the HCR rats in which the normoxic bout was the first run of the day showed VO₂ max (ml STPD·min⁻¹·kg⁻¹) of 70.0 ± 2.5 (n = 5) when the normoxic run was the second of the day, VO₂ max was 68.4 ± 1.8 (n = 5; NS). For LCR, the values of normoxic VO₂ max were 45.8 ± 1.9 (n = 5) and 49.7 ± 2.2 (n = 5) ml STPD·min⁻¹·kg⁻¹ in the first and second runs of the day, respectively (NS). A similar lack of statistically significant difference was observed between first and second runs of both HCR and LCR under hypoxic conditions. These data and previous observations using this experimental protocol (10, 13, 14, 19, 20, 34) suggest that the values of O₂ transport variables observed using this experimental design adequately represent those present in normal physiological conditions.

Body weights at the time of the experiments were significantly lower in HCR than in LCR. Artificial selection has produced differences in body weight as a correlated trait in this model: in general, HCR has become increasingly lighter and LCR heavier with each generation (29). Multiple regression analysis using weight and generation as predictor of running capacity show that differences in body weights contribute to only 7% of the variation in maximal distance run by HCR and LCR females (44). Nevertheless, if VO₂ max values obtained in this study are not normalized for body weight, the differences between lines in absolute values of VO₂ max and derived values are much smaller than those presented in Tables 1–3. It could be hypothesized that the weight difference between lines is due to a larger proportion of body fat in LCR, with similar muscle mass in both lines. If this were the case, similar amounts of O₂ would be consumed by a similar mass of contracting muscle in both lines because during maximal exercise most of the O₂ is consumed by the contracting muscles (32). This would mean that the difference between lines in the O₂ transport variables reported in Tables 1–3 would be artificially high. Several lines of evidence indicate that this is not the case. First, weight of the gastrocnemius is significantly smaller in HCR, and the gastrocnemius-to-body weight ratio is the same in both lines. In the rats of generation 7, smaller body and gastrocnemius weights

**Table 2. Hemodynamic variables during maximal exercise**

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<th>Normoxic Exercise</th>
<th>Hypoxic Exercise</th>
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<tr>
<td></td>
<td>HCR</td>
<td>LCR</td>
</tr>
<tr>
<td></td>
<td>Qmax, ml·min⁻¹·kg⁻¹</td>
<td>487±24†</td>
</tr>
<tr>
<td></td>
<td>HRmax, beats/min</td>
<td>520±9</td>
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<tr>
<td></td>
<td>SVmax, ml/kg BW</td>
<td>0.93±0.04†</td>
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<tr>
<td></td>
<td>SVmax, ml/g HW</td>
<td>0.260±0.011†</td>
</tr>
<tr>
<td></td>
<td>MABP, mmHg</td>
<td>123±3*</td>
</tr>
<tr>
<td></td>
<td>MABP/Qmax, mmHg/l·min⁻¹·kg⁻¹</td>
<td>258±13†</td>
</tr>
<tr>
<td></td>
<td>HW, mg</td>
<td>704±15†</td>
</tr>
<tr>
<td></td>
<td>HW/BW, mg/g</td>
<td>364±5†</td>
</tr>
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</table>

Qmax, maximal cardiac output; HRmax, maximal heart rate; SVmax, maximal stroke volume; MABP, mean systemic arterial blood pressure; HW, heart weight; HW/BW, heart-to-body weight ratio. *P < 0.05 normoxic exercise vs. hypoxic exercise; †P < 0.05 HCR vs. LCR.

**Table 3. Tissue O₂ transport variables during maximal exercise**

<table>
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<th></th>
<th>Normoxic Exercise</th>
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<tr>
<td></td>
<td>HCR</td>
<td>LCR</td>
</tr>
<tr>
<td></td>
<td>Pao₂, Torr</td>
<td>124.0±1.1*</td>
</tr>
<tr>
<td></td>
<td>PvO₂, Torr</td>
<td>30.4±2.0*</td>
</tr>
<tr>
<td></td>
<td>Pcapo₂, Torr</td>
<td>53.2±2.4*</td>
</tr>
<tr>
<td></td>
<td>Dto₂, ml·min⁻¹·Torr⁻¹·kg⁻¹</td>
<td>1.37±0.07†</td>
</tr>
<tr>
<td></td>
<td>O₂ER</td>
<td>0.732±0.033</td>
</tr>
<tr>
<td></td>
<td>Standard Pso₂, Torr</td>
<td>36.8±0.3</td>
</tr>
</tbody>
</table>

Pao₂, partial pressure of O₂ in arterial blood; PvO₂, partial pressure of O₂ in mixed venous (pulmonary arterial) blood; Pcapo₂, mean partial pressure of O₂ in the systemic capillaries; Dto₂, tissue diffusing capacity; O₂ER, tissue O₂ extraction ratio = C(a-v) O₂/CaO₂. *P < 0.05 normoxic exercise vs hypoxic exercise; †P < 0.05 HCR vs. LCR.
Convective blood O2 delivery to the tissue capillaries is determined by the balance between tissue O2 diffusive and perfusive conductances. The higher \( \dot{V}O_2_{max} \) of HCR is the result of a greater rate of blood O2 convection (Table 1). O2 extraction, on the other hand, was not different between lines (Table 3).

**Determinants of \( \dot{V}O_2_{max} \) in HCR and LCR of generation 15.** Conceptually, \( \dot{V}O_2_{max} \) can be viewed as the result of the interaction between two major processes: the rate of O2 delivery to the tissues by blood on one hand, and the extraction of O2 from blood by the tissues on the other. The rate of convective blood O2 delivery to the tissue capillaries is determined by pulmonary and cardiovascular function, as well as by blood O2-carrying capacity. The effects of artificial selection on pulmonary function will be described in a separate paper.

**Convective blood O2 delivery.** The greater \( \dot{V}O_2_{max} \) of HCR ultimately resulted from a greater SV\(_{max} \) in this line (Table 2). SV is influenced by structural factors such as heart size, as well as by the interaction of HR, ventricular preload and afterload, and myocardial contractility. When normalized for body weight, heart weight was significantly larger in HCR (Table 2). There is a positive correlation between the individual values of heart and body weight in both lines; however, the relationship is shifted toward larger heart weights for a given body weight in the HCR line (Fig. 2). These data suggest that the enhanced capacity to deliver O2 to the tissues during maximal exercise may be mediated in part by anatomical differences between lines. On the other hand, the SV generated per each gram of heart was also larger in HCR (Table 2), suggesting that the greater SV of HCR may be the result of a more effective cardiovascular performance during exercise. This is consistent with previous observations in the same animal model that showed that, under similar HR, preload, and afterload values, SV normalized per gram of isolated working hearts was higher in HCR (26) and that both systolic and diastolic function of isolated left ventricular cells were enhanced in HCR with respect to LCR (26). The differences observed between lines in isolated working hearts and cardiac cells suggest an enhanced myocardial performance in HCR.

**Tissue O2 extraction.** In the present study whole body O2 consumption was measured; however, during maximal exercise in quadrupeds, most of the O2 is consumed in skeletal muscle (32). Accordingly, \( O_2_{ER} \) under these conditions largely reflects the extraction of O2 by skeletal muscle. Although the
rate of O₂ delivery to the exercising skeletal muscles was significantly higher in HCR than in LCR, the amount of O₂ extracted by the tissues in proportion to the O₂ supplied was similar in both lines. This occurred despite the fact that one of the determinants of tissue O₂ extraction, tissue O₂ diffusive conductance, was substantially higher in HCR. Figure 3 shows a plot of \( V_{O2\text{max}} \) as a function of mean capillary PO₂. During maximal exercise, mitochondrial PO₂ is essentially zero (12, 38); accordingly, the mean capillary PO₂ is an adequate representation of the gradient for O₂ diffusion between capillary and mitochondrion. Thus the slope of the line relating \( V_{O2\text{max}} \) and mean capillary PO₂ represents the average tissue O₂ diffusing capacity (i.e., \( V_{O2\text{max}}/P_{O2} \) gradient = \( D_{To2} \)), a composite parameter determined by all the processes involved in the flow of O₂ from the capillary to the mitochondrion. The average \( D_{To2} \) value over the entire PO₂ range investigated is 1.29 ± 0.03 in HCR vs. 0.90 ± 0.04 ml·min⁻¹·Torr⁻¹·kg⁻¹ in LCR (\( P < 0.01 \)).

Several observations indicate that a major determinant of skeletal muscle diffusive O₂ conductance is the area of interface between fibers and capillaries (21, 22, 33). The findings of generation 7 support this notion (25): the smaller gastrocnemius size of HCR rats of generation 7 was the result of smaller fiber size in the presence of equal number of fibers and capillaries compared with the LCR line. By reducing the muscle fiber cross-sectional area without change in the number of capillaries, the selective process resulted in a larger number of capillaries per unit of muscle area. The greater capillary density indicates a larger area of interface between capillaries and muscle fibers in the HCR line, which support the greater tissue O₂ diffusive conductance of this line (25).

Despite the greater \( D_{To2} \), \( O_{2ER} \) was not significantly greater in HCR than in LCR. The reason for this apparent discrepancy is that \( O_{2ER} \) is determined by the interaction between tissue diffusive and perfusive conductances expressed by the ratio \( D_{To2}/(\beta Q) \) (37) where \( \beta \) is the slope of the blood Hb-O₂ dissociation curve and \( Q \) is the blood flow. This relationship illustrates that, whereas an increase in convective O₂ delivery will tend to elevate \( V_{O2\text{max}} \) by making more O₂ available to exercising muscles, it tends, on the other hand, to limit \( V_{O2\text{max}} \) by reducing the proportion of delivered O₂ that can be extracted by the muscles. This explains why, as pointed out previously (40, 42, 43), changes in the rate of convective O₂ delivery are not translated into proportionate changes in \( V_{O2\text{max}} \).

Comparison between generations 7 and 15. The higher \( V_{O2\text{max}} \) of HCR in the rats of generation 7 was solely the result of a greater O₂ extraction ratio by the tissues mediated by a larger tissue O₂ diffusive conductance (20). This was accompanied by higher capillary density and increased activity of oxidative enzymes of skeletal muscle (25), pointing to a structural and metabolic basis for the enhanced capacity for transport and utilization of O₂ by skeletal muscle in this line. At that point in the artificial selection process, no differences between lines were observed in the ability of the cardiopulmonary system to deliver O₂ to the tissues during maximal exercise. The present data show that \( V_{O2\text{max}} \) continues to diverge as the selection process continues. A comparison of key O₂ transport and hemodynamic variables between both lines is shown in Fig. 4. As \( D_{To2} \) in HCR increased along generations, this improvement was accompanied by increased rate of blood O₂ convection. This supports the idea that a continuing enhancement of the capacity of skeletal muscle to transport and utilize O₂ requires improvement in the rate of delivery of O₂ to the tissues.

The greater tissue diffusive O₂ conductance, supported by an augmented capacity for blood O₂ convection observed in HCR reveals a strategy for increasing exercise capacity similar to that observed in some selected populations characterized by high aerobic capacity. Human elite athletes, for instance, as well as animal athletes such as race horses, are able to generate high Q values during maximal exercise (7, 27). Interestingly, in both cases exercise-induced hypoxemia may develop as a result, in part, of pulmonary O₂ diffusion limitation (8). Greater skeletal muscle capillarity, accompanied by higher metabolic enzyme capacity, allows the maintenance of tissue O₂ extraction, which, in the case of the horse, is remarkably high (7).

Figure 4 illustrates another important observation, namely that the divergence in \( V_{O2\text{max}} \) along generations of artificial selection is the combined result of improvement in HCR as well as deterioration in LCR: whereas \( V_{O2\text{max}} \) increased in HCR by ∼8%, it decreased in LCR by about 20% from generation 7 to 15. The increasing difference in \( V_{O2\text{max}} \) between lines was the result of diverging changes in the same O₂ transport variables. The declining rate of O₂ supply to the tissues in LCR was ultimately due to a smaller SV in generation 15 compared with generation 7. The lower SV\(_{max}\) was accompanied by a greater systemic vascular resistance, pre-
senting a mirror image to that seen in the evolution of the changes in cardiovascular function of HCR. Tissue O2 diffusing capacity and O2ER of LCR remained relatively unchanged from generation 7 to 15, implying that the major changes during the selection process occurred in the systems involved in the delivery of O2 to the exercising muscles. This deterioration in cardiovascular function during maximal exercise in LCR is accompanied by evidence of increased risk of cardiovascular disease, such as higher daytime and nighttime arterial blood pressure, increased insulin resistance, and deterioration of mitochondrial function (44).

The divergence between lines in the capacity to deliver O2 to the tissues appears to be the result of opposite changes in the same physiological processes. This suggests that artificial selection is causing segregation of contrasting alleles for major physiological complexes. On the other hand, whereas DTO2 continues to improve in HCR, it does not deteriorate along generations in LCR. The different behavior in processes involved in convective O2 delivery and tissue O2 diffusion may reflect different underlying genetic determinants.

In summary, artificial selection of rats for exercise endurance results in lines with diverging capacities for systemic O2 transport. Because both HCR and LCR practically remain sedentary throughout their lifetimes, differences between lines reflect differences in intrinsic exercise capacity that are at least partially determined by genetic factors. Early in selection (generation 7), the greater VO2 max of HCR is the result of a greater capacity to extract O2 by the exercising muscles.
is mediated by greater capillary density and enhanced oxidative capacity. As artificial selection continues, \( V_{\text{O2 max}} \) continues to diverge as a result of improvement in HCR and deterioration in LCR. The main determinant of this divergence is the opposite divergence as a result of improvement in HCR and deterioration in LCR.

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**REFERENCES**


