Hemodynamics and O\textsubscript{2} uptake during maximal knee extensor exercise in untrained and trained human quadriceps muscle: effects of hyperoxia

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Mourtzakis, M., J. González-Alonso, T. E. Graham, and B. Saltin. Hemodynamics and O\textsubscript{2} uptake during maximal knee extensor exercise in untrained and trained human quadriceps muscle: effects of hyperoxia. J Appl Physiol 97: 1796–1802, 2004. First published June 18, 2004; doi:10.1152/japplphysiol.00169.2004.—To elucidate the potential limitations on maximal human quadriceps O\textsubscript{2} capacity, six subjects trained (T) one quadriceps on the single-legged knee extensor ergometer (1 h/day at 70% maximum workload for 5 days/wk), while their contralateral quadriceps remained untrained (UT). Following 5 wk of training, subjects underwent incremental knee extensor tests under normoxic (inspired O\textsubscript{2} fraction = 21%) and hyperoxic (inspired O\textsubscript{2} fraction = 60%) conditions with the T and UT quadriceps. Training increased quadriceps muscle mass (2.9 ± 0.2 to 3.1 ± 0.2 kg), but did not change fiber-type composition or capillary density. The T quadriceps performed at a greater peak power output than UT, under both normoxic (101 ± 10 vs. 80 ± 7 W; P < 0.05) and hyperoxic (97 ± 11 vs. 81 ± 7 W; P < 0.05) without further increases with hyperoxia. Similarly, thigh peak O\textsubscript{2} consumption, blood flow, vascular conductance, and O\textsubscript{2} delivery were greater in the T vs. the UT thigh (1.4 ± 0.2 vs. 1.1 ± 0.1 l/min, 8.4 ± 0.8 vs. 7.2 ± 0.8 l/min, 42 ± 6 vs. 35 ± 4 ml·min\textsuperscript{-1}·mmHg\textsuperscript{-1}, 1.71 ± 0.18 vs. 1.51 ± 0.15 l/min, respectively) but were not enhanced with hyperoxia. Oxygen extraction was elevated in the T vs. the UT thigh, whereas arteriovenous O\textsubscript{2} difference tended to be higher (78 ± 2 vs. 72 ± 4%, P < 0.05; 160 ± 8 vs. 154 ± 11 ml/L, respectively; P = 0.098) but again were unaltered with hyperoxia. In conclusion, the present results demonstrate that the increase in quadriceps muscle O\textsubscript{2} uptake with training is largely associated with increases in blood flow and O\textsubscript{2} delivery, with smaller contribution from increases in O\textsubscript{2} extraction. Furthermore, the elevation in peak muscle blood flow and vascular conductance with endurance training seems to be related to an enhanced vasodilatory capacity of the vasculature perfusing the quadriceps muscle that is unaltered by moderate hyperoxia.

O\textsubscript{2} delivery; muscle blood flow; muscle vascular conductance

IT IS WELL DOCUMENTED THAT exercise training increases maximal aerobic capacity (VO\textsubscript{2 max}) in humans by improving the capacity of the systemic circulation to deliver O\textsubscript{2}, as well as enhancing the ability of skeletal muscle to utilize O\textsubscript{2} (6, 11, 13, 21, 26, 29). The controversial issue, however, is whether VO\textsubscript{2 max} of trained and untrained muscle is determined by a fixed or variable relationship in the systemic supply of O\textsubscript{2}, diffusive O\textsubscript{2} transport from the circulation into myocytes, or oxidative capacity of mitochondria. During dynamic exercise using a large muscle mass in trained human subjects, there is evidence that supports a predominant O\textsubscript{2} supply limitation (8, 9, 29, 32, 33). By reducing the fraction of O\textsubscript{2} inspired (F\textsubscript{I O\textsubscript{2}}) or challenging cardiac output during maximal exercise with or without heat stress, several studies have demonstrated that a decrease in O\textsubscript{2} supply can reduce whole body and exercising muscle O\textsubscript{2} uptake (VO\textsubscript{2}) through impaired O\textsubscript{2} delivery (8, 9, 14, 21, 24, 25). Thus these studies suggest that O\textsubscript{2} availability is crucial to VO\textsubscript{2 max} in trained subjects (8, 9, 14, 21, 24, 25). In untrained human subjects, however, it is suggested that VO\textsubscript{2 max} is not limited by O\textsubscript{2} supply; rather, it is limited by the capacity of mitochondria to utilize O\textsubscript{2} for ATP production (20, 24, 25). This implies that there is a potential transition from a limitation in O\textsubscript{2} utilization in mitochondria to convective O\textsubscript{2} supply with training (15, 20–22, 32, 33).

A different approach to directly investigate the factors limiting maximal O\textsubscript{2} capacity in trained and untrained muscle in humans is to use a functionally isolated muscle group, such as the quadriceps muscle during knee extensor exercise. Central circulatory limitations do not play a major role in this model, because cardiac output does not reach maximal capacity and arterial blood pressure does not decline (23, 27, 28). By training one quadriceps while the contralateral quadriceps remains untrained, whereby dominant and nondominant thighs are randomly assigned to the training regimen, it is possible to directly compare the effects of training on O\textsubscript{2} delivery as well as the effects of enriched arterial oxygenation. Two previous studies used the knee extensor model to examine the effects of hyperoxia on trained muscle and observed an increased peak power output and peak VO\textsubscript{2} (VO\textsubscript{2 peak}) with enhanced O\textsubscript{2} supply, resulting from elevated blood flow and arterial O\textsubscript{2} content (Ca\textsubscript{O\textsubscript{2}}) (21, 22). However, no study to date has compared the effects of hyperoxia on untrained and trained muscle in the same individual.

Therefore, the aim of this study was to test the following hypotheses in trained and untrained quadriceps muscle from healthy subjects performing maximal knee extensor exercise: 1) training would result in elevated knee extensor peak work capacity and VO\textsubscript{2} primarily driven by enhanced O\textsubscript{2} delivery, and 2) within an individual, the trained quadriceps would demonstrate enhanced VO\textsubscript{2 peak} and work capacity compared with a lack of improvement in the untrained quadriceps during hyperoxia.

METHODS

Eight healthy, recreationally active male subjects were recruited to participate in this study, but only six were able to complete the training and experimental protocols. The mean ± SE age, body mass, and height of the six subjects tested was 23 ± 1 yr, 84.7 ± 8.9 kg, and 185 ± 4 cm, respectively. Subjects were informed, verbally and in writing, of the nature of the study and were informed of the potential risks and benefits of the project. All subjects had signed informed consent forms approved by the University of Guelph Human Ethics Review Board (September 6, 2002). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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writing, of the purpose of the study as well as the procedures and risks involved with the experiment. The experimental protocol was approved by the Copenhagen and Frederiksberg Ethics Committee in Denmark.

**Experimental protocol.** Selection of the quadriceps to be trained was randomized to avoid potential effects of dominance. The maximum workload for both quadriceps muscles was predetermined before training sessions. Subjects underwent supervised training sessions using the one-legged knee extensor model, which allows the exercise to be confined to the quadriceps muscle (1). The training protocol consisted of 1-h training at ~70% of the predetermined maximum work rate for approximately five sessions per week. After a training period of 5 wk, the subjects underwent the testing protocol. The testing procedure involved an incremental maximal test in which the work rate was increased every 2 min by a predetermined amount, based on the untrained maximal test performed before training.

For 2 days before the testing day, subjects were provided with a standard mixed diet that matched their individual daily caloric intake. Subjects arrived on the day of the experiment following an overnight fast. Catheters were inserted, under local anesthesia, in the femoral artery of one leg and the femoral vein of both legs. The femoral artery was cannulated at ~2 cm below the inguinal ligament and set proximally ~10 cm above which was connected to a blood pressure transducer and thermistor. The femoral venous catheter was placed ~4 cm below the inguinal ligament and forwarded ~10 cm in the distal direction. A thermistor was placed in the femoral veins of each leg to measure blood flow. After the insertion of the catheters, subjects rested for 30 min before resting blood samples were drawn.

Our aim was to compare the untrained (UT) with the trained (T) quadriceps at the same absolute work rates (i.e., compare the UT absolute peak work rate in the UT and T quadriceps), in addition to comparing the respective peak work rates in both quadriceps. To accurately compare these cardiovascular responses, it was necessary to carry out an incremental maximal test on the UT quadriceps, first to repeat the same work rates with the T quadriceps and subsequently to continue the incremental test until maximal work rate in the T quadriceps was attained. Blood samples and blood flow measurements were then taken at the same time points for each thigh. Whereas this creates a potential order effect, this protocol enabled us to compare the T quadriceps at a work rate that is equivalent to the peak work rate of the UT quadriceps.

Maximal work rates were attained by incrementally increasing the workload during the knee extensor exercise. Once the exercise was completed in the UT quadriceps, there was a 15-min rest period before testing the T quadriceps. During the maximal dynamic knee extensor exercise tests, subjects were encouraged to increase their work rates to achieve the greatest work rate possible for each quadriceps. Femoral venous blood flow measurements were made by using the thermodilution technique (1, 10), which is largely representative of the quadriceps blood flow during the knee extensor exercise. To avoid contamination of blood flow from the lower leg, an occlusion cuff was placed just below the knee and was inflated to >240 mmHg for 30 s before infusing cold saline through the thermistor. Thigh blood flows were measured at every 40 and 90 s following a change in work rate and were then calculated by using the heat balance equation. Blood samples were taken 60 s following the change of work rate. These procedures were carried out first under normoxic conditions (FiO₂ = 21%) and subsequently under hypoxic conditions (FiO₂ = 60%). This order of testing conditions was maintained to avoid any contamination of the potential hyperoxic effects onto the normoxic conditions. Following the maximal work of the T quadriceps, there was a 15-min rest period before the inhalation of the 60% oxygen mixture. Subjects continuously inhaled this mixture for 10 min before starting to exercise the UT quadriceps. As such, each quadriceps rested ~40 min before carrying out a subsequent bout of exercise. Previous work has demonstrated that repeated, intense exercise does not result in altered ATP turnover and quadriceps VO₂ (3). Moreover, alternative designs, such as testing on different days, would have been ethically inappropriate regarding catheterization and unsuitable due to potential day-to-day changes in training status that may occur in the quadriceps.

**Blood analyses.** Heparinized syringes were used to collect blood samples for measuring blood pH, PCO₂, and PO₂ (ABL5, Radiometer, Denmark), Hb, oxyhemoglobin fraction (HbO₂; OSM3 hemoximeter, Radiometer, Denmark), and hematocrit. Hematocrit was measured in triplicate following microcentrifugation. Whole blood lactate was also collected in heparinized syringes and measured (EML105, Radiometer, Denmark).

**Measurements of the muscle mass.** MRI were obtained from the patella to the anterior inferior iliac spine. Twenty-eight scans were taken whereby each scan was 3 mm thick with a distance of 17.1 mm between each scan. To calculate the muscle volume of the quadriceps, the area of each scan was multiplied by the pixel area and was then multiplied by the distance between sections. These scans were then summed to attain the total volume and were multiplied by 1.04 kg/l, which is assumed to be the density of muscle tissue. These muscle mass data were used to calculate VO₂ peak relative to the quadriceps muscle mass for each thigh. Capillary analysis and fiber typing were also carried out on each thigh by using the methods outlined by Qu et al. (19).

**Calculations.** Oxygen content (ml/dl) was calculated as ([1.39 × [Hb] × HbO₂] + (0.003 × PO₂)), where [Hb] is the total hemoglobin concentration (g/dl) and HbO₂ is the fraction of HbO₂ in blood. HbO₂ was calculated as [O₂ sat × (100 – COHb – MetHb)]/100, where O₂ sat is the O₂ saturation and COHb and MetHb are the fractions of carboxyhemoglobin and methemoglobin, respectively. Arteriovenous (a-v) difference was calculated by subtracting the venous values from the arterial values. This difference was then divided by arterial O₂ concentration to give O₂ extraction. Oxygen delivery was calculated by multiplying blood flow and CaO₂, and thigh vascular conductance was calculated by dividing blood flow by mean arterial pressure (MAP). To obtain an index of O₂ diffusion across the thigh, O₂ conductance was calculated by dividing VO₂ peak by venous PO₂. To calculate VO₂, the (a-v)O₂ difference was multiplied by blood flow; similarly, lactate flux was calculated by multiplying lactate a-v difference with blood flow. Power output developed by the quadriceps muscle during knee extensor exercise (the knee flexor muscles are inactive because a weight placed on the flywheel brings the leg backwards) was approximately multiplied by the external workload on the ergometer by the cadence recorded continuously in the MacLab data-acquisition system (1, 10).

Due to technical challenges, we were unable to attain blood-gas measurements under hyperoxia when the T thigh was exercising at the UT peak work rate. Because work rates were the same, we assumed that VO₂ for the T thigh at the UT peak work rate also remained unchanged. During hyperoxia, we can also assume that arterial oxygen saturation is 100% so that we could estimate (a-v)O₂ difference in the T thigh when exercising at the UT peak work rate.

**Statistics.** Values are expressed as means ± SE. Statistical differences between the UT and T quadriceps at peak and at the same work rates were analyzed by using one-way repeated-measures ANOVA for the O₂ parameters as well as the lactate data. Rest and peak measurements between the T and UT quadriceps were analyzed by using two-way repeated-measures ANOVA for the analysis of the catecholamine data. Statistical significance was accepted at P < 0.05, and Tukey’s post hoc test was used for further analysis.

**RESULTS**

Training effects on quadriceps muscle mass, fiber-type distribution, and capillary density. Following 5 wk of endurance training with the one-legged knee extensor model, muscle mass of the T quadriceps was elevated by ~0.2 kg (~7%) compared with the UT quadriceps (3.09 ± 0.21 vs. 2.89 ± 0.21 kg; P < 0.05). In UT vs. T quadriceps, there were no statistical differ-
the UT quadriceps during normoxia (99 H11006 5,631
Peak work rate was UT quadriceps.

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fibers (56
ences in the percent area and cross-sectional area of type I
fibers (56 ± 4 vs. 61 ± 5% and 5,567 ± 403 vs. 6,092 ± 510

mm², respectively) or type II fibers (43 ± 4 vs. 39 ± 5% and
5,631 ± 323 vs. 5,976 ± 360 nm², respectively). Furthermore,
capillary density was not different between the UT and T
quadriceps (UT = 460 ± 33 vs. T = 468 ± 28 capillaries/

mm²) and no training effects on the number of capillaries
around a fiber (UT = 2.7 ± 0.2 vs. T = 3.0 ± 0.2).

Work capacity and cardiovascular responses of the T and
UT quadriceps. Peak work rate was ~24% greater in the T vs.
the UT quadriceps during normoxia (99 ± 10 vs. 80 ± 7 W,
P < 0.05) and hyperoxia (97 ± 11 vs. 81 ± 7 W; P < 0.05;
Tables 1 and 2). In parallel to the increase in peak power,

\( \dot{V}O_2 \) peak was elevated ~23% in the T thigh compared with the
UT thigh (e.g., normoxia 1.4 ± 0.2 vs. 1.1 ± 0.1 l/min, P <
0.05; Fig. 1). \( \dot{V}O_2 \) peak relative to the quadriceps muscle mass in
the T thigh was greater than that in the UT thigh by ~17% (UT
= 0.38 ± 0.04 vs. T = 0.43 ± 0.04 l·min⁻¹·kg⁻¹ of
quadriceps, P < 0.05). The increase in \( \dot{V}O_2 \) peak in the T thigh is
largely associated with elevated thigh blood flow (~21%) and
O₂ delivery (~17%) because (a–v)O₂ differences did not
increase significantly (UT = 153 ± 11 vs. T = 160 ± 8 ml/l,

Table 1. Oxygen parameters and arterial catecholamines in the untrained and trained thigh when exposed
to normoxia and hyperoxia

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hyperoxia</th>
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<tbody>
<tr>
<td></td>
<td>Untrained thigh peak work rate</td>
<td>Trained thigh peak work rate</td>
</tr>
<tr>
<td>Work rate, W</td>
<td>80±7</td>
<td>81±7</td>
</tr>
<tr>
<td>Hb, g/l arterial</td>
<td>155±4</td>
<td>148±5*</td>
</tr>
<tr>
<td>venous</td>
<td>155±4</td>
<td>146±5*</td>
</tr>
<tr>
<td>( P_O_2 ), Torr</td>
<td>118±4</td>
<td>110±2*</td>
</tr>
<tr>
<td>arterial</td>
<td>22±2</td>
<td>20±1</td>
</tr>
<tr>
<td>venous</td>
<td>26.5±3.6</td>
<td>21.8±1.5</td>
</tr>
<tr>
<td>( O_2 ) saturation, % arterial</td>
<td>96.2±0.4</td>
<td>96.0±0.2</td>
</tr>
<tr>
<td>venous</td>
<td>211±6</td>
<td>201±6*</td>
</tr>
<tr>
<td>( O_2 ) content, ml/l arterial</td>
<td>57±6</td>
<td>45±2</td>
</tr>
<tr>
<td>venous</td>
<td>5.5±1.0</td>
<td>6.7±0.7</td>
</tr>
<tr>
<td>Norepinephrine, nmol/l arterial</td>
<td>2.2±0.6</td>
<td>2.8±0.6</td>
</tr>
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</table>

Values are means ± SE for 6 subjects. Arterial and venous values are from femoral artery and vein. [Hb], Hb concentration. *Significantly different from untrained, P < 0.05. †Significantly higher than normoxia, P < 0.05.

Table 2. Individual data for work rate, blood flow, and (a–v)O₂ difference

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th>Hyperoxia</th>
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<tbody>
<tr>
<td></td>
<td>Untrained thigh peak work rate</td>
<td>Trained thigh peak work rate</td>
</tr>
<tr>
<td>Work rate, W</td>
<td>80±7</td>
<td>81±7</td>
</tr>
<tr>
<td>Subject 1</td>
<td>71</td>
<td>71</td>
</tr>
<tr>
<td>Subject 2</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Subject 3</td>
<td>53</td>
<td>55</td>
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<tr>
<td>Subject 4</td>
<td>96</td>
<td>96</td>
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<tr>
<td>Subject 5</td>
<td>84</td>
<td>91</td>
</tr>
<tr>
<td>Subject 6</td>
<td>96</td>
<td>93</td>
</tr>
<tr>
<td>Blood flow, l/min</td>
<td>7.2±0.8</td>
<td>6.6±0.4</td>
</tr>
<tr>
<td>Subject 1</td>
<td>8.1</td>
<td>7.2</td>
</tr>
<tr>
<td>Subject 2</td>
<td>5.4</td>
<td>5.1</td>
</tr>
<tr>
<td>Subject 3</td>
<td>6.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Subject 4</td>
<td>10.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Subject 5</td>
<td>6.0</td>
<td>7.1</td>
</tr>
<tr>
<td>Subject 6</td>
<td>7.1</td>
<td>7.2</td>
</tr>
<tr>
<td>(a–v)O₂ difference, ml/l</td>
<td>153±11</td>
<td>156±8</td>
</tr>
<tr>
<td>Subject 1</td>
<td>105</td>
<td>127</td>
</tr>
<tr>
<td>Subject 2</td>
<td>171</td>
<td>172</td>
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<td>Subject 3</td>
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<td>149</td>
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<td>Subject 4</td>
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<td>152</td>
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<tr>
<td>Subject 5</td>
<td>168</td>
<td>156</td>
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<tr>
<td>Subject 6</td>
<td>180</td>
<td>180</td>
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</table>

Values are means ± SE for 6 subjects. a–v, Arterial and venous from femoral artery and vein. *Significantly different from untrained (P < 0.05).
P = 0.098; Fig. 1). At the same work rate (UT = 80 ± 7 vs. T = 81 ± 7 W), VO₂ was not different between the UT and T thighs (UT = 1.1 ± 0.1 vs. T = 1.0 ± 0.1 l/min; Fig. 1). In addition, thigh blood flow and O₂ delivery remained similar when the T thigh exercised at the same work rate as the UT thigh under both normoxic and hyperoxic conditions (Fig. 1). When the T thigh increased exercise intensity from ~80 and ~100 W, Cao₂, venous O₂ content, and O₂ extraction were not altered, whereas blood flow and O₂ delivery increased. Hyperoxia resulted in significantly greater arterial PO₂ (Pao₂) and O₂ saturation, which resulted in elevated Cao₂ (Table 1); however, thigh blood flow, VO₂ peak, O₂ delivery, and O₂ extraction in the T vs. UT muscle were not enhanced (Fig. 1).

MAP and heart rate rose from resting levels during normoxia and hyperoxia for both the UT and T thigh (Fig. 1). However, when exercising at peak work rate of the UT thigh under normoxia and hyperoxia (~80 W), the T thigh performed with a lower MAP and heart rate (i.e., normoxia = 182 ± 6 vs. 205 ± 7 mmHg and 153 ± 5 vs. 125 ± 7 beats/min; hyperoxia = 179 ± 7 vs. 198 ± 9 mmHg and 144 ± 3 vs. 120 ± 6 beats/min, respectively; P < 0.05, Fig. 1). Moreover, hyperoxia tended (P = 0.10) to reduce MAP and heart rate.
Thigh vascular conductance at peak exercise during normoxia and hyperoxia tended to be or was elevated in the T compared with the UT thigh (e.g., normoxia, 42 ± 6 vs. 35 ± 4 ml·min⁻¹·mmHg⁻¹, respectively; \( P = 0.068 \); Fig. 1).

*Plasma catecholamines and thigh lactate release.* In the T compared with the UT thigh under normoxic conditions, peak plasma norepinephrine and epinephrine (Table 2) and thigh lactate release (26.6 ± 3.4 vs. 22.1 ± 4.0 mmol/min; \( P = 0.25 \)) tended to be higher. When hyperoxia was compared with normoxia, plasma catecholamines tended to be lower both in the T and UT thigh (Table 1); however, peak thigh lactate release was unaffected (26.3 ± 2.0 and 20.6 ± 2.2 mmol/min for T and UT thigh, respectively).

**DISCUSSION**

The key findings of the present study demonstrated that, following 5 wk of training the knee extensors, the T quadriceps muscle was larger than the UT quadriceps by ~7%; however, its fiber-type composition and capillary density remained unchanged. During both normoxia and hyperoxia, the T quadriceps performed ~24% more work than the UT quadriceps, whereby Vo₂peak paralleled the increase in power output but was not improved with hyperoxia, despite the concomitant increase in CaO₂. During normoxia, increased Vo₂peak in the T quadriceps was primarily linked to elevated blood flow (21%) and O₂ delivery (17%), with a smaller contribution from elevated O₂ extraction and (a-v)O₂ difference (6%). Elevated peak blood flow in the T quadriceps was associated with a paralleled increase in vascular conductance compared with the UT quadriceps. When the T quadriceps exercised at the UT peak power output, heart rate and MAP were reduced, suggesting that training the quadriceps muscle (~3 kg) resulted in central cardiovascular effects. Together, these findings in T and UT human quadriceps muscles reveal that enhanced Vo₂peak in T muscle is predominantly associated with elevated blood flow and O₂ delivery resulting from the local vasodilatory adaptations of the existing microvasculature.

After the 5 wk of endurance training, there was ~23–24% difference in peak power output and Vo₂ between the UT and T quadriceps. Our findings are consistent with previous studies examining the cardiovascular effects of training one leg while the contralateral leg remained untrained in the same individual (13, 27). In general agreement with these studies (4, 13, 24, 27), we observed that the elevation in Vo₂peak for the T quadriceps in the present study was largely associated with elevated blood flow and O₂ delivery and, to a lesser extent, increased O₂ extraction. Unlike training studies that involve all leg muscles (13, 24, 27), the present study isolated the training exercise to the knee extensors. Interestingly, if we assumed that both the T and UT quadriceps muscles were recruited maximally at peak power output, the increase in peak thigh Vo₂, blood flow, and vascular conductance per unit of mass would be rather similar (17, 15, and 16%, respectively). This suggests an important role of enhanced blood flow and vasodilatation for improved Vo₂max in the T quadriceps. In support of this interpretation, we also observed a marginal increase in O₂ extraction and (a-v)O₂ difference in congruence with a minimal elevation in mitochondrial enzyme activities (e.g., 2-oxo-glutarate dehydrogenase and succinate dehydrogenase). Together, these data reveal that improved blood flow and O₂ delivery accounted for most of the increase in Vo₂peak in the T quadriceps.

The elevated peak blood flow and O₂ delivery in the T quadriceps were potentially driven by enhanced vasodilatory capacity of the microvasculature perfusing this muscle. Whereas CaO₂ and blood flow determine O₂ delivery, the training-induced increase in O₂ delivery predominantly resulted from enhanced blood flow, because CaO₂ was essentially unchanged. The similar increases in thigh blood flow and vascular conductance suggest that the T quadriceps had an enhanced ability to vasodilate compared with the UT quadriceps. As the same systemic circulation perfused both muscles, it would be reasonable to suggest that a local phenomenon accounted for the ~1.2 l/min increase in peak blood flow in the T quadriceps. Three possible mechanisms could increase maximal quadriceps blood flow with training: 1) increased capillary number (5), 2) increased capillary recruitment, and 3) increased maximal vasodilatory capacity of the existing microvasculature perfusing the quadriceps muscle (14, 27). Because 5 wk of endurance training did not increase capillary density, enhanced peak quadriceps blood flow may have been derived from increased vasodilatation of existing capillaries and/or an increased capillary recruitment. Reports have demonstrated an enhanced vasodilatory capacity of feed arteries and arterioles from trained skeletal muscle with the infusion of the nitric oxide donor sodium nitroprusside or acetylcholine, which clearly support this notion (12, 16, 30, 35). Thus elevated Vo₂peak was likely driven by thigh blood flow as a result of local vasodilatory training adaptations.

The present study also depicted a significant increase in maximal O₂ extraction in the T quadriceps. However, in both the T and UT quadriceps, Vo₂peak and, subsequently, fatigue were associated with high levels of O₂ in the femoral venous circulation and much lower O₂ extraction values than those observed across the exercising leg during maximal bicycle exercise (i.e., 73–77 vs. 91%) (9, 10, 14). These observations during maximal knee extensor exercise could suggest that there are considerable O₂ diffusion limitations in the exercising quadriceps. Although the latter possibility cannot be ruled out, it is likely that reduced maximal O₂ extraction across the functionally isolated quadriceps compared with the whole exercising leg is, in part, due to contamination from oxygenated blood stemming from the largely inactive knee flexor muscles (31). By assuming equal blood flow distribution between the knee extensor and knee flexor muscles during passive exercise and unchanged O₂ saturation in the hamstring muscles during knee extensor exercise, Bangsbo et al. (2) estimated a ~12% increase in (a-v)O₂ difference and O₂ extraction across the quadriceps that is attributed to oxygenated blood from the inactive knee flexors. Therefore, future studies are needed to measure these differences by maximally exercising both the knee extensor and knee flexor muscles.

Another aim of this study was to test the importance of O₂ delivery on Vo₂peak in T and UT muscle by increasing CaO₂ by having subjects breath a 60% O₂ gas mixture. Although this approach has been previously used during whole body (15, 34) and knee extensor exercise (21, 22), the novel aspect of this study was the use of hyperoxia on T and UT quadriceps within the same human subject. In contrast to previous work, hyperoxia did not increase peak power output or Vo₂peak in T muscle (17, 21, 22, 24, 25) during knee extensor exercise, despite the
fact that the T quadriceps was exposed to a greater PaO₂. Hence, an increased diffusion gradient between the arterial blood and the muscle was achieved in this study with hyperoxia at 60%, without increasing O₂ delivery or altering O₂ unloading and muscle Vo₂. This agrees with previous results using 60% O₂ gas mixture in nontrained muscle during whole body (34) and knee extensor exercise (18), indicating that the increase in blood-to-muscle O₂ gradient alone does not result in enhanced muscle Vo₂. With higher levels of hyperoxia, however, there is evidence of enhanced work capacity and peak Vo₂ during knee extensor exercise (21, 22). Although the subjects in the present study and the previous two studies (21, 22) demonstrated similar T muscle Vo₂_peak subjects in the studies by Richardson et al. (21, 22) inspired 100% O₂ which significantly elevated CaO₂. Moreover, blood flow to the contracting quadriceps was somewhat elevated (~0.2–0.3 l/min), which, together with the enhanced CaO₂, led to a significant rise in O₂ delivery compared with control. In contrast, the present study did not result in elevated blood flow or O₂ delivery, which is in agreement with reports showing an unchanged (7, 15, 21, 22) or decreased limb blood flow (8, 34) and unaltered O₂ delivery. Thus our results indicate that elevated CaO₂ using 60% O₂ gas mixture was insufficient in increasing O₂ delivery and Vo₂_peak.

MAP and heart rate were elevated to a similar extent at peak exercise in both UT and T thighs during normoxia. Training, however, reduced the MAP and heart rate responses at a given absolute work rate (~80 W) without altering local thigh hemodynamic responses. Saltin et al. (27) showed that training of one leg while the contralateral leg remained untrained resulted in an attenuated heart rate at a submaximal workload, and Klausen et al. (13) similarly demonstrated a relationship that predicted reduced heart rate and mean blood pressure at the same Vo₂. This central effect that resulted from training a small muscle group may explain the lack of local thigh hemodynamic responses for the T muscle at this work rate. Because catecholamine responses were unaltered, it is unlikely that the reduced MAP was due to metabolic adaptations from training. Rather, it is possible that neural adaptations resulted, with training, to decrease MAP at the same absolute work rate in the T muscle compared with the UT muscle, which further supports an enhanced vasodilatory capacity in the T muscle.

Although this is the only study to examine the effects of hyperoxia in UT and T muscle within the same individual, careful interpretation of the data is required. The order of the experiments may affect the hyperoxia data because normoxia experiments were conducted before hyperoxia. Although there is no evidence that suggests FlO₂ = 60% would affect subsequent normoxic exercise, we avoided the risk of any contamination with prior inhalation of a hyperoxic mixture. Also, prior exercise has not been shown to affect ATP turnover and Vo₂_peak (2, 3), and there was sufficient rest between testing periods. The exercise bouts consisted of 12-min incremental exercise protocols, where peak work rate was only sustained for 2 min and catecholamines were minimally affected due to the small muscle mass (~3 kg) being tested. Furthermore, there may be some concern regarding the small sample size that was used. Although eight subjects were recruited, seven subjects successfully completed the training protocol, and six were capable of undergoing the test protocol, reflecting the complexity of the study. Small sample sizes (n = 5 and 7) were also utilized in studies investigating hyperoxia in knee extensor exercise that were comparable to the invasiveness of the present study (21, 22), demonstrating the level of complexity of such work. Although these data demonstrate novel implications for training and hyperoxia, future investigations are needed to further examine these effects.

In summary, the quadriceps muscle of healthy men performed at a higher work rate following 5 wk of endurance training that also resulted in elevated Vo₂_peak compared with the UT quadriceps. Hyperoxia did not further elevate peak Vo₂, despite the elevated PaO₂, O₂ saturation, and O₂ content. Enhanced quadriceps Vo₂_peak resulting from training was largely due to the increase in O₂ delivery and blood flow. It appeared that the increase in blood flow with training was a result of a local adaptation in the quadriceps muscle, as seen with the increase in local vascular conductance in the T thigh.

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