Continued divergence in \( V\dot{O}_2 \text{max} \) of rats artificially selected for running endurance is mediated by greater convective blood O2 delivery

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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 \( V\dot{O}_2 \text{max} \) of rats artificially selected for running endurance is mediated by greater convective blood O2 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 O2 uptake (\( V\dot{O}_2 \text{max} \)) was 12% greater in high-capacity (HCR) than in low-capacity runners (LCR). This difference was due exclusively to a greater O2 uptake and utilization by skeletal muscle of HCR, without differences between lines in convective O2 delivery to muscle by the cardiopulmonary system (\( Q\dot{O}_2 \text{max} \)). The present study in generation 15 (G15) female rats tested the hypothesis that continuing improvement in skeletal muscle O2 transfer must be accompanied by augmentation in \( Q\dot{O}_2 \text{max} \) to support \( V\dot{O}_2 \text{max} \) of HCR. Systemic O2 transport was studied during maximal normoxic and hypoxic exercise (inspired PO2 = 70 Torr). \( V\dot{O}_2 \text{max} \) divergence between lines increased because of both improvement in HCR and deterioration in LCR; normoxic \( V\dot{O}_2 \text{max} \) was 50% higher in HCR than LCR. The greater \( V\dot{O}_2 \text{max} \) in HCR was accompanied by a 41% increase in \( Q\dot{O}_2 \text{max} \); 96.1 ± 4.0 in HCR vs. 68.1 ± 2.5 ml \( \text{STPD} \) O2 \( \text{min}^{-1} \cdot \text{kg}^{-1} \) in LCR \( (P < 0.01) \) during normoxia. The greater G15 \( Q\dot{O}_2 \text{max} \) of HCR was due to a 48% greater stroke volume than LCR. Although tissue O2 diffusive conductance continued to increase in HCR, tissue O2 extraction was not significantly different from LCR at G15, because of the offsetting effect of greater HCR blood flow on tissue O2 extraction. These results indicate that continuing divergence in \( Q\dot{O}_2 \text{max} \) between lines occurs largely as a consequence of changes in the capacity to deliver O2 to the exercising muscle.

intrinsic exercise capacity; genetic determinants; tissue O2 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 O2 uptake (\( V\dot{O}_2 \text{max} \)) of a group of generation 7 females was 12% higher in HCR than in LCR (20). The higher \( V\dot{O}_2 \text{max} \) of HCR was entirely due to a greater ability to transport O2 from the atmosphere to the tissue capillaries in HCR (25). The greater tissue O2 extraction and utilization in HCR was the result of increased tissue O2 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 O2 delivery from the tissue capillaries in HCR (20).

Because the higher \( V\dot{O}_2 \text{max} \) of HCR of generation 7 was exclusively due to a greater capacity of skeletal muscle to extract and utilize O2, the question arises whether it would be possible to maintain a continually expanding capacity for O2 uptake and utilization at the skeletal muscle level without an accompanying increase in the ability to deliver O2 to the muscle. The studies presented here were designed to test the hypothesis that an increased capacity for tissue O2 transfer would have to be accompanied by a parallel augmentation in the capacity to transport O2 to the tissue capillaries to maintain \( V\dot{O}_2 \text{max} \). To test this hypothesis, systemic O2 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 O2 diffusive conductance between HCR and LCR is accompanied by significant differences between lines in the capacity to deliver O2 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 in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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 (VO2) and CO2 production (VCO2) 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 VO2max was reached. VO2max was defined as the VO2 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 VO2 and VCO2 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. Inflowing and outflowing O2 concentrations and outflowing CO2 concentration were measured continuously and simultaneously via a Perkin-Elmer model 1100 mass spectrometer (Pomona, CA). VO2 and VCO2 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·m⁻¹·min⁻¹) was calculated from the maximal speed, treadmill angle, and body weight.

Arterial and mixed venous blood samples were analyzed for pH, PO2, and PCO2 in an Instrumentation Laboratory Synthesis 45 blood-gas analyzer (Lexington, MA) with use of appropriate electrodes at 37°C. Hb concentration and O2 saturation of Hb 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, PO2, 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 PO2 from all samples were used to estimate standard hemoglobin P50. Cardiac output (Q; in ml·min⁻¹·kg⁻¹) was calculated as the ratio VO2/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 milliliters per kilogram body wt and milliliters per gram heart weight.

The rate of convective blood O2 delivery, QO2 (ml·min⁻¹·kg⁻¹), was calculated as the product of O2 times C(a-v)O2. The O2 extraction ratio, O2ER, was calculated as C(a-v)O2/CaO2. Mean tissue capillary PO2 (Tor) and the corresponding value for tissue O2 conductance (DTO2, 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 VO2max, 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 VO2max 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. $\dot{V}O_2_{\text{max}}$ 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 $V\dot{O}_2_{\text{max}}$ and maximal 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 $V\dot{O}_2_{\text{max}}$ in HCR was accompanied by a larger rate of convective blood $O_2$ delivery to the tissues: $\dot{Q}O_2_{\text{max}}$, the product of maximal ($Q_{\text{max}}$) and arterial blood $O_2$ content, was 41% higher in HCR than in LCR in normoxia and 25% higher in hypoxia (Table 1). There were no differences in arterial blood $O_2$ contents between groups in either hypoxia or normoxia (Table 1); accordingly, the higher $Q\dot{O}_2_{\text{max}}$ of HCR was entirely the result of greater $Q_{\text{max}}$ (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 ($SV_{\text{max}}$) 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 as well as in hypoxic exercise (Table 2). There were no significant differences in mean arterial blood pressure between groups in either normoxic or hypoxic exercise; however, hypoxia resulted in similar reduction of blood pressure with respect to normoxia in both lines (Table 2). The ratio $\text{MABP}/Q_{\text{max}}$, an index of systemic vascular resistance, was significantly lower in HCR than LCR only during normoxia. There was no significant effect of hypoxia on $\text{MABP}/Q_{\text{max}}$ 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 $O_2$ at the tissue level. The expected decreases in arterial, mixed venous, and mean capillary blood $P_O_2$ were observed during hypoxia; there were no significant differences between lines in any of these values. Tissue $O_2$ diffusing capacity was significantly higher in HCR than in LCR. Hypoxic exercise was associated with an increase in $\text{DT}_{O_2}$ in both groups; however, the increases were similar, such that $\text{DT}_{O_2}$ was ~50% higher in HCR than LCR in both hypoxia and normoxia. Although there was a tendency for a larger $O_2ER$ 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 $O_2ER$ 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 $V\dot{O}_2_{\text{max}}$ of HCR is due to both a larger rate of convective blood $O_2$ delivery to the tissues and a greater tissue $O_2$ diffusing capacity. However, although tissue $O_2$ diffusive conductance is greater in HCR, its effect on the tissue $O_2$ extraction ratio $V\dot{O}_2_{\text{max}}/Q\dot{O}_2_{\text{max}}$ is offset by the larger tissue $O_2$ perfusive conductance such that $O_2$ extraction by the tissues is similar in both lines. This combination of greater rate of $O_2$ delivery to the tissues in HCR and similar tissue $O_2$ extraction in both lines supports a larger $V\dot{O}_2_{\text{max}}$ in HCR.

**Table 1. $V\dot{O}_2_{\text{max}}$ and convective $O_2$ delivery variables during maximal exercise**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HCR (n = 10)</th>
<th>LCR (n = 10)</th>
<th>HCR (n = 9)</th>
<th>LCR (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal work rate, kg·m⁻¹·min⁻¹</td>
<td>6.94±0.25†</td>
<td>3.60±0.35</td>
<td>4.78±0.15†</td>
<td>2.91±0.34</td>
</tr>
<tr>
<td>$V\dot{O}<em>2</em>{\text{max}}$, ml⁻¹·kg⁻¹</td>
<td>69.2±2.00*</td>
<td>46.3±1.1*</td>
<td>50.3±1.1†</td>
<td>38.6±2.1</td>
</tr>
<tr>
<td>$Q\dot{O}<em>2</em>{\text{max}}$, ml⁻¹·kg⁻¹</td>
<td>96.1±4.00*</td>
<td>68.1±2.5*</td>
<td>64.2±2.4†</td>
<td>55.0±3.7</td>
</tr>
<tr>
<td>$C_{A_2O_2}$, ml/dl</td>
<td>19.9±0.5*</td>
<td>19.9±0.4*</td>
<td>14.1±0.8</td>
<td>15.2±0.7</td>
</tr>
<tr>
<td>$C_{V_2O_2}$, ml/dl</td>
<td>5.4±0.7*</td>
<td>6.3±0.4*</td>
<td>3.0±0.3</td>
<td>4.5±0.6</td>
</tr>
<tr>
<td>Blood [Hb], g/dl</td>
<td>15.3±0.4</td>
<td>15.1±0.3</td>
<td>15.1±0.5</td>
<td>14.9±0.6</td>
</tr>
</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. $V\dot{O}_2_{\text{max}}$, maximal rate of $O_2$ consumption; $Q\dot{O}_2_{\text{max}}$, maximal rate of convective $O_2$ delivery = cardiac output × arterial blood $O_2$ content; $C_{A_2O_2}$, arterial blood $O_2$ content; $C_{V_2O_2}$, mixed venous (pulmonary arterial) blood $O_2$ content; blood [Hb], hemoglobin concentration in blood. *P < 0.05 normoxic exercise vs. hypoxic exercise; †P < 0.05 HCR vs. LCR.
Table 2. Hemodynamic variables during maximal exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>HCR</th>
<th>LCR</th>
<th>HCR</th>
<th>LCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q(_{\text{max}}), ml (\text{min}^{-1}\text{kg}^{-1})</td>
<td>487±24(\dagger)</td>
<td>343±15</td>
<td>465±24</td>
<td>363±20</td>
</tr>
<tr>
<td>HR(_{\text{max}}), beats/min</td>
<td>520±9</td>
<td>529±12</td>
<td>519±8</td>
<td>526±5</td>
</tr>
<tr>
<td>SV(_{\text{max}}), ml/kg BW</td>
<td>0.93±0.04(\dagger)</td>
<td>0.63±0.02(\ast)</td>
<td>0.91±0.06(\dagger)</td>
<td>0.69±0.04</td>
</tr>
<tr>
<td>SV(_{\text{max}}), ml/g HW</td>
<td>0.260±0.01(\dagger)</td>
<td>0.209±0.009</td>
<td>0.258±0.008(\dagger)</td>
<td>0.220±0.007</td>
</tr>
<tr>
<td>MABP, mmHg</td>
<td>123±3(\dagger)</td>
<td>117±2(\ast)</td>
<td>110±2</td>
<td>104±4</td>
</tr>
<tr>
<td>MABP/Q(_{\text{max}}), mmHg (\text{min}^{-1}\text{kg}^{-1})</td>
<td>258±13(\dagger)</td>
<td>357±18(\ast)</td>
<td>245±17</td>
<td>297±26</td>
</tr>
<tr>
<td>HW, mg</td>
<td>704±15(\dagger)</td>
<td>787±18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>364±5(\dagger)</td>
<td>300±4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(Q_{\text{max}}\), maximal cardiac output; HR\(_{\text{max}}\), maximal heart rate; SV\(_{\text{max}}\), maximal stroke volume; MABP, mean systemic arterial blood pressure; HW, heart weight; HW/BW, heart-to-body weight ratio. \(\ast P < 0.05\) normoxic exercise vs. hypoxic exercise; \(\dagger P < 0.05\) 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 O\(_2\). The experimental model used here allowed us to obtain an accurate measure of the variables involved in the transport of O\(_2\) from the atmosphere to the tissue capillaries and therefore to determine which of the linked conductances that compose the O\(_2\) 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 O\(_2\) limitation and to establish the O\(_2\) dependence of pulmonary and tissue diffusive O\(_2\) conductances.

Vascular catheter implantation is necessary to measure systemic O\(_2\) 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). Values of Q\(_{\text{max}}\), maximal HR, and SV\(_{\text{max}}\) obtained in HCR in the present studies, as well as in experiments using the 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 \(\dot{V}O_2\) \(_{\text{max}}\) (ml \(\text{min}^{-1}\text{kg}^{-1}\)) of 70.0±2.5 (\(n=5\)); when the normoxic run was the second of the day, \(\dot{V}O_2\) \(_{\text{max}}\) was 68.4±1.8 (\(n=5\); NS). For LCR, the values of normoxic \(\dot{V}O_2\) \(_{\text{max}}\) were 45.8±2.0 (\(n=5\)) and 49.7±2.2 (\(n=5\)) ml \(\text{min}^{-1}\text{kg}^{-1}\) 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 O\(_2\) 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 \(\dot{V}O_2\) \(_{\text{max}}\) values obtained in this study are not normalized for body weight, the differences between lines in absolute values of \(\dot{V}O_2\) \(_{\text{max}}\) and derived variables 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 O\(_2\) would be consumed by a similar mass of contracting muscle in both lines because during maximal exercise most of the O\(_2\) is consumed by the contracting muscles (32). This would mean that the difference between lines in the O\(_2\) 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 3. Tissue O\(_2\) transport variables during maximal exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>HCR</th>
<th>LCR</th>
<th>HCR</th>
<th>LCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>(P_{\text{aO}_2}), Torr</td>
<td>124.0±1.1(\ast)</td>
<td>121.5±2.1(\ast)</td>
<td>52.2±1.1</td>
<td>51.0±1.7</td>
</tr>
<tr>
<td>(P_{\text{vO}_2}), Torr</td>
<td>30.4±2.0(\ast)</td>
<td>31.3±1.1(\ast)</td>
<td>19.2±1.0</td>
<td>22.6±0.8</td>
</tr>
<tr>
<td>(P_{\text{capO}_2}), Torr</td>
<td>53.2±2.4(\ast)</td>
<td>52.7±1.4(\ast)</td>
<td>34.4±1.0</td>
<td>35.6±0.8</td>
</tr>
<tr>
<td>(D_{\text{toO}_2}), ml (\text{min}^{-1}\text{Torr}^{-1}\text{kg}^{-1})</td>
<td>1.37±0.07(\dagger)</td>
<td>0.88±0.03(\ast)</td>
<td>1.58±0.07(\dagger)</td>
<td>1.06±0.03</td>
</tr>
<tr>
<td>(O_{2\text{ER}})</td>
<td>0.732±0.033</td>
<td>0.686±0.019</td>
<td>0.787±0.014</td>
<td>0.709±0.030</td>
</tr>
<tr>
<td>Standard (P_{\text{sO}_2}), Torr</td>
<td>36.8±0.3</td>
<td>36.2±0.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(P_{\text{aO}_2}\), partial pressure of O\(_2\) in arterial blood; \(P_{\text{vO}_2}\), partial pressure of O\(_2\) in mixed venous (pulmonary arterial) blood; \(P_{\text{capO}_2}\), mean partial pressure of O\(_2\) in the systemic capillaries; \(D_{\text{toO}_2}\), tissue diffusing capacity; \(O_{2\text{ER}}\), tissue O\(_2\) extraction ratio = \(C_{(a-v)}\) \(O_2\)/\(CaO_2\). \(\ast P < 0.05\) normoxic exercise vs. hypoxic exercise; \(\dagger P < 0.05\) HCR vs. LCR.

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were also observed in HCR, without differences in the muscle-to-body weight ratio (25). The smaller muscle weight of the HCR rats of generation 7 was due to a smaller fiber size without a difference in the number of fibers between lines (25). Thus the weight difference between lines is due, at least in part, to a smaller muscle mass in HCR. This is not unusual: smaller mass and reduced fiber size are seen in muscles with high aerobic capacity such as the flight muscles of the hummingbird (33). Mice artificially selected for spontaneous wheel running capacity show locomotory muscles ("mighty mini-muscles") that are characterized by smaller size and high mass-specific mitochondrial and glycolytic enzymatic activity (11, 24). It has been postulated that the loss of capacity for power development secondary to the reduced muscle mass may be compensated by the increased capacity for oxidative ATP generation (24) and may reduce the energy costs of locomotion (24, 41). The observation that skeletal muscle oxidative enzyme concentration of the HCR rats of generation 7 was greater than in LCR is consistent with these findings (25).

A second reason that suggests that the weight difference is not the result of different proportion of body fat in the presence of similar muscle mass in both lines is that heart size was markedly lower in LCR. If similar absolute values of $V_O2_{max}$ per rat were due to similar absolute muscle mass in both lines, it would be expected that the work rate would be similar as well. Work rate in this case is determined by speed and body weight; the heavier LCR rats would achieve a similar work rate at the expense of a lower speed compared with HCR. However, Table 1 shows that maximal work rate of LCR is slightly over one half that of HCR. Figure 1 shows that the lower work rate of LCR is accompanied by a proportionate reduction in $V_O2_{max}$ compared with HCR. Taken together, these observations suggest that it is unlikely that the different body weights are the result of different proportions of body fat and similar muscle mass in the two lines. Accordingly, normalization of $O_2$ transport variables for body weight is a legitimate approach to compare the $O_2$ transport system characteristics of the two lines.

**Determinants of $V_O2_{max}$ in HCR and LCR of generation 15.** Conceptually, $V_O2_{max}$ can be viewed as the result of the interaction between two major processes: the rate of $O_2$ delivery to the tissues by blood on one hand, and the extraction of $O_2$ from blood by the tissues on the other. The rate of convective blood $O_2$ delivery to the tissue capillaries is determined by pulmonary and cardiovascular function, as well as by blood $O_2$-carrying capacity. The effects of artificial selection on pulmonary function will be described in a separate paper. The extraction of $O_2$ by the tissues, on the other hand, is determined by the balance between tissue $O_2$ diffusive and perfusive conductances.

The higher $V_O2_{max}$ of HCR is the result of a greater rate of blood $O_2$ convection (Table 1). $O_2$ extraction, on the other hand, was not different between lines (Table 3). The physiological determinants of blood $O_2$ convection and tissue $O_2$ extraction are discussed below.

**Convective blood $O_2$ delivery.** The greater $V_O2_{max}$ 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 $O_2$ 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.

It is possible that the response of the peripheral circulation to maximal exercise may also contribute to the larger SV of HCR. In the present study, systemic vascular resistance during normoxic exercise was significantly lower in HCR (Table 2). This could reflect a larger peripheral vasodilatory response during maximal exercise in this line. The observation that endothelial-dependent vasodilation of isolated carotid arteries is higher in HCR supports this possibility (30, 44). A lower systemic resistance would tend to attenuate possible elevations in afterload as $Q$ increases and would contribute to a larger ventricular ejection in maximal exercise. Future research is necessary to determine the relative contribution of structural vs. functional characteristics in determining the differences in cardiovascular performance between HCR and LCR, as well as the mechanisms underlying these differences.

**Tissue $O_2$ extraction.** In the present study whole body $O_2$ consumption was measured; however, during maximal exercise in quadrupeds, most of the $O_2$ is consumed in skeletal muscle (32). Accordingly, $O_2_{ER}$ under these conditions largely reflects the extraction of $O_2$ by skeletal muscle. Although the
rate of $O_2$ delivery to the exercising skeletal muscles was significantly higher in HCR than in LCR, the amount of $O_2$ extracted by the tissues in proportion to the $O_2$ supplied was similar in both lines. This occurred despite the fact that one of the determinants of tissue $O_2$ extraction, tissue $O_2$ diffusive conductance, was substantially higher in HCR. Figure 3 shows a plot of $V_{O_2\text{max}}$ as a function of mean capillary $P_{O_2}$. During maximal exercise, mitochondrial $P_{O_2}$ is essentially zero (12, 38); accordingly, the mean capillary $P_{O_2}$ is an adequate representation of the gradient for $O_2$ diffusion between capillary and mitochondrion. Thus the slope of the line relating $V_{O_2\text{max}}$ and mean capillary $P_{O_2}$ represents the average tissue $O_2$ diffusing capacity (i.e., $V_{O_2\text{max}}/P_{O_2}$ gradient = $D_{T_O2}$), a composite parameter determined by all the processes involved in the flow of $O_2$ from the capillary to the mitochondrion. The average $D_{T_O2}$ value over the entire $P_{O2}$ 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 $O_2$ 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 $O_2$ diffusive conductance of this line (25).

Despite the greater $D_{T_O2}$, $O_2ER$ 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 $D_{T_O2}/(\beta Q)$ (37) where $\beta$ is the slope of the blood Hb-$O_2$ dissociation curve and $Q$ is the blood flow. This relationship illustrates that, whereas an increase in convective $O_2$ delivery will tend to elevate $V_{O_2\text{max}}$ by making more $O_2$ available to exercising muscles, it tends, on the other hand, to limit $V_{O_2\text{max}}$ by reducing the proportion of delivered $O_2$ that can be extracted by the muscles. This explains why, as pointed out previously (40, 42, 43), changes in the rate of convective $O_2$ delivery are not translated into proportionate changes in $V_{O_2\text{max}}$. In the present case, although $D_{T_O2}$ was 50% higher in HCR than LCR, $Q_{\text{max}}$ was ~40% higher in HCR during normoxic exercise. In the presence of similar blood Hb concentration and Hb-$O_2$ affinity in both lines, i.e., similar values of $\beta$, the high $Q$ of HCR attenuated the effect of the elevated $D_{T_O2}$ on tissue $O_2$ extraction, such that $O_2ER$ of HCR was only 7% greater than in LCR. This self-limiting effect of $Q_{\text{max}}$ changes on $V_{O_2\text{max}}$ has been demonstrated experimentally after increases in blood flow (10), $C_aO_2$ (19, 34), and a combination of both (10, 13).

**Comparison between generations 7 and 15.** The higher $V_{O_2\text{max}}$ of HCR in the rats of generation 7 was solely the result of a greater $O_2$ extraction ratio by the tissues mediated by a larger tissue $O_2$ 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 $O_2$ 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 $O_2$ to the tissues during maximal exercise. The present data show that $V_{O_2\text{max}}$ continues to diverge as the selection process continues. A comparison of key $O_2$ transport and hemodynamic variables between both lines is shown in Fig. 4. As $D_{T_O2}$ in HCR increased along generations, this improvement was accompanied by increased rate of blood $O_2$ convection. This supports the idea that a continuing enhancement of the capacity of skeletal muscle to transport and utilize $O_2$ requires improvement in the rate of delivery of $O_2$ to the tissues.

The greater tissue diffusive $O_2$ conductance, supported by an augmented capacity for blood $O_2$ 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 $O_2$ diffusion limitation (8). Greater skeletal muscle capillarity, accompanied by higher metabolic enzyme capacity, allows the maintenance of tissue $O_2$ extraction, which, in the case of the horse, is remarkably high (7).

Figure 4 illustrates another important observation, namely that the divergence in $V_{O_2\text{max}}$ along generations of artificial selection is the combined result of improvement in HCR as well as deterioration in LCR: whereas $V_{O_2\text{max}}$ increased in HCR by ~8%, it decreased in LCR by about 20% from generation 7 to 15. The increasing difference in $V_{O_2\text{max}}$ between lines was the result of diverging changes in the same $O_2$ transport variables. The declining rate of $O_2$ supply to the tissues in LCR was ultimately due to a smaller SV in generation 15 compared with generation 7. The lower $SV_{\text{max}}$ was accompanied by a greater systemic vascular resistance, pre-

![Graph showing VO2 max as a function of mean capillary PO2](http://jap.physiology.org/)
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. This
is mediated by greater capillary density and enhanced oxidative capacity. As artificial selection continues, VO_{2max} continues to diverge as a result of improvement in HCR and deterioration in LCR. The main determinant of this divergence is the opposite diverge as a result of improvement in HCR and deterioration in LCR. The main determinant of this divergence is the opposite 

direct direction of the changes in the capacity to deliver O_2 to the exercising muscles. The continuing improvement in tissue O_2 diffusion capacity of HCR is accompanied by enhancement of the rate of tissue O_2 delivery; on the other hand, the decline in the capacity to deliver O_2 to the exercising muscles in LCR is reflected in a reduced VO_{2max} as selection continued. The contrasting evolution of the O_2 transport system in both lines points to an effect of artificial selection on the genetic determinants of cardiovascular performance.

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