The assumption that cellular oxygen pressure (PO2) is close to zero in maximally exercising muscle is essential for the hypothesis that O2 transport between blood and mitochondria has a finite conductance that determines maximum O2 consumption. The unique combination of isolated human quadriceps exercise, direct measures of arterial, femoral venous PO2, and 1H nuclear magnetic resonance spectroscopy to detect myoglobin desaturation enabled this assumption to be tested in six trained men while breathing room air (normoxic, N) and 12% O2 (hypoxic, H). Within 20 s of exercise onset partial myoglobin desaturation was evident even at 50% of maximum O2 consumption, was significantly greater in H than N, and was then constant at an average of 51 +/- 3% (N) and 60 +/- 3% (H) throughout the incremental exercise protocol to maximum work rate. Assuming a myoglobin PO2 where 50% of myoglobin binding sites are bound with O2 of 3.2 mmHg, myoglobin-associated PO2 averaged 3.1 +/- .3 (N) and 2.1 +/- .2 mmHg (H). At maximal exercise, measurements of arterial PO2 (115 +/- 4 [N] and 46 +/- 1 mmHg [H]) and femoral venous PO2 (22 +/- 1.6 [N] and 17 +/- 1.3 mmHg [H]) resulted in calculated mean capillary PO2 values of 38 +/- 2 (N) and 30 +/- 2 mmHg(H). Thus, for the first time, large differences in PO2 [...]

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Myoglobin O₂ Desaturation during Exercise
Evidence of Limited O₂ Transport

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Abstract

The assumption that cellular oxygen pressure (P₀₂) is close to zero in maximally exercising muscle is essential for the hypothesis that O₂ transport between blood and mitochondria has a finite conductance that determines maximum O₂ consumption. The unique combination of isolated human quadriceps exercise, direct measures of arterial, femoral venous P₀₂, and ¹H nuclear magnetic resonance spectroscopy to detect myoglobin desaturation enabled this assumption to be tested in six trained men while breathing room air (normoxic, N) and 12% O₂ (hypoxic, H). Within 20 s of exercise onset partial myoglobin desaturation was evident even at 50% of maximum O₂ consumption, was significantly greater in H than N, and was then constant at an average of 51±3% (N) and 60±3% (H) throughout the incremental exercise protocol to maximum work rate. Assuming a myoglobin P₀₂ where 50% of myoglobin binding sites are bound with O₂ of 3.2 mmHg, myoglobin-associated P₀₂ averaged 3.1±3 (N) and 2.1±2 mmHg (H). At maximal exercise, measurements of arterial P₀₂ (115±4 [N] and 46±1 mmHg [H]) and femoral venous P₀₂ (22±1.6 [N] and 17±1.3 mmHg [H]) resulted in calculated mean capillary P₀₂ values of 38±2 (N) and 30±2 mmHg (H). Thus, for the first time, large differences in P₀₂ between blood and intracellular tissue have been demonstrated in intact normal human muscle and are found over a wide range of exercise intensities. These data are consistent with an O₂ diffusion limitation across the 1–5-μm path-length from red cell to the sarcolemma that plays a role in determining maximal muscle O₂ uptake in normal humans. (J. Clin. Invest. 1995; 96:1916–1926). Key words: quadriceps • blood flow • diffusional conductance • myoglobin • magnetic resonance spectroscopy

Introduction

The complete oxygen cascade from the air to mammalian tissue has been challenging to study in vivo. The final two levels in this cascade, capillary oxygen pressure (P₀₂) and intracellular P₀₂, are probably of most interest and have remained the most elusive. Recently, however, the ability to detect myoglobin desaturation using proton magnetic resonance spectroscopy (MRS) has made it possible to estimate intracellular P₀₂ (1). Proton MRS, unlike most previous techniques that address tissue oxygenation, is noninvasive, is without deleterious effects, and therefore is suitable for in vivo human studies (2). Effluent venous P₀₂ reflects mean end-capillary P₀₂ in an isolated muscle (3), thus if both arterial and venous P₀₂ are measured, a value for mean capillary P₀₂ can be computed, and from this a diffusional conductance (D₀₂) can be determined assuming mitochondrial P₀₂ = 0 and residual venous P₀₂ reflects only diffusion limitation of O₂ transport (4). The functional isolation of the quadriceps muscle group has previously been used to study exercising muscle in humans (5) and allows the measurement of effluent (femoral) venous P₀₂ and the calculation of mean capillary P₀₂ (6).

By combining the isolated quadriceps muscle model (5) and proton MRI technology to detect myoglobin saturation (1), it is possible to study the complete series of oxygen gradients from ambient air to human muscle in vivo during dynamic exercise. The present study uses this unique combination of methodologies to test the hypothesis that during exercise intracellular P₀₂ is much lower than capillary P₀₂, which would confirm the importance of the blood to mitochondrial O₂ conductance pathway in determining maximum oxygen uptake (V₀₂max). Specifically, MRS techniques were used to detect simultaneously proton and phosphorous levels in the quadriceps muscle group during dynamic incremental knee-extensor exercise breathing both 21 and 12% O₂. Proton MRS allowed the determination of myoglobin saturation, and ³¹P data were collected to characterize the muscles metabolic activity during this mode of incremental exercise and to act as an independent measure of metabolic demand. 2 wk before these MRS measurements, using femoral vein and radial artery catheterization, arterial P₀₂, femoral venous P₀₂, and muscle blood flow were directly measured in the same subjects during identical work profiles to relate intravascular P₀₂ to intramuscular values.

Methods

Subjects. All six healthy, nonsmoking male subjects were competitive athletes who regularly bicycled 200–400 miles per wk. Health histories

1. Abbreviations used in this paper: CaO₂, arterial oxygen concentration; CvO₂, venous oxygen concentration; D₀₂, oxygen diffusional conductance; FID, free-induction decay; H, hypoxic; Hb, hemoglobin; MRS, magnetic resonance spectroscopy; Mb, myoglobin; N, normoxic; Pₐₒₒ, oxygen pressure where 50% of myoglobin binding sites are bound with oxygen; Pₐₐₐₐₒₒ, capillary P₀₂; Pₐₐₐₒₒₐₒₒ, mitochondrial P₀₂; Pₐₐₐₐₒₒ, myoglobin P₀₂; Pₒₒ, oxygen pressure; PCr, creatine phosphate; Pi, inorganic phosphate; Q, blood flow; Vₒₒ, oxygen uptake; Vₒₒₒₒₐₓₐₐ, maximum Vₒₒ; WR, work rate; WRₐₓₐₐₐₐₐₐ, maximum WR.

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and a physical examination were completed on each subject. All subjects were without history of cardiopulmonary disease and upon physical examination were within normal limits. As this research was performed in two laboratories, informed consent was obtained according to both the University of California San Diego and the University of Pennsylvania Institutional Review Board for Human Subjects Research Committees.

**Overview of experimental protocol.** This research studied one group of subjects in two locations: subjects were initially studied in San Diego, CA, and were then transported to Philadelphia, PA and studied again. In San Diego, to facilitate the learning of the unfamiliar knee-extensor exercise, all subjects performed five to six training bouts on the knee-extensor apparatus before data collection. The final two practices included a graded maximal knee-extensor exercise test and a protocol that simulated the planned experiment. On the final San Diego test day, after the catheterization of the femoral vein and radial artery (explained in detail below), two bouts of exercise were performed: left leg quadriceps exercise breathing room air (21% O2) and left leg quadriceps exercise breathing 12% O2. The sequence of these exercise bouts across subjects was reversed in three subjects to avoid ordering artifacts. For each bout, exercise work rate (WR) was increased from 25 to 50 to 75 and then to 90 and 100% of normoxic (N) maximum WR (WRMAX) with data obtained at each level. After a 2–3 min period at each work rate, sufficient to attain constant pulmonary oxygen uptake (VO2) (7), the sequence of events at each work rate was as follows: (a) 3-ml blood samples were taken, and (b) femoral venous blood flow was measured. Duplicate measurements of all variables were then taken without delay, to keep total exercise time to a minimum. Each incremental exercise bout was completed in 10–16 min.

Within 2 wk of completing the final San Diego study, subjects traveled by airplane to Philadelphia. In Philadelphia, the 7-cm surface coil was strapped to the anterior portion of the quadriceps. Initial resting measurements (2 min) were made and then ischemic measurements (10 min) were made through the inflation of a thigh cuff to 270 mmHg (proximal to the surface coil) with the subject positioned in the bore of the magnet, as for exercise (Fig. 1). After the removal of the thigh cuff and a 30-min recovery period, subjects reproduced both the normoxic and hypoxic (H) exercise protocols performed in San Diego, but without vascular catheterization. Exercise loads, WRs, and times were set to exactly reproduce the exercise profile achieved in San Diego. Throughout rest and exercise both proton MRS and 31P MRS data were collected with 20-s resolution.

**Specifics of exercise study with blood flow (Q) and blood gas measurements in San Diego.** Two catheters (radial artery and left femoral vein) and a thermocouple (left femoral vein) were emplaced using sterile technique as previously reported (8, 9). A 20-gauge, 3.2-cm long arterial catheter was inserted percutaneously under local anesthesia (1% lidocaine) in the radial artery of the nondominant hand for arterial blood sampling. Subsequently, a catheter 1.25 mm in external diameter (DSA 400L; Cook, Bloomington, IN) was introduced percutaneously into the left femoral vein 2 cm below the inguinal ligament and advanced 7 cm distally. This catheter has an open end and also 10 pinhole side ports in the distal 2.5 cm oriented in all directions around the catheter. This ensures that during injection of cold saline for thermomodulation flow measurement, thin streams are ejected at all orientations into the vein facilitating mixing across the vein lumen. This mixing was confirmed during in vitro model testing of the technique, with colored dye injection and video analysis. The second catheter consisted of a thin (0.64-mm diameter) polyethylene-coated thermocouple (IT-18; Physitemp Instruments, Clifton, NJ) that was advanced from approximately the same location proximally 10 cm into the left femoral vein toward the crux. Each catheter was attached to the skin using adhesive tape and positioned so as to minimize the risk of movement or creasing. During exercise, iced saline was infused through the femoral venous catheter at flow rates sufficient to decrease blood temperature at the thermocouple by ≈ 1°C. Infusions were continued for 15–20 s until femoral vein temperature had stabilized at its new lower value. Saline injection rate was measured by weight change in a reservoir bag suspended from a force transducer which was calibrated before and after each experiment. The calculation of blood flow was performed on thermal balance principles as detailed by Andersen and Saltin (10). This method of measuring blood flow has been used on several occasions in research from this and other laboratories (8–13). In each of these experiments and in this study, the ultimate criterion of validity (which currently in intact humans can only be whether the measurements of blood flow and arterial–venous differences yield physiological values for oxygen uptake [VO2] for the awake subject achieved based on the VO2-WR relationship. In a previous paper from this group (9), a detailed consideration of putative sources of error and steps which reduce their influence was published. Precautions noted therein were carefully followed throughout this study.

The samples of arterial and venous blood were used to measure PO2, CO2 pressure, pH, O2 saturation, and hemoglobin. All measurements were made on a blood gas analyzer (IL 1306; Instrumentation Laboratories, Inc., Lexington, MA) and a CO-oximeter (I. B. 282; Instrumentation Laboratories). Between each sample, electrodes were calibrated and demonstrated acceptable reproducibility (SD of repeated determinations: PO2 and CO2 pressure, 1.5 mmHg; pH, 0.003). Blood lactate concentration was determined using a blood lactate analyzer (1500; Yellow Springs Instrument Co., Yellow Springs, OH). Blood O2 concentration was calculated as 1.39 × [hemoglobin (Hb)] × measured O2 saturation + 0.003 × measured PO2. Arterial tissue (Vo2) difference was calculated from the difference in radial artery and femoral venous oxygen concentration. This difference was then divided by arterial concentration to give O2 extraction. Leg VO2 was calculated as the product of arterial–venous O2 concentration difference and blood flow.

Throughout testing in San Diego, subjects breathed through a low resistance two-way breathing valve (2700; Hans-Rudolph Inc., Kansas City, MO). Expired gas passed into a 7.2-liter heated, low resistance mixing chamber and was continuously sampled through a 0.8 cm-thick aerodynamic (8) flow nozzle by an MGA 1100 mass spectrometer (Perkin-Elmer Corp., Norwalk, CT) (inflow rate, 20 ml·min−1). Expired gas flow was measured by a pneumotachograph (Fleisch No. 3; Hans-Rudolph Inc.). Electrical signals from the mass spectrometer and pneumotachograph were logged at 100 Hz by use of a 12-bit analogue-to-digital converter for a determination of ventilation (body temperature, pressure, saturated with water vapor) and gas exchange (VO2 and CO2 uptake) (standard temperature, pressure, dry) by a commercially available software package (Con- sensus Technologies, Salt Lake, UT). VO2 and respiratory exchange ratio were displayed graphically, averaged over 30 s, for real-time monitoring of gas exchange. Repeated calibrations with this system established both volumetric and gas exchange accuracy to within 2%, confirmed by Douglas bag collections of expired gas and Tissot spirometry.

**Specifics of exercise study with MRS measurements in Philadelphia.** Within 2 wk of the San Diego exercise study with leg Q and blood gas measurements, subjects were transported to Philadelphia where the exercise protocol was reproduced in a 2.0 Tesla imaging magnet with custom-built spectrometer (Oxford Instruments, Cambridge, UK) (Fig. 1). A 7-cm diameter surface coil double-tuned to proton (85.45 MHz) and phosphorus (34.59 MHz) was placed over the rectus femoris portion.
of the quadriceps group, ~ 20–25 cm proximal to the knee (14). Spectra were collected from the muscle region beneath the surface coil. This region, as determined from scout images (data not shown) and previous calculations of surface coil sensitivity is largely confined to a semispherical volume defined by the coil circumference and penetrating to a depth equal to the radius of the coil. For these studies, this "sensitive region" was 100 cm³, which isolated signal detection predominantly from the region of the rectus femoris.

Details of the theory behind oxygen-sensitive myoglobin (Mb) signals have been published previously (15). The heme iron exhibits oxygen-dependent spin states that in turn influence nearby protons. The Nδ proton on proximal histidine P8, one of the ligands coordinated to the iron, is particularly sensitive to these changes. When oxygen is bound to the active site, the resonance of this proton is hidden beneath the dominant water signal. However, when myoglobin becomes deoxygenated, changes in the iron spin-state shift this peak to a temperature-dependent position that is clearly distinct from all other resonances. At physiological temperature, this peak resonates ~ 73 ppm downfield from the water resonance. This oxygen-dependent signal can be calibrated to obtain values for myoglobin desaturation; these values can be converted to intracellular oxygen tensions using oxygen-binding curves for myoglobin.

After placing the subject in the magnet, the coil was centered in the homogeneous portion of the magnetic field by aligning the water resonance in the center of applied x, y, and z gradient fields. The main magnetic field was then shimmed until the linewidth of the water resonance at half-height decreased to < 20 Hz (17.8±0.5 Hz, mean±SD). After this, the nominal 90° hard pulses were calibrated at both frequencies. They averaged 0.153±0.004 ms for proton and 0.143±0.005 ms for phosphorus. This indicated that the B1 fields for the two frequencies differed by < 7%, which indicated that the proton and phosphorus signals arose from matching sample volumes.

Proton and phosphorus spectra were obtained using a modified supraspinale WEPT water suppression sequence (Noyes, G. E., R. Reddy, Z. Wang, and J. S. Leigh, manuscript in preparation). For proton data, water and fat resonances were inverted using a 12-ms hyperbolic secant pulse with inversion bandwidth = 2 kHz. 65 ms later, the remaining spins were excited with a 0.5-ms gaussian pulse centered 6,650 Hz downfield from the water resonance frequency. This gaussian pulse had previously been calibrated on the water resonance to achieve the same signal intensity as the nominal 90° proton-hard pulse. Proton free-induction decays (FIDs) were then sampled over a spectral bandwidth = 20 kHz using 512 points and dwell time = 50 μs. Total repetition time = 130 ms for this sequence. This sequence was paused every 4 s to collect phosphorus data in an interleaved manner. After excitation with a nominal 90° hard pulse (0.143±0.005 ms), phosphorus FIDs were sampled over a spectral bandwidth = 2 kHz using 512 points and dwell time = 500 μs. 24 proton FIDs were collected for every phosphorus FID. For each 20-s time point this corresponded to 120 proton signal averages and 5 phosphorus signal averages.

During the entire study and particularly during the exercise, precautions were taken to minimize unnecessary movement that could alter signal intensity. The subject was securely fastened at the shoulders, waist, and knee before data collection. Subjects were instructed to minimize any movement beyond the intended exercise. In addition, to determine if motion was a problem, the water resonance was checked periodically for possible changes in position or linewidth. The resonance did not move by > 0.1 ppm or increase in width > 2 Hz for any of the subjects, indicating that motion did not degrade the signal during the study.

After acquisition, proton and phosphorus FIDs were apodized with exponentially weighted functions: for proton this corresponded to 100 Hz of line broadening in the frequency domain while for phosphorus the line broadening was 5 Hz. The FIDs were then Fourier transformed and manually phased to generate the frequency spectra. For the phosphorus spectra, areas were determined through integration of the peaks arising from inorganic phosphate (Pi), creatine phosphate (PCr), and b-ATP resonances. Conversion to concentration units was then accomplished by normalizing signal areas to the ATP value at rest, assuming resting ATP = 8.2 mM (16). Pi/PCr values were also computed as an index of the bioenergetic state of the muscle tissue (17). Intracellular pH was calculated from the parts per million difference between Pi and PCr peaks, using the formula cited by Taylor et al. (18).

For the proton spectra, areas were obtained under the peak resonating ~ 73 ppm downfield from the residual water signal (Fig. 2). Before analysis, the outer 128 points on either side of the spectrum were removed. The remaining portion was baseline corrected by fitting the spectrum to a fifth-order polynomial in the frequency domain, which was then subtracted from the spectrum. To ensure that deoxy-Mb resonances were not altered, the spectrum in the region 65–80 ppm downfield from the water resonance was removed before the baseline routine. After baseline correction, spectra were manually phased, and areas were obtained through integration of the peak arising 73 ppm from the water resonance. The peak position remained relatively constant throughout the protocol, averaging 73.2±0.1 ppm during the last 2 min of cuffing, 73±0.1 ppm during the normoxic exercise, and 72.8±0.1 ppm during the hypoxic bout. No other peaks were visible within 10 ppm of the deoxy-Mb resonance at any time. Although deoxy-Hb in solution gives rise to signals in the region of the deoxy-Mb peak (19), it has previously been shown that the visibility of deoxy-Hb in vivo is greatly reduced compared to that of deoxy-Mb (20).
Fraction deoxy-Mb was determined by normalizing signal areas to the average signal obtained during the 9th and 10th min of a cuff ischemia at suprasystolic pressure (265 mmHg). At rest, intramuscular oxygen depletes within 6–8 min of occlusion (21). Therefore the signals obtained during the last 2 min of the cuff represent complete deoxygenation of myoglobin and may be used to estimate total Mb content within the muscle. Conversion to PO2 values were then calculated from the well-known oxygen-binding curve for myoglobin:

$$PO2 = \frac{(1 - f)}{f} \cdot Pao$$  

where 1 – f is the fraction of myoglobin that is oxygenated, f is the fraction of myoglobin that is not oxygenated, and Pao is the oxygen pressure where 50% of the myoglobin binding sites are bound with oxygen. The temperature-dependent myoglobin Pao of 3.2 mmHg was used (22), based on an approximate muscle temperature of 39°C (23). However, as we report both saturation and myoglobin associated PO2, the latter can be recalcualted at any Pao.

Exercise model. The knee-extensor ergometer was designed and constructed in the Johnson Foundation (Philadelphia, PA) for the left (instrumented) leg to replicate the device reported in previous research (5, 8, 10, 13) (Fig. 1). This ergometer was constructed from nonmetallic materials to allow its use in both the human physiology lab in San Diego and the MRS facility in Philadelphia. The subject lay supine on a padded bed with the knee-extensor ergometer placed in front of him. The resistance to knee-extension was provided via a fiberglass bar attached to the crank of the ergometer and to a specially designed shin brace worn by the subject (Fig. 1). This ergometer was a prototype, and as such WR could not be measured in conventional units of work, but WR was prescribed and measured as a percentage of the maximum resistance (weight [g], resisting the fly-wheel turning, Fig. 1) at the end of the preliminary graded maximal test. 60 dynamic contractions of the knee-extensor muscles per min were performed. Contractions of the quadriceps femoris muscle caused the lower part of the leg to extend from ~90–170° flexion. Throughout the exercise the thigh remained immobile. This stability was enhanced by the 45° angle of the exercising leg and the harness worn by each subject (Fig. 1). The momentum of the flywheel returned the relaxed leg to the start position. This passive movement of the leg after contraction, anecdotal reports from subjects, and force tracings from a force transducer placed between the ergometer and the subject during this exercise (8) support the conclusion that active contraction was limited to the quadriceps muscles. The subjects were constrained in the bed by a safety belt which anchored the hips and shoulders (Fig. 1).

Mean capillary PO2 and muscle DO2. Using the measured intracellular PO2 values, mean capillary PO2 was calculated as described previously (4). Because these calculations used the measured myoglobin-associated PO2, they were no longer burdened by the assumption of a low intracellular PO2 at WRmax (4). As a result, DO2 calculations were performed for each WR using the corresponding intracellular PO2 measurement and not just at 100% of WRmax, as in previous studies. A numerical integration procedure is used to determine that value of DO2, assumed constant along the capillary, that produces the measured femoral venous PO2, given the measured arterial PO2. Additional explicit assumptions of this calculation are: the only explanation of O2 remaining in the femoral venous blood is diffusion limitation of O2 efflux from the muscle microcirculation, Perfusion/VO2 heterogeneity, and perfusion or diffusion shunt are considered negligible. To the extent that these phenomena contribute O2 to femoral venous blood, the parameter DO2 is a conductance coefficient that expresses the diffusional conductance that would be required to achieve the measured VO2 assuming only diffusion limitation. This assumption cannot be avoided currently for the lack of specific means for detecting perfusion/VO2 heterogeneity and shunt. Mean capillary PO2 is the average of all PO2 values computed at equal time intervals along the capillary from the arterial to the venous end. While mean capillary PO2 is useful for graphical purposes (see Results), our conclusions come from statistical comparisons of DO2 among the two experimental conditions.

Quadriceps femoris muscle mass. As suggested by Andersen and Saltin and others (8, 10, 24), thigh length, circumference, and skinfold measurements were used to determine thigh volume. This permitted an estimate of quadriceps muscle mass. It should be recognized that this calculation of muscle mass and the consequent normalizing of Q and VO2 assume that this is the only muscle mass involved in the knee-extensor exercise.

Statistical analyses. Least-squares regression, repeated measures ANOVA (Tukey post hoc), and Student’s t test analyses were computed using a commercially available software package (PC+; SPSS Inc., Chicago, IL). Variables were considered significantly different when P < 0.05. Data are presented as the mean ± SE throughout the manuscript.

Results

Subject characteristics and screening tests. The physical characteristics of the subjects were as follows (average ± SE): age, 23.3 ± 1.7 yr; height 181.2 ± 1.7 cm; and weight 73.8 ± 2.4 kg. As a part of this screening process, subjects performed a conventional incremental cycle ergometry test (100 W initial WR increased by 25 W · min⁻¹ until fatigue), and their VO2max averaged 4.4 ± 0.1 liters · min⁻¹ or 60.0 ± 1.7 ml · kg⁻¹ · min⁻¹ in this test.

Leg blood flow, leg VO2, and WR. During both normoxic and hypoxic exercise, the instrumented left leg demonstrated a
Table I. Major Physiological Variables Measured During Incremental Knee-extensor Exercise

<table>
<thead>
<tr>
<th>Measured variable</th>
<th>Exercise level 1 (N)</th>
<th>Exercise level 2 (N)</th>
<th>Exercise level 3 (N)</th>
<th>Exercise level 4 (N)</th>
<th>Exercise level 5 (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of normoxic leg VO2max</td>
<td>50.0±4.5</td>
<td>64.0±4.8</td>
<td>77.0±5.0</td>
<td>95.3±3.8</td>
<td>100±0±0</td>
</tr>
<tr>
<td>CaO2 (ml · 100 ml⁻¹)</td>
<td>18.1±0.2</td>
<td>18.3±0.1</td>
<td>18.5±0.2</td>
<td>18.7±0.2</td>
<td>18.7±0.5*</td>
</tr>
<tr>
<td>Cvo2 (ml · 100 ml⁻¹)</td>
<td>14.9±0.4†</td>
<td>14.8±0.4†</td>
<td>15.2±0.2</td>
<td>15.2±0.5†</td>
<td>—</td>
</tr>
<tr>
<td>Mean capillary Pvo2</td>
<td>6.7±0.9</td>
<td>6.1±0.8</td>
<td>5.7±0.8</td>
<td>4.9±0.7</td>
<td>4.7±0.7*</td>
</tr>
<tr>
<td>Diffusional conductance (mmHg)</td>
<td>4.5±0.6†</td>
<td>4.1±0.5†</td>
<td>3.8±0.5†</td>
<td>3.4±0.5†</td>
<td>—</td>
</tr>
<tr>
<td>Leg PVO2 (mmHg)</td>
<td>26.3±1.3</td>
<td>25.1±1.5</td>
<td>24.4±1.5</td>
<td>22.6±1.4</td>
<td>22.1±1.6*</td>
</tr>
<tr>
<td>Mean capillary PO2 (mmHg)</td>
<td>42.0±1.5</td>
<td>42.4±1.6</td>
<td>40.1±1.7</td>
<td>38.4±1.5</td>
<td>37.5±1.6*</td>
</tr>
<tr>
<td>Diffusional conductance (ml O2·min⁻¹·mmhg⁻¹)</td>
<td>15.7±0.2</td>
<td>21.0±0.3</td>
<td>24.9±0.4</td>
<td>33.7±0.3</td>
<td>35.3±0.3</td>
</tr>
<tr>
<td>Deoxy-Mb signal (percentage of maximum cuff signal)</td>
<td>48±5</td>
<td>56±4</td>
<td>50±5</td>
<td>50±4</td>
<td>51±3*</td>
</tr>
<tr>
<td>Mb-associated PO2 (mmHg)</td>
<td>3.5±0.4</td>
<td>2.5±0.2</td>
<td>3.2±0.4</td>
<td>3.2±0.4</td>
<td>3.1±0.3*</td>
</tr>
<tr>
<td>Leg Q (liters·min⁻¹)</td>
<td>5.6±0.3</td>
<td>6.8±0.5</td>
<td>7.8±0.6</td>
<td>9.2±0.6</td>
<td>9.4±0.6*</td>
</tr>
<tr>
<td>Leg O2 delivery (liters·min⁻¹)</td>
<td>6.1±0.5</td>
<td>6.9±0.6</td>
<td>7.8±0.6</td>
<td>8.3±0.6</td>
<td>—</td>
</tr>
<tr>
<td>Leg VO2 (liters·min⁻¹)</td>
<td>0.9±0.1†</td>
<td>1.2±0.1</td>
<td>1.4±0.1</td>
<td>1.7±0.1</td>
<td>1.7±0.1*</td>
</tr>
<tr>
<td>Leg O2 extraction (%)</td>
<td>62.9±4.7</td>
<td>66.6±4.3</td>
<td>69.3±4.1</td>
<td>73.6±3.5</td>
<td>74.8±3.4</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>69.5±4.4†</td>
<td>72.1±3.4†</td>
<td>75.1±0.4</td>
<td>77.6±3.3</td>
<td>—</td>
</tr>
<tr>
<td>Venous pH</td>
<td>7.372±0.01</td>
<td>7.369±0.01</td>
<td>7.363±0.01</td>
<td>7.370±0.02</td>
<td>7.374±0.02</td>
</tr>
<tr>
<td>Arterial lactate (mM)</td>
<td>1.4±0.1</td>
<td>2.0±0.1</td>
<td>2.6±0.2</td>
<td>3.7±0.3</td>
<td>4.3±0.3</td>
</tr>
<tr>
<td>Pulmonary VO2 (liters·min⁻¹)</td>
<td>0.9±0.04</td>
<td>1.12±0.06</td>
<td>1.35±0.09</td>
<td>1.76±0.13</td>
<td>2.11±0.10</td>
</tr>
<tr>
<td>Respiratory exchange ratio</td>
<td>0.97±0.04</td>
<td>1.15±0.05</td>
<td>1.63±0.11</td>
<td>2.04±0.08</td>
<td>—</td>
</tr>
<tr>
<td>Heart rate (beats·min⁻¹)</td>
<td>111.0±3.0</td>
<td>123.0±4.0</td>
<td>137.0±6.0</td>
<td>153.0±5.0</td>
<td>165.0±4.0</td>
</tr>
</tbody>
</table>

Mean (±SE) percentage of normoxic leg VO2; arterial (CaO2) and femoral venous (Cvo2) O2 content; leg venous partial pressure of O2 (PVO2); mean capillary partial pressure of O2 (PO2); diffusional conductance of O2; deoxy-myoglobin (deoxy-Mb) saturation; myoglobin (Mb) associated partial pressure of oxygen (PO2); leg blood flow (Q); O2 delivery; leg VO2; O2 extraction; arterial pH; venous pH; arterial lactate concentration; pulmonary VO2; respiratory exchange ratio, and heart rate during knee-extensor exercise in normoxic (N) and hypoxic (H) conditions. * Significantly different from normoxic values at maximal exercise (P ≤ 0.05). † Significantly different relationship from normoxic values across all exercise intensities (P ≤ 0.05).

linear rise in both leg Q and leg VO2 as WR increased towards WRmax (Fig. 3, Table I). Leg Q and leg VO2 rose to high mass-specific levels corresponding quantitatively with previous reports (6, 8, 25) (Fig. 3, Table I). At submaximal work rates there was no significant difference in the relationship between leg Q and WR or leg VO2 and WR between the two conditions (Fig. 3, Table I). However, maximal leg Q, leg VO2max, and WRmax were all significantly reduced in hypoxia (P ≤ 0.05) (Fig. 3, Table I). It is important to note that this prototype nonmetallic ergometer required an appreciable amount of work during "unweighted" exercise (Fig. 3). As this internal work of the ergometer was not measurable, the metabolic cost of producing each WR (increased by the addition of weight to a belt around the flywheel) was related in a different fashion to conventional cycle ergometry (i.e., 25% of WRmax = 50% of leg VO2max, 50% of WRmax = 65% of leg VO2max, etc., Table 1). To avoid incorrect implications of the present results, in relation to a percentage of WRmax and other exercise ergometers, all data have been expressed in relation to the percentage of normoxic single leg VO2max.

Intravascular PO2 and muscle DO2. In hypoxia at submaximal leg VO2max, arterial PO2, venous PO2, and calculated mean capillary PO2 were also significantly lower than in normoxia (P ≤ 0.05) (Table 1, Fig. 4). At the reduced hypoxic leg VO2max
both venous $P_O_2$ and calculated mean capillary $P_O_2$ were also significantly lower than $V_{O_2_{max}}$ in normoxia ($P < 0.05$) (Table I, Fig. 4). In both hypoxia and normoxia the calculated tissue $D_O_2$ increased as the intensity of work increased (Fig. 5 A). At each submaximal WR, $< 90\%$, the calculated $D_O_2$ was elevated in hypoxia in comparison to normoxia ($P < 0.05$) (Table I, Fig. 5 A), but due to the greater continued rise in normoxic tissue $D_O_2$ there was no significant difference in $D_O_2$ between normoxia and hypoxia at $W_{R_{max}}$ (Table I, Fig. 5). This was the case no matter whether mean capillary or femoral venous $P_O_2$ was used to illustrate muscle $D_O_2$ (Fig. 5 B).

**Intracellular $P_O_2$.** Under resting conditions the proton resonances from myoglobin lay under the water peak, and the deoxy-myoglobin signal was not evident (Fig. 2 A). After inflation of the thigh cuff, proximal to the surface coil, the deoxy-myoglobin signal increased for the initial 6 min and plateaued for the final 4 min as in previous research (1) (Figs. 2 C and 6 A). Based upon this plateau in the deoxy-Mb signal amplitude (Figs. 2 C and 6 A) and a previous MRS study of anoxic resting skeletal muscle (21), this signal averaged over minutes 9 and 10 of vascular occlusion was considered to be 100% of the deoxy-Mb signal. Before commencing the exercise protocol there was again no discernible deoxy-myoglobin signal. During unweighted knee-extensor exercise the deoxy-myoglobin signal rose to an average of 38% of the maximal deoxy-myoglobin signal ($P_MbO_2 = 5$ Torr) (Figs. 4 and 7). In normoxia, as exercise progressed the deoxy-myoglobin signal increased rapidly (within 20 s to $\approx 50\%$ of the maximum signal ($P_MbO_2 = 3.1$ mmHg) and maintained this value until $W_{R_{max}}$ (Figs. 2 B, 4, 6, and 7). During hypoxic exercise the deoxy-myoglobin signal also increased rapidly to $\approx 60\%$ of the maximum signal ($P_MbO_2 = 2.1$ mmHg) and maintained this value through.
WRMAX (Figs. 2 B, 4, 5, and 6). In both conditions, the cessation of exercise, after reaching WRMAX, produced a rapid reduction in the deoxy-myoglobin signal and therefore a large increase in PmO2 within 20 s (Fig. 6 A).

O₂ delivery and O₂ extraction. In normoxic and hypoxic exercise, arterial O₂ content was independent of increasing work rate (P > 0.05) but was lower in hypoxia as expected (Table I). Femoral venous O₂ content decreased as work rate was increased (P > 0.05) (Table I). As the leg Q to WR relationship was similar in normoxia and hypoxia, muscle O₂ delivery to the leg was significantly reduced in hypoxia in comparison to normoxia (P < 0.05) (Table I). Throughout submaximal exercise, muscle O₂ extraction [(CaO₂-CvO₂)/CaO₂] was significantly higher in hypoxia than normoxia (P < 0.05) (Table I), where CaO₂ is arterial oxygen concentration and CvO₂ is venous oxygen concentration.

Muscle metabolism. Initial levels of PCr and Pi at rest were consistent with previous research (26) and were rapidly affected by the beginning of exercise. At the initial WR, which elicited ≈ 50% of leg VO₂max, PCr fell and Pi rose to levels previously associated with this level of exercise intensity (26) (Table II, Fig. 6). The PCr/Pi index fell to a near nadir at ≈ 65% of leg VO₂max, illustrating the high intensity of the muscular work during knee-extensor exercise. There were no significant differences in these variables between exercise in normoxia and hypoxia (Table II).

Intravascular and intracellular pH. Arterial pH was maintained at close to resting values in both normoxic and hypoxic conditions and was not significantly different in either condition during submaximal or maximal WRs. Venous pH fell with increasing exercise intensities in both conditions and was not different between hypoxia and normoxia (Table II). Intracellular pH also fell to low levels with increasing leg VO₂ and was also not significantly different in hypoxia than in normoxia (Table II).

Quadriiceps femoris weight. Estimated average quadriiceps femoris weight was 2.5 ± 0.14 kg. This allowed the calculation of mass specific Q and VO₂ in normoxia and hypoxia which were 375 ± 24 and 333.1 ± 26 ml·min⁻¹·100 g⁻¹ and 52.0 ± 4.4 and 39.3 ± 3.8 ml·min⁻¹·100 g⁻¹, respectively, at WRMAX.

Discussion

The results of this study, in terms of leg VO₂ and leg Q, revealed high mass-specific values similar to previous studies in trained subjects exercising from submaximal to maximum levels with the quadriiceps (6, 8) (Table I, Fig. 3). As in these previous studies, arterial PO₂ remained relatively constant in both hypoxia and normoxia as WR was increased and venous PO₂ fell progressively (6, 8) (Table I). From these data, mean capillary values were calculated to be 37 ± 2 (N) and 30 ± 2 mmHg (H) (Table I). Proton MRS measurements revealed a rapid desaturation of myoglobin, and thus myoglobin associated PO₂ fell quickly to an average of 3.1 ± 3 (N) and 2.1 ± 2 mmHg (H) which was then maintained through submaximal to maximal WRs (Table I).

These data represent the first experimental evidence of a steep O₂ gradient from blood to intracellular sites in human skeletal muscle in vivo. These independent numerical estimates

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Table II. Indices of Muscle Metabolism During Incremental Knee-extensor Exercise

<table>
<thead>
<tr>
<th>Measured variable</th>
<th>No exercise</th>
<th>Exercise level 1</th>
<th>Exercise level 2</th>
<th>Exercise level 3</th>
<th>Exercise level 4</th>
<th>Exercise level 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of normoxic leg VO₂max</td>
<td>N</td>
<td>Rest</td>
<td>50.0 ± 4.5</td>
<td>64.0 ± 4.8</td>
<td>77.0 ± 5.0</td>
<td>95.3 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>Rest</td>
<td>48.5 ± 3.8</td>
<td>56.5 ± 2.6</td>
<td>67.5 ± 1.0</td>
<td>75.1 ± 1.8</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>38.2 ± 2.5</td>
<td>13.0 ± 1.9</td>
<td>6.4 ± 1.1</td>
<td>3.4 ± 0.5</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>38.0 ± 1.4</td>
<td>13.1 ± 2.0</td>
<td>7.0 ± 1.9</td>
<td>3.7 ± 1.0</td>
<td>3.4 ± 0.8</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>3.3 ± 0.6</td>
<td>23.4 ± 1.5</td>
<td>28.5 ± 1.5</td>
<td>30.3 ± 1.7</td>
<td>29.9 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>2.8 ± 0.5</td>
<td>21.6 ± 1.9</td>
<td>25.1 ± 1.3</td>
<td>27.0 ± 0.9</td>
<td>26.5 ± 0.8</td>
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<td></td>
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<tr>
<td></td>
<td>N</td>
<td>11.5 ± 4.1</td>
<td>0.6 ± 1.3</td>
<td>0.2 ± 0.7</td>
<td>0.1 ± 0.4</td>
<td>0.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>13.6 ± 3.6</td>
<td>0.6 ± 1.1</td>
<td>0.3 ± 1.5</td>
<td>0.1 ± 1.1</td>
<td>0.1 ± 1.0</td>
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<td></td>
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<td></td>
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<tr>
<td>Intracellular pH</td>
<td>N</td>
<td>7.073 ± 0.017</td>
<td>6.867 ± 0.036</td>
<td>6.745 ± 0.014</td>
<td>6.617 ± 0.017</td>
<td>6.554 ± 0.032</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>7.119 ± 0.044</td>
<td>6.691 ± 0.076</td>
<td>6.535 ± 0.094</td>
<td>6.464 ± 0.084</td>
<td>6.394 ± 0.147</td>
</tr>
</tbody>
</table>

Mean (±SE) percentage of maximum normoxic VO₂max, intracellular creatine phosphate (PCr), intracellular inorganic phosphate (Pi), intracellular bioenergetic state (PCr/Pi), and intracellular pH.
reveal that O₂ pressure in the blood during exercise was 11–12-fold higher than in the cytoplasm at submaximal WRs (50% leg VO₂max) and remained 8–10-fold higher even at WRmax. This equates to a PO₂ gradient from mean capillary blood to myoglobin of 39 (N) and 29 mmHg (H) at submaximal WRs, which fell slightly to 35 (N) and 27 mmHg (H) at WRmax. The determination of intravascular and intracellular PO₂ in this study allows an analysis of muscle diffusivity, less burdened by assumptions, throughout a range of exercise intensities in normoxia and hypoxia.

**Muscle diffusional conductance.** Muscle diffusivity can be described by Fick’s law of diffusion:

\[ \dot{V}O_2 = D_02 (P_{CAPO2} - P_{MITO2}) \]  

(2)

Where \( D_02 \) is muscle O₂ diffusional conductance and \( P_{CAPO2} \) is mean muscle capillary PO₂. \( P_{MITO2} \) is mitochondrial PO₂. It should be noted that the substitution of \( P_{CAPO2} \) for effluent venous PO₂ (which closely approximates mean end capillary PO₂ [27]) eliminates uncertainties about the calculation of mean capillary PO₂ but does not qualitatively affect the conclusions of this study (Fig. 5 B).

Based on direct measurements of intracellular PO₂ (28) and mathematical modeling (29) in maximally exercising muscle, previous analyses of diffusivity, not knowing \( P_{MITO2} \), have limited their calculations to \( V_{O2max} \) where it was assumed cellular PO₂ was very low (1–3 mmHg range) and so could be neglected (4, 6, 30, 31). The present study has demonstrated that the assumption of a low intracellular PO₂ at \( V_{O2max} \), relied upon in these initial investigations, was valid and in fact is the case even at submaximal exercise intensities eliciting only 50% of \( V_{O2max} \) (Fig. 4), at least in this particular form of exercise.

Figure 5 A illustrates the progressive increase in diffusional conductance in both hypoxia and normoxia from submaximal to maximal WRs. As described above, until this unique measurement of both intracellular and intravascular PO₂, it was considered invalid to perform this analysis at any point other than \( V_{O2max} \) because of uncertainty about intracellular PO₂. At these submaximal levels diffusional conductance was greater in hypoxia, but as WRmax was approached both calculated mean capillary and femoral venous PO₂ in hypoxia and normoxia exhibited proportionality to the achieved leg \( V_{O2max} \) (Fig. 5 B, Table 1). This single level of diffusional conductance at WRmax is consistent with other studies (8, 30, 31) and the theory that diffusion limitation may play a role in determining \( V_{O2max} \) in exercising muscle (4).

The effective diffusivity at submaximal WRs in hypoxia was markedly elevated in comparison to normoxic values. At ≈ 65% of \( V_{O2max} \), the diffusional conductance in hypoxia was already 85% of the maximum recorded value, whereas in normoxia at a comparable VO₂ conductance was only 59% of the maximum value (Table I, Fig. 5 A). This difference may be the result of the increased concentration of available O₂ carrier within the muscle tissue (deoxy-Mb) (32) (Fig. 7).

**The role of VO₂/Q heterogeneity and the spatial averaging of the deoxy-Mb signal.** When considering the importance of O₂ diffusivity in determining the large O₂ gradient from blood to myoglobin-associated PO₂ (Fig. 4, Table 1), it is important to address the role of VO₂/Q heterogeneity as a phenomenon which can produce similar results (33). To objectively quantify the influence of VO₂/Q heterogeneity under these experimental conditions, we considered the possible scenario in which all venous PO₂ was the result of only heterogeneity, with no diffusion limitation. In this situation, when there is zero heterogeneity, VO₂ is equal to the product of Q and CaO₂ (O₂ supply) and venous PO₂ must be zero. Thus, with this conceptual starting point and the data collected during maximal normoxic exercise, we calculated the necessary change in VO₂/Q distribution (as estimated using log SDQ, the second moment of the perfusion distribution on a log scale) that would result in a venous PO₂ of 22.1 mmHg, as measured in the femoral vein at maximal exercise, Table 1). The log SDQ which satisfied this measurement venous PO₂ was 0.55. To put this figure in perspective, we used the same SDQ calculations to produce an estimate of muscle mass/Q distribution in a maximally exercising in situ canine gastrocnemius preparation (34) (modified to reduce surgical procedures and tissue traumatization [35]), injected with colored microspheres, and sectioned into 12 pieces after exercise. The log SDQ for this preparation was only 0.13. Hence, we had to theoretically impose a fourfold greater maldistribution of Q than recorded in the surgically prepared canine muscle preparation to account for the venous PO₂ in our subjects through heterogeneity alone. Consequently, although this analysis can not quantitatively determine or discount the role of heterogeneity in producing the measured O₂ gradients, it does suggest that it is unlikely that these healthy subjects would demonstrate a VO₂/Q heterogeneity that is significantly greater than that observed in the surgically prepared, electrically stimulated, in situ canine gastrocnemius preparation.

The deoxy-Mb spectra collected represent the spatial average signal across the region beneath the surface coil and therefore cannot provide insight into the distribution of deoxy-myoglobin at the intracellular level. Research at the cellular level has suggested that O₂ gradients within the cell are shallow and no anoxic loci have been found during exercise (36). Any heterogeneity in myoglobin saturation is minimized by the arrangement of the microvascular units (37) and the facilitated diffusion and redistribution of O₂ performed by myoglobin itself (38).

**Rapid myoglobin desaturation at submaximal exercise.** The maximum deoxy-myoglobin signal was apparent even at 50% of \( V_{O2max} \) (Fig. 7) and this desaturation occurred very rapidly (within a 20 s time frame, Fig. 6 A). The latter observation was highlighted during a single data collection period in the magnet by the rapid disappearance of the deoxy-myoglobin signal (resaturation) when exercise was ceased for < 45 s to adjust a subject’s shin brace. Upon recommencement of the knee-extensor exercise the deoxy-myoglobin signal was again apparent and had returned to its previous value within the time resolution of the MRS system (20 s). This emphasizes that it would be inappropriate to attempt to measure intracellular PO₂ via myoglobin saturation with MRS by placing a subject in a magnet immediately after exercise. Additionally, this rapid myoglobin desaturation to 50–60% is indicative of the immediate use of at least half of the myoglobin O₂ stores. Thus, the speed and magnitude of this response may have several functions. First, the immediate availability of 50% of the stored myoglobin-associated O₂ may be an important O₂ source for the increased oxidative metabolism at the start of exercise. Second, this myoglobin desaturation reduces the carrier-depleted region, maximizing the PO₂ gradient from blood to cell. Consequently, the passive transport system responsible for O₂ influx into the muscle cell is facilitated by this rapid desaturation, even during light exercise. The greater deoxy-myoglobin signal in hypoxia (Fig. 7) and the concurrent elevation in the O₂ diffu-
sional conductance in hypoxia throughout submaximal exercise (Fig. 5), substantiates these observations.

Unaltered deoxy-myoglobin associated PO$_2$ from submaximal to maximal exercise. Both intuition and theoretical modeling (39) suggest it is reasonable to expect intracellular PO$_2$ to fall as the intensity of exercise increases to enhance the O$_2$ flux into the muscle cell (Eq. 2). The present data do not illustrate a fall in myoglobin saturation and thus intracellular PO$_2$ beyond that achieved in moderate exercise. However, as Eq. 2 illustrates, DO$_2$ also regulates O$_2$ flux. A major determinant of DO$_2$ is the effective surface area available for diffusion (40). If DO$_2$ increases in response to capillary recruitment and increased dynamic hematocrit (41-43), myoglobin-associated PO$_2$ need not change with increasing exercise intensity. In fact, consistent with this analysis, the calculated DO$_2$ for the present data did increase with each increased WR (Fig. 6). Thus, this study reveals that the O$_2$ conductance is recruited proportionally as VO$_2$ increases (Fig. 5 A), but PMO$_2$ remains constant after an initial fall during exercise eliciting submaximal VO$_2$.

Muscle bioenergetics. The present MRS data represent the first continuous analyses of intramuscular bioenergetics in the rectus femoris during knee-extensor exercise. These data are similar to previous findings using other exercise modalities (17, 44). However, as recognized earlier, because the lowest intensity of knee-extensor exercise performed equated to $\approx 50\%$ of VO$_{2\text{max}}$, these data reveal a rapid change in muscle metabolism and pH during initial submaximal exercise. In fact, PCR fell from normal initial resting values to almost total depletion at WR$_{\text{MAX}}$ and muscle pH fell to very low levels in both hypoxia and normoxia (Table II, Fig. 6 B and D). The concentration of Pi demonstrated a concomitant rise from resting levels (Table II, Fig. 6 C). These indicators of muscle metabolism, collected concurrently with the myoglobin spectra, are of significant importance as they illustrate that the proton MRS data were collected under conditions of high metabolic demand. Additionally, as these $^{31}$P data are similar to previous measurements, made in this (45) and other human muscles with different exercise modalities (26), it is probable that the concurrent measurements of myoglobin saturation are not unique to this form of exercise and may be generalizable to other forms of dynamic exercise in humans.

Myoglobin PO$_2$ as a determinant of cellular respiration. This study has determined that myoglobin becomes significantly desaturated at submaximal exercise levels ($\approx 50\%$ of VO$_{2\text{max}}$) and remains at this level of desaturation even as leg VO$_2$ is increased to maximum (Fig. 7). However, a secondary observation was that in hypoxia the degree of myoglobin desaturation was significantly greater than in normoxia and that this difference in O$_2$ tension was still evident at leg VO$_{2\text{max}}$ (Table I). It is not apparent from these data why, at normoxic WR$_{\text{MAX}}$, myoglobin-associated PO$_2$ did not fall to the level reached in hypoxia and why under both conditions desaturation was far less at WR$_{\text{MAX}}$ than under conditions of cuff occlusion. In an effort to reconcile these questions, Fig. 8 illustrates that the present data support the hypothesis that leg VO$_{2\text{max}}$ is dependent on myoglobin-associated PO$_2$ (Figs. 5 B and 8 B) and may represent an in vivo correlate (in myocytes) of the effect of O$_2$ tension on cellular respiration rate (in kidney cells) as previously described in vitro by Wilson et al. (46) (Fig. 8 A). The proportional relationship illustrated between leg VO$_2$ and measured myoglobin-associated PO$_2$ and that this relationship, if continued, would pass through the origin (Fig. 8 B) are similar to the findings of Wilson et al. (46) who found a hyperbolic relationship between O$_2$ tension and cellular respiratory rate in kidney cells (Fig. 8 A). It is speculated that these findings may represent initial data in the development of a hyperbolic relationship between in vivo muscle VO$_2$ and intracellular PO$_2$, supporting the concept that maximal respiratory rate ($\dot{V}O_{2\text{max}}$) is limited by O$_2$ supply (Fig. 8 B). We hypothesize that our data may describe a similar relationship to those of Wilson et al. (46) by reconciling the hyperbolic expression of O$_2$ utilization in Fig. 8 A with the linear expression of O$_2$ transport in Eq. 2, as theoretically illustrated in Fig. 8 C. Thus, Eq. 2, when VO$_2$ is plotted against P$_{\text{MET}}$, is a straight line of similar slope (DO$_2$) in normoxia and hypoxia but with a lower intercept in hypoxia due to the lower P$_{\text{CAP}}$ at VO$_{2\text{max}}$ (Table I). The intersection of these lines with the intrinsic mitochondrial VO$_2$/PO$_2$ hyperbolic relationship shows how the present myoglobin-associated PO$_2$ data fit with O$_2$ supply dependence of VO$_{2\text{max}}$ in intact normal humans. The conclusions are identical, but the data are essentially independent of those relating VO$_{2\text{max}}$ to mean capillary PO$_2$ in Fig. 5 B.
**Oxygen cascade from air to tissues.** Although theoretical figures similar in form to Fig. 9 are common, often used as teaching aids (47), the ability to construct Fig. 9 from actual data has potential diagnostic use. These in vivo data collected in normal humans illustrate the potential clinical use of combining techniques to further elucidate the underlying pathophysiology of disease states which affect this oxygen cascade by identifying where the compromise in O₂ transport occurs.

**Summary.** The combination of isolated human quadriceps exercise and MRS technology in this study allowed the unique analysis of O₂ gradients from the air to tissue in humans. These data provide evidence of a substantial O₂ gradient from blood to tissue, suggesting a resistance to the diffusion of O₂ between red cell and sarcolemma, and that this is present even during submaximal exercise. As WR is increased and O₂ demand is elevated, O₂ flux can be increased despite this diffusion limitation, presumably by increasing the area available for diffusion by increasing both blood flow and capillary recruitment. Diffusional conductance was consistently elevated in hypoxia, as was the level of deoxy-myoglobin. Thus, influenced heavily by the increased magnitude of myoglobin-facilitated O₂ flux in hypoxia, the reduction in intracellular resistance from sarcolemma to cell interior may account for the enhanced diffusional conductance at submaximal WRs. As maximal VO₂ was approached in normoxia, diffusional conductance continued to rise and match the diffusional conductance in hypoxia at WRₘₐₓ, despite no increase in myoglobin desaturation. This was probably made possible by the continued ability to perform work in normoxia and the resulting increase in blood flow that this dictates. The ability to measure tissue PO₂ in hypoxia and normoxia reveals that at muscle VO₂ₘₐₓ tissue O₂ diffusional conductance is equal and mean capillary PO₂, femoral venous PO₂, myoglobin PO₂, and maximal VO₂ are each reduced proportionately in hypoxia supporting the concept that O₂ supply plays a role in determining VO₂ₘₐₓ.

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