Genetic Models in Applied Physiology

Selected Contribution: Skeletal muscle capillarity and enzyme activity in rats selectively bred for running endurance

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Howlett, Richard A., Norberto C. Gonzalez, Harrieth E. Wagner, Zhenxing Fu, Steven L. Britton, Lauren G. Koch, and Peter D. Wagner. Selected Contribution: Skeletal muscle capillarity and enzyme activity in rats selectively bred for running endurance. J Appl Physiol 94: 1682–1688, 2003.—To attempt to explain the difference in intrinsic (untrained) endurance running capacity in rats selectively bred over seven generations for either low (LCR) or high running capacity (HCR), the relationship among skeletal muscle capillarity, fiber composition, enzyme activity, and O2 transport was studied. Ten females from each group [body wt: 228 g (HCR), 247 g (LCR); P = 0.03] were studied at 25 wk of age. Peak normoxic maximum O2 consumption and muscle O2 conductance were previously reported to be 12 and 33% higher, respectively, in HCR, despite similar ventilation, arterial O2 saturation, and a cardiac output that was <10% greater in HCR compared with LCR. Total capillary and fiber number in the medial gastrocnemius were similar in HCR and LCR, but, because fiber area was 37% lower in HCR, the number of capillaries per unit area (or mass) of muscle was higher in HCR by 32% (P < 0.001). A positive correlation (r = 0.92) was seen between capillary density and muscle O2 conductance. Skeletal muscle enzymes citrate synthase and β-hydroxyacyl-CoA dehydrogenase were both ~40% higher (P < 0.001) in HCR (12.4 ± 0.7 vs. 8.7 ± 0.4 and 3.4 ± 0.2 vs. 2.4 ± 0.2 mmol·kg−1·min−1, respectively), whereas phosphofructokinase was significantly (P = 0.02) lower in HCR (27.8 ± 1.2 vs. 35.2 ± 2.5 mmol·kg−1·min−1) and hexokinase was the same (0.65 ± 0.04 vs. 0.65 ± 0.03 mmol·kg−1·min−1). Resting muscle ATP, phosphocreatine, and glycogen contents were not different between groups. Taken together, these data suggest that, in rats selectively bred for high-endurance exercise capacity, most of the adaptations for improved O2 utilization occur peripherally in the skeletal muscles and not in differences at the level of the heart or lung.

oxidative capacity; vascularization; genetic variation; mitochondria; aerobic exercise; genetic models

THE ABILITY OF AN ORGANISM to perform endurance exercise is determined by the interplay of a multitude of physiological factors, such as maximal sustainable power output, lactate threshold, efficiency, and skeletal muscle oxidative capacity, among many others, and the relative contribution of each to improved endurance performance after training can be variable (10, 24, 41). One factor that has been studied extensively and has been shown to correlate with endurance performance is maximal O2 consumption (VO2 max) capacity. Although many studies have attempted to determine the one limiting factor determining VO2 max (for review, see Ref. 47), it is clear that the mechanisms determining VO2 max are many and of varied importance (48). Whereas some of these factors can be altered via extrinsic processes, such as training or ergonomic aids (8), others are determined genetically in any given individual. The genetic component of endurance performance in humans has long been recognized, and studies that have attempted to quantify the contribution have estimated its heritability to be as high as 70–90% (7, 28). Therefore, it is assumed that there is a large genetic component to variation in O2 utilization and metabolic capacity and implied that these determinant phenotypes of endurance performance can be altered by artificial selection.

In 1996, divergent artificial selection was initiated in the heterogeneous N:NIH rat stock to produce selected lines of low- (LCR) and high-capacity runners (HCR) (29). Selection was based on maximal distance run on a motorized treadmill with the use of a velocity-ramped protocol. This approach has resulted in diverging endurance capacity with each successive generation of offspring, and, at generation 6, the LCR and HCR differed by 171% in treadmill running distance. Systemic O2 transport during maximal exercise was studied in these rats from both lines (17) before the subsequent measurements on the same animals were completed for the present study. That previous study reported that the HCR rats demonstrated a greater normoxic VO2 max relative to the LCR. However, this increase was achieved despite ventilation and arterial saturation that were not different between LCR and HCR and a cardiac output during normoxic testing...
<10% greater in HCR. Interestingly, that previous study demonstrated that total skeletal muscle \( \text{O}_2 \) conductance was \( \geqslant 30\% \) higher in HCR compared with LCR. These findings suggest that the greater \( \text{VO}_{2\text{max}} \) seen in HCR is achieved more by peripheral (skeletal muscle) than central (cardiopulmonary) adaptations.

Based on the higher efficacy of \( \text{O}_2 \) transfer at the tissue level exhibited in the HCR rats, it was hypothesized that these rats would possess both structural and biochemical adaptations that could explain greater \( \text{O}_2 \) conductance and utilization. Specifically, this study investigated whether the previously reported functional peripheral adaptations in HCR rats were caused by changes in muscle fiber type, increases in skeletal muscle capillarity, and/or increases in cellular oxidative enzyme activities. Because the same animals were used in both investigations, it was possible to match each individual animal’s measured functional variables to morphometric indexes and muscle metabolic capacity.

### METHODS

**Animal subjects.** Adult female rats (\( n = 20 \)) selectively bred for high (\( n = 10 \), HCR) or low (\( n = 10 \), LCR) endurance treadmill running capacity, as outlined (29), were used for this study. The LCR were 25.5 ± 0.7 wk old, and the HCR were 25.0 ± 0.7 wk old at terminal study. Mean body weight was significantly (\( P = 0.03 \)) lower in HCR than in LCR (229 vs. 247 g).

**Preexperimental testing.** Subjects were tested for endurance running capacity by using the ramp test to exhaustion that was previously described (17, 29). Rats began running on a 15° incline at 10 m/min and continued with increases in velocity of 1 m/min every 2 min until they could no longer maintain the required speed. HCR rats ran 1,590 ± 77 m (62.8 ± 2.0 min), whereas LCR rats ran 222 ± 17 m (16.2 ± 0.96 min) at exhaustion.

**Animal preparation.** Subjects were prepared for cardiopulmonary measurements reported in a previous study, as described previously (17). Briefly, after anesthesia, catheters were introduced into the aortic arch and pulmonary artery, exteriorized, and sealed. Animals were then recovered for subsequent exercise testing on later days.

**Exercise testing.** The exercise testing that was utilized for the cardiopulmonary measurements reported by Henderson et al. (17) consisted of ramp tests to \( \text{VO}_{2\text{max}} \) completed in both normoxic (inspired \( \text{PO}_2 \sim 145 \text{Torr} \)) and hypoxic (inspired \( \text{PO}_2 \sim 70 \text{Torr} \)) conditions separated by ~3 h. The treadmill protocol consisted of runs beginning at 10 m/min on a 10° incline and continuing with increases in speed of 4 m/min every 90–120 s until \( \text{VO}_{2\text{max}} \) was reached. Testing was done in an airtight Lucite chamber to allow for measurement of gas exchange, and blood sampling was done via the implanted catheters.

**Determination of \( \text{O}_2 \) diffusive conductance.** Skeletal muscle \( \text{O}_2 \) conductance was estimated as previously described (1, 45).

**Tissue preparation.** Within 10 min of completing both exercise bouts, each rat was anesthetized with pentobarbital sodium (60 mg/kg iv), and both left and right gastrocnemius muscles were removed intact. Each muscle was divided into lateral and medial portions and weighed individually. An entire transverse slice from the widest point of the middle belly portion of either the left or right medial gastrocnemius muscle was excised and frozen in precooled isopentane (–140°C) and stored at –80°C until further processing. Transverse 8-μm serial sections were cut on a cryomate (Cryostat) at −26°C and mounted on slides for histochemical analysis of capillary number and fiber type.

The contralateral gastrocnemius was prepared for enzyme and metabolite measurements. To attempt to ensure a representative mix of gastrocnemius muscle fiber types, the entire muscle was ground to a fine powder under liquid nitrogen. Subsequent aliquots of wet muscle were removed for enzyme measurements. Samples weighing ~100 mg were removed and freeze-dried for measurement of muscle metabolites.

**Capillary staining.** A combined protocol of alkaline phosphatase (AP) and dipeptidylpeptidase (DPP) reactions was used to stain the capillaries (15, 31). The sections were presoaked in a precooled (~20°C) 1:1 mixture of acetic acid and chloroform at room temperature for 5 min and then allowed to air dry. The slides were transferred to an incubation mixture containing 0.08% gly-pro 4-methoxy-β-napthylamid e and 0.054% fast blue in 0.1 M phosphate buffer at pH 7.2. Sections were incubated for 1 h at 37°C. Slides were briefly rinsed in phosphate buffer before transfer to a mixture containing 0.04% naphthol ASMX phosphate and 0.21% variance blue in 0.1 M Tris buffer, pH 9.2. Sections were incubated for 2 h at 37°C. The treatment with AP stains the arterial ends of the capillary segments blue, whereas the DPP stains the venous ends of the capillary segments red (36). The sections were air-dried overnight before mounting with Permount.

**Fiber typing by myosin-ATPase reaction.** A modified procedure of Ogilvie and Feeback (37) was used to delineate the muscle fiber types. Sections were preincubated for 8 min in a medium containing 0.49% potassium acetate and 0.26% calcium chloride at pH 4.4 and then briefly rinsed in 0.1 M Tris buffer at pH 7.8. Sections were then incubated at room temperature for 30 min in a medium containing 0.4% glycine, 0.42% calcium chloride, 0.38% sodium chloride, 0.19% sodium hydroxide, and 0.15% ATP at pH 9.4. Slides were rinsed in 1% calcium chloride and stained in 0.1% toluidine blue for 1 min, rinsed in distilled H\(_2\)O, dehydrated in ethanol, cleared in Hemo-De, and mounted with Permount.

**Morphometry.** The stained sections were viewed under a light microscope at magnification ×25. The entire muscle cross section was digitally imaged (each rectangular image being 1.15 mm × 0.86 mm), and MATLAB 5.3 was used to perform morphometric measurements on the whole cross section, image by image. Total capillary number was determined from images of the AP-DPP slides prepared as above, and total number of fibers were counted from the myosin-ATPase-stained sections. The myosin-ATPase slides were also used to determine the percentage of type I fibers. Finally, the total cross-sectional area of the muscle was measured from the entire set of digital images. From these data, we calculated mean capillary-to-fiber ratio (C/F), mean capillary density, mean fiber area, and percentage of type I fibers over the entire cross section.

**Skeletal muscle enzyme activities.** All enzyme measurements were performed at 20°C on a Beckman model 64 spectrophotometer. To assay citrate synthase (CS) and β-hydroxyacyl-CoA dehydrogenase (β-HAD) activities, whole muscle homogenates were prepared by using 6–10 mg of pulverized wet tissue. Samples were homogenized in 100 volumes (wt/vol) of buffer (175 mM KCl, 2 mM EDTA; pH 7.4) with a Polytron mixer for 35–45 s and frozen in liquid N\(_2\) before undergoing three cycles of freezing and thawing in liquid N\(_2\). Before use, the homogenates were thawed a final
Table 1. Skeletal muscle morphometry and capillarity for the medial gastrocnemius cross-section from both HCR and LCR groups of rats

<table>
<thead>
<tr>
<th></th>
<th>HCR</th>
<th>LCR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total muscle area, mm²</td>
<td>20.4 ± 1.2</td>
<td>27.9 ± 1.9</td>
<td>0.01</td>
</tr>
<tr>
<td>Total fiber no.</td>
<td>4,681 ± 210</td>
<td>5,235 ± 598</td>
<td>NS</td>
</tr>
<tr>
<td>Mean fiber area, μm²</td>
<td>4,356 ± 112</td>
<td>5,428 ± 354</td>
<td>0.02</td>
</tr>
<tr>
<td>Type I fibers, %</td>
<td>12.9 ± 0.6</td>
<td>11.4 ± 1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Total capillary no.</td>
<td>8,239 ± 314</td>
<td>8,552 ± 393</td>
<td>NS</td>
</tr>
<tr>
<td>Mean capillary density, mm⁻²</td>
<td>408 ± 15</td>
<td>309 ± 14</td>
<td>0.009</td>
</tr>
<tr>
<td>Capillary/fiber ratio</td>
<td>1.78 ± 0.06</td>
<td>1.68 ± 0.12</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. HCR, high-capacity runners; LCR, low-capacity runners; NS, not significant.

Table 2. Whole skeletal muscle enzyme activities from the mixed gastrocnemius for both HCR and LCR groups of rats

<table>
<thead>
<tr>
<th></th>
<th>HCR</th>
<th>LCR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrate synthase</td>
<td>12.4 ± 0.7</td>
<td>8.7 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>β-HAD</td>
<td>3.4 ± 0.2</td>
<td>2.4 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Phosphofructokinase</td>
<td>27.8 ± 1.2</td>
<td>35.2 ± 2.5</td>
<td>0.009</td>
</tr>
<tr>
<td>Hexokinase</td>
<td>0.65 ± 0.04</td>
<td>0.65 ± 0.03</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. All enzyme activities are expressed as mmol·kg wet weight⁻¹·min⁻¹. β-HAD, β-hydroxyacyl-CoA dehydrogenase.

Table 3. Whole skeletal muscle metabolite contents at rest from the mixed gastrocnemius for both HCR and LCR groups of rats

<table>
<thead>
<tr>
<th></th>
<th>HCR</th>
<th>LCR</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>31.5 ± 0.8</td>
<td>31.2 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>PCr</td>
<td>100.0 ± 3.8</td>
<td>101.2 ± 2.2</td>
<td>NS</td>
</tr>
<tr>
<td>Glycogen</td>
<td>162.6 ± 12.2</td>
<td>151.9 ± 11.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. All metabolites are expressed as mmol/kg dry wt. PCr, phosphocreatine.

RESULTS

Physical characteristics. Both groups of rats were age matched, but the LCR rats had a significantly greater (P = 0.03) mean body weight than the HCR at the time of testing (247 vs. 229 g). In the maximal exercise test, the HCR rats achieved a significantly higher work rate than the LCR, as previously reported by Henderson et al. (17).

Muscle morphometry. The morphometric characteristics of the gastrocnemius muscle are shown in Table 1. Total fiber number for the medial gastrocnemius cross section was not significantly different between HCR and LCR. However, because the mean cross-sectional area of each fiber was significantly greater (by 25%) in the LCR compared with HCR, the total muscle area was significantly greater in LCR. The percentage of total fibers that were type I was not different between the two groups.

Capillarity. Table 1 also shows the capillary measurements for the medial gastrocnemius cross section for both HCR and LCR. Total capillary number, determined by counting all of the capillaries in the medial gastrocnemius cross section, was similar between the two groups. Therefore, the mean C/F was not different between LCR and HCR. However, because the HCR group showed a significantly lower muscle area, the mean capillary density was significantly higher (by 32%) in HCR compared with LCR.

Muscle enzyme activities. The whole mixed gastrocnemius muscle enzyme activities for various oxidative and glycolytic enzymes are shown in Table 2. The whole muscle activities of oxidative enzymes CS and β-HAD were significantly higher in HCR than LCR. For glycolytic enzyme activities, PFK was significantly higher in LCR, whereas HK was not significantly different between the two groups.

Muscle metabolites. There were no differences in the resting skeletal muscle contents of ATP, phosphocreatine, or glycogen between the HCR and LCR groups (Table 3).

Oxygen transport. Figure 1 shows the relationship between mean capillary density (total capillary number/total muscle cross-sectional area) and functional O₂ conductance reported previously (17) for both groups of rats. There was a highly significant positive correlation (r = 0.92) between the two measures. There was no relationship between C/F and O₂ conductance (r = 0.30).

DISCUSSION

The results of this study demonstrate that rats selectively bred (but not trained) for high-endurance running capacity (HCR) differ from those bred for low capacity for running endurance (LCR) in their ability to utilize O₂, manifest primarily at the level of skeletal muscle. As shown previously by Henderson et al. (17) in these same rats, neither ventilation, arterial O₂ saturation, nor hemoglobin concentration was significantly different between these groups. Whereas HCR rats had a slightly (~10%) higher peak cardiac output, total systemic O₂ delivery was not statistically different between groups. What was different was a higher
O₂ extraction facilitated by a >30% higher total muscle O₂ conductance. The present study has added further evidence of the peripheral muscle adaptations via muscle morphometry and metabolic enzyme activities. HCR rats had lower gastrocnemius muscle mass, but the same total number of both capillaries and fibers. Capillary-to-fiber ratios were, therefore, similar, but capillary density was considerably greater in HCR. Functional muscle O₂ conductance correlated closely (r = 0.92) with overall muscle capillary density. There was augmentation of metabolic enzyme activity evident in the oxidative (CS and β-HAD) but not glycolytic components (PFK and HK) to support the higher capacity for O₂ utilization in HCR.

Comparison with other specially selected groups. Previous studies on unique populations have shown varied adaptation strategies to increase O₂ utilization. Elite human athletes have been shown to have peak ventilation and diffusing capacity of the lung that are not substantially different from nonathletic subjects, and arterial O₂ saturation can be lower than in sedentary subjects (12), whereas fractional extraction is often only marginally higher. Therefore, cardiac output (and thus muscle blood flow) remains as the major difference between the elite athlete and more sedentary subjects (5). Of course, to make use of much higher muscle blood flow (and thus higher muscle O₂ delivery), it is evident that both muscle O₂ conductance (44) and metabolic enzyme capacity (11, 23, 39) must be increased, and this is indeed found.

In horses, selectively bred for normoxic exercise performance for thousands of years, lung function lags that of the rest of the O₂ transfer system dramatically, resulting in hypercapnia, severe O₂ desaturation, and extreme pulmonary hypertension (9). Cardiovascular function is, on the other hand, remarkable, with peak cardiac output values of 0.8 l·min⁻¹·kg⁻¹ seen commonly (corresponding values in the average human athlete are just 0.3 l·min⁻¹·kg⁻¹). Despite high blood flow, muscle O₂ extraction is very high (27), due to extremely high muscle capillarity coupled to high metabolic capacity (25).

Humans resident at altitude for many generations show yet a different response to a relative need for O₂. Of course, here the stimulus is not increased demand but reduced availability, but the responses are primarily in the lungs (increased lung volumes and diffusing capacity, reduced ventilation at a given exercise level) and in the blood [increased hemoglobin concentration (22)]. There is also little increase in peak O₂ consumption (V̇O₂) when ambient hypoxia is abolished acutely (in contrast to sea-level residents when inspired O₂ fraction is altered), suggesting a downregulation of metabolic scope matched to O₂ availability (22).

In the present study, genetic selection on intrinsic aerobic capacity alone without endurance training showed a quite different outcome: mostly adaptive differences at the level of skeletal muscle. Overall, essentially none of the common central factors, such as increased cardiac output, ventilation, or heart rate, which can augment O₂ transport in response to greater O₂ need in these settings, was seen as differences between these same LCR and HCR rats in a previous study (17). This suggests that long-term training acts to stimulate adaptation for O₂ transfer in some fundamentally different ways than from genetic selection alone when endurance performance is the selected outcome. In particular, cardiopulmonary adaptation for V̇O₂max appears to be a major response to long-term endurance training but is seen to a much lesser degree with just selection on intrinsic capacity alone, although this conclusion does come from interpreting differences in structure and function between humans and rats. In addition, it must be taken into consideration that, although the cardiopulmonary measures are made directly during exercise, the estimation of muscle O₂ diffusive conductance is interpolated from blood values that represent the sum of the working muscles and that the morphometric and enzymatic measurements of peripheral adaptation were performed in only one representative locomotory muscle.

Muscle capillarity and muscle O₂ conductance. August Krogh developed the idea that diffusion of O₂ was the mechanism responsible for transport of O₂ from the muscle microcirculation to the mitochondria. His model (30) suggested that distance from the capillary was paramount, but, since then, many studies have produced data that strongly suggest that the size of the capillary-to-fiber interface rather than diffusion distance is the structural factor that mostly determines O₂ conductance (13, 20, 34).

Intuitively, the present results might appear at variance with this earlier body of work, because these data show a close relationship between capillary density and O₂ conductance (Fig. 1), but no such relationship with the C/F (Table 1). However, there are at least two potential strategies to increase the effective size of the capillary-to-fiber surface and hence increase O₂ flux potential. First, an increase in the C/F should increase
capillary-to-fiber surface, if the average fiber cross-sectional area remains unchanged (19). C/F was shown to increase with endurance training in older humans, concurrent with no change in fiber area, resulting in an increase in the size of the capillary-to-fiber interface (21). Conversely, at a given C/F, a decrease in mean fiber area should increase the capillary-to-fiber surface contact. This has been shown to be the case in very aerobic muscle (hummingbird flight muscle) that actually has an unremarkable C/F but very high capillary-to-fiber surface due to very small fiber areas (34). The present data are consistent with the latter case in that C/F is not different between groups, but capillary density is greater due to a decrease in fiber size.

Consistent with their increases in V\textsubscript{O\textsubscript{2}}\textsubscript{max} and O\textsubscript{2} conductance are increases in oxidative enzyme activities (see below). Therefore, the relative increase in capillary density was supported by an increase in the ability to utilize delivered O\textsubscript{2}. It has been shown that increased capillary-to-fiber surface area is well correlated with effective mitochondrial density, both in highly aerobic muscle (34) and with training (40). Conversely, several previous studies that have demonstrated a decrease in fiber area with hypoxic exposure (26) or detraining (20) have also resulted in a decrease in oxidative capacity, resulting in a matching between capillarity and mitochondrial density. This is also seen in high-altitude natives, who demonstrate decreased capillarity concurrent with decreased oxidative capacity (26). However, high-altitude natives and elite high-altitude climbers, while having a decreased fiber area, demonstrate a high V\textsubscript{O\textsubscript{2}}\textsubscript{max}-to-mitochondrial density ratio, suggesting that conductance is higher in these populations (26).

Similarly, when high-altitude natives are exercise trained, they undergo large increases in capillary density, C/F, and muscle oxidative capacity without changes in fiber area, resulting in increases in V\textsubscript{O\textsubscript{2}}\textsubscript{max} (26). Taken together, the increased capillary density, decreased fiber size, and increased oxidative enzymes in the HCR group of the present study represent a suite of adaptations that interact to increase O\textsubscript{2} conductance. It may be postulated that, had metabolic capacity not been higher in the HCR rats, any lower muscle mass per se would have eventually been matched by a corresponding loss of capillaries, such that capillary density would have equalled that in the LCR group. That postulate is consistent with the long-term effects of limb immobilization, where capillary rarefaction follows fiber atrophy (20).

The results of the present study thus support the hypothesis that capillary supply is regulated to match the capacity for O\textsubscript{2} utilization (19). This would seem to make inherent sense, because intracellular O\textsubscript{2} levels, reflecting the balance between O\textsubscript{2} supply and demand, could provide regulatory signals for capillary growth via the hypoxia inducible factor-VEGF axis (38).

Enzyme activities. The large differences in oxidative enzyme (represented by CS and \beta-HAD) activities between the HCR and LCR in the present study are consistent with numerous studies of endurance-trained athletes. Many cross-sectional studies have demonstrated that trained endurance athletes possess skeletal muscle oxidative enzyme activities that are much greater than their sedentary counterparts (4, 23, 32). Similarly, longitudinal studies on both human and animal subjects have demonstrated that endurance training results in increases in oxidative enzyme activities over time (23, 24, 39). These increases in oxidative capacity are due to an increase in skeletal muscle mitochondrial content via mitochondrial biogenesis (14) and are consistent with the previously shown relationship between capillarity and mitochondrial content (24, 34, 40). However, the question of whether increased oxidative capacity is absolutely a prerequisite for improved endurance performance or merely a result still remains.

It is often suggested that cardiac output or bulk O\textsubscript{2} transport alone will limit V\textsubscript{O\textsubscript{2}}\textsubscript{max} and that skeletal muscle oxidative enzyme activities are generally far in excess of what is required to support a given V\textsubscript{O\textsubscript{2}}\textsubscript{max}. In athletes, increasing O\textsubscript{2} delivery through raised inspired O\textsubscript{2} fraction or superfusion of the muscle will result in greater utilization of O\textsubscript{2}, suggesting that they are “supply” limited and that their oxidative capacity is in excess of normal O\textsubscript{2} delivery capacity (42). However, sedentary subjects can often show no improvement in V\textsubscript{O\textsubscript{2}}\textsubscript{max} with increasing O\textsubscript{2} delivery, suggesting that they are “demand” limited or that normal O\textsubscript{2} provision is already in excess of oxidative capacity.

Robinson et al. (43) suggested that increased mitochondrial content is necessary for increased skeletal muscle V\textsubscript{O\textsubscript{2}}\textsubscript{max} and McAllister and Terjung (35) demonstrated that rat skeletal muscle peak V\textsubscript{O\textsubscript{2}}\textsubscript{max} is reduced with a reduction in electron transport capacity. Similarly, Blomstrand et al. (6) showed that skeletal muscle V\textsubscript{O\textsubscript{2}}\textsubscript{max} was correlated with the maximal activity of 2-oxoglutarate dehydrogenase, one of the lowest activity mitochondrial oxidative enzymes, during intense leg extensor exercise. These studies suggest that muscle enzyme activities can be limiting to V\textsubscript{O\textsubscript{2}}\textsubscript{max} in some cases.

Finally, it has been shown that increases in performance after training can be greater than increases in absolute V\textsubscript{O\textsubscript{2}}\textsubscript{max} (24, 41), suggesting that increases in oxidative capacity and/or capillarity may contribute more to a greater submaximal sustained work rate than to whole body O\textsubscript{2} utilization.

Fiber types. One adaptation often seen in both humans and animals in response to endurance training is a shift in fiber type toward a greater percentage of slow-twitch oxidative (type I) fibers. One consequence of this shift is a decrease in mean fiber area. In the present study, there was no difference in the percentage of the medial gastrocnemius classified as type I fibers between HCR and LCR. It is interesting that there is a large difference in mean fiber cross-sectional area (i.e., lower by ~20% in HCR compared with LCR) without any shift in fiber type. It has been suggested that the fiber-type distribution is fairly homogeneous within a species and that, although remarkable plasticity is possible, the changes are dependent on the imposed stressor(s), such as endurance training.
Summary. Rats bred for high intrinsic endurance capacity demonstrated peripheral skeletal muscle adaptations consistent with their improved $O_2$ conductance compared with their low-endurance counterparts. Muscle capillary density and oxidative enzyme activities were significantly higher in HCR rats compared with LCR rats. These changes are only in part similar to adaptations to stressors of $O_2$ transport, such as endurance training and hypoxia. Thus the changes consequent to selective breeding for intrinsic endurance capacity may be different from those in response to environmental influences.

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