國立體育學院九十五學年度研究所博士班入學考試試題

（本試題共9頁）

體育運動論文評論（運動保健組）

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J Appl Physiol 98: 1177–1184, 2005;
doi:10.1152/japplphysiol.00591.2004.

Effect of acute hypoxia on microcirculatory and tissue oxygen levels in rat cremaster muscle

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Submitted 9 June 2004; accepted in final form 29 November 2004

IN RECENT YEARS, THE PHYSIOLOGICAL and pathological effects of brief periods of hypoxia (≤1 min) have come under increasing scrutiny due to the growing interest in sleep apnea. Animal studies have demonstrated an acute increase in sympathetic nerve activity (12) and local vasodilator mechanisms (6, 22) during short periods of hypoxia. With chronic intermittent hypoxia over a period of weeks, experimental animals exhibit sustained elevation of sympathetic nerve activity and arterial pressure and a decrease in cardiac function (2, 12, 20, 30). Similar changes in blood pressure and cardiac function have been seen in patients with sleep apnea (15).

As the responses to brief periods of hypoxia have become increasingly well documented, there is a need for information on the changes in oxygen levels in the organism that elicit these responses. Studies in animals have described the time course of arterial desaturation during induced apnea (13, 39), whereas studies in humans have documented the changes in arterial blood saturation during voluntary apnea (11) or sleep apnea (5). However, information on the time course and magnitude of changes in oxygen tension at the microcirculatory and tissue levels is lacking. Such information would be useful in assessing the effects of arterial desaturation on microcirculatory P_{O_2} and oxyhemoglobin (HbO_2) and on tissue P_{O_2} levels during episodes of sleep apnea.

Using a 1-min exposure of anesthetized rats to 7% O_2 in N_2, we examined the rate and magnitude of the fall in P_{O_2} in arterial blood, the microcirculation, and tissue sites at the venous end of the capillary network where tissue P_{O_2} is believed to be lowest (23). This drop in inspired oxygen fraction ($F_{I_{O_2}}$) is at least as large as would be expected in sleep apnea (5, 13). We tested the hypothesis that the time course of change in P_{O_2} and HbO_2 saturation in microcirculatory vessels and in tissue P_{O_2} during hypoxia is slower than in arterial blood. We also tested the hypothesis that the tissue P_{O_2} during hypoxia would fall into the range where a shift from aerobic to anaerobic metabolism is known to occur.

METHODS

Animal preparation. Studies were performed on 33 fasted male Sprague-Dawley rats (165 ± 2 g body wt) anesthetized with pentobarbital sodium (60 mg/kg, Nembutal, Abbott Laboratories) administered by intraperitoneal injection. Supplemental anesthesia consisting of Nembutal was administered through a catheter in the right jugular vein to maintain a surgical level of anesthesia as determined by appropriate criteria. The studies were approved by the University of California, San Diego Animal Subjects Committee. The right carotid artery was cannulated to obtain arterial blood samples and to measure systemic arterial pressure. A tracheal tube was inserted to maintain a patent airway. In the microcirculatory studies, the cremaster muscle was prepared in a manner similar to that described in a previous report (7) based on the original description by Baez (1). During surgery, the muscle was continuously irrigated with physiological salt solution and subsequently covered with polyvinyl film (Saran Wrap), and the muscle and animal were placed on a heated Lucite platform. The animal was then mounted on a microscope stage for viewing and measurement of microcirculatory variables and oxygen levels. A 30-min period of equilibration on the stage was allowed before the first protocol was initiated.

Systemic measurements. Arterial pressure was measured with a Becton Dickinson pressure transducer (model DTX Plus TNF-R) and recorded on a Biopac model MP150CE (Biopac Systems, Santa Barbara, CA). Arterial P_{CO_2} and P_{O_2} during the control and experimental periods were measured on 100-μl blood samples taken from the carotid artery using a model 248 CHIRON Diagnostics (Halstead, UK) blood gas analyzer.

Microscope and oxygen measurement systems. The microscope system used in these studies has been used previously for in vivo determination of oxygen levels in microcirculatory vessels and parenchymal tissue (35). The system has the capability for repeated mea-

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surement of oxygen tension at localized sites and measurement of vessel dimensions and red cell velocity of selected vessels. Briefly, the system consists of an inverted microscope IMT2 (Olympus, New Hyde Park, NY), including a Hg arc for transillumination of the tissue and a video camera and tape recorder for monitoring and recording the visual field. A strobe light source and photomultiplier tube are incorporated to measure the time decay of phosphorescence of an oxygen-sensitive dye. A ×20 objective (numerical aperture = 0.46, Olympus) was used to obtain a final magnification of 180 μm on the horizontal axis and 140 μm on the vertical axis of the video monitor.

Because the microscopic field could not be visualized during oxygen measurements, data on vessel diameter and flow were obtained only during the control period. Diameter measurements were taken from the video image using a Digital Video Image Shearing Monitor model 908 (Vista Electronics, San Diego, CA). Velocity measurements were made using a Fiber Optic Photo Diode Pickup and Velocity Tracker model 102B (Vista Electronics) and corrected to mean red cell velocity as described previously (35).

To determine oxygen levels, the microscope system includes a model 2601 strobe light source (EG & G, Salem, MA) for epi-illumination and excitation of a phosphorescent dye, a photomultiplier tube and amplifier for recording the phosphorescence decay, and custom software for calculating the mean P_{O_2} and standard deviation of the measurement. The method of P_{O_2} measurement was developed first by Vanderkooi et al. (38) and adapted in our laboratories for microcirculatory studies (16, 35). The method has been used by a number of other laboratories to measure P_{O_2} in microcirculatory vessels as reviewed in Ref. 36. In respect to measurements in tissue where dye concentration is lower than blood, simultaneous P_{O_2} measurements in tissue with our system and the Whalen oxygen microelectrode showed no significant difference between the two methods (36).

Procedure for microcirculatory oxygen measurement. To measure oxygen tension in the microcirculation and tissue, Pd-*meso*-tetra(4-carboxyphenyl)porphine (Porphyrin Products, Logan, UT) was prepared as described previously (25) and injected intravenously at a dose of 15 mg/kg body wt. The dye distributes rapidly in the bloodstream and diffuses into the extracellular tissue spaces in sufficient concentration to allow measurement of oxygen levels in interstitial fluid as well as microcirculatory vessels. Phosphorescence was excited by the strobe light source that delivered a total of 10 flashes at 10 Hz. Phosphorescence intensity was measured in a rectangular window of variable size according to the dimensions of the measurement site. Sites selected for study were arterioles and venules of 16- to 42-μm inner diameter and tissue sites in the venous capillary network. In all instances, sites were chosen that were devoid of large vessels that might affect the P_{O_2} reading.

To estimate the HbO_2 saturation from P_{O_2} measurements, the characteristic HbO_2 saturation curve for rats of this strain (Sprague-Dawley) and supplier (Simonson, Gilroy, CA) was determined as described previously (37). The P_{O_2} necessary to obtain 50% oxygen saturation (P_{50}) was 32.4 Torr and Hill's n_{50} was 2.61 (n = 2). These values are similar to those found for albino rats of 35.4 ± 1.3 and 2.56 ± 0.09 (means ± SD), respectively, by Teisseire et al. (33). The latter investigators obtained a value of 0.56 for the Bohr affect, which was used in this study to correct for pH effects on the P_{O_2}-HbO_2 relationship. The pH of arteriolar, venous capillary, and venular blood was assumed to be 0.13 unit less than arterial blood based on the study of Kobayashi and Takizawa (18). It was assumed that the pH change in microcirculatory vessels during hypoxia was the same as that in arterial blood. HbO_2 saturation was determined from the P_{50} and n_{50} values using Hill's equation.

Hypoxia system. The hypoxia system consists of parallel circuits for directing either air or 7% oxygen in nitrogen through a T tube attached to the tracheal catheter. Rapid switching was achieved by means of a manually controlled three-way valve. Flowmeters allowed adjustment of the flow rate to 2 l/min for both gases. In several experiments, a FOXY spectrophotometer with AL300 probe (Ocean Optics, Dunedin, FL) was used to monitor the time course of change in oxygen concentration at the entrance to the tracheal cannula.

Exposure protocol. Hypoxia was induced by switching from air to the 7% oxygen mixture for a period of 1 min. We selected a 1-min period of exposure to fully cover the range of change in oxygen level expected to occur in voluntary or sleep apnea (5, 27). In the group used for arterial blood-gas determinations, blood samples were taken while the animal breathed room air, beginning at 7, 27, or 54 s after switching to 7% oxygen and at the end of the recovery period. Arterial blood samples required a period of 6 s. To minimize blood loss from repetitive sampling, the recovery value after a hypoxic period was taken as the control for the subsequent period if that value was not different from the preceding control value.

In the animals used for microscopic determinations of oxygen tension, arterial pressure was recorded along with the oxygen level at the chosen site (an arteriole, venule, or tissue site at the venous end of the capillary network). During a 1-min control period, three oxygen measurements were obtained at 0, 30, and 60 s followed by measurements at 10, 30, and 60 s of hypoxia after which ventilation with air was restored. Measurements were continued at 30-s intervals for 2 min into the recovery period. After an intervening period of ~10 min, the protocol was repeated at a different site.

Capillary P_{O_2} could not be measured directly due to low signal level with our system in vessels this size. To obtain an estimate of capillary P_{O_2} for the purpose of evaluating capillary HbO_2 saturation, we first determined the difference between the P_{O_2} in postcapillary venules (<13-μm inner diameter), where the signal level was sufficient, and in adjacent tissue sites. Measurements were made during a control period, after 1 min of exposure to 10% oxygen, and after 2 min of recovery. These data were then used together with the P_{O_2} in tissue sites near the capillaries to estimate capillary P_{O_2} and HbO_2 saturation as described in RESULTS.

Statistics and data analysis. All values are presented as means ± SE. The statistical significance of changes in arteriolar, venular, and tissue P_{O_2} during the experimental procedure was determined by the repeated-measures one-way ANOVA followed by the Student-Newman-Kuels test. Comparisons between groups and between arterial blood values at different time points utilized a one-way ANOVA followed by the Student-Newman-Kuels test. A P value of <0.05 was considered significant. The coefficient of variation (CV; standard deviation/mean) was calculated for P_{O_2} values obtained from arterial blood, arteriolar, venular, and tissue sites for each time point in the study. To determine the CV of the measurement procedures, repeated determinations of arterial blood P_{O_2} were made on a single arterial blood sample and repeated determinations with the phosphorescence technique on a single blood sample in a microhematocrit tube. The CV for arterial blood P_{O_2} with the CHIRON blood gas-analyzer was 1.3% (n = 10) and 2.1% for P_{O_2} with the phosphoresence technique (n = 10). A two-way ANOVA was used to compare the CV in the microcirculation and tissue during control and hypoxia.

RESULTS

Hemodynamic measurements. Mean arterial pressure in the control period averaged 130 ± 2.4 mmHg. Mean red cell velocity in the control period averaged 8.0 ± 0.99 mm/s in arterioles and 2.2 ± 0.60 mm/s in venules. Arterial pressure fell to 81 ± 5 mmHg at 1 min of hypoxia and returned to 129 ± 4 mmHg 2 min after $F_{I_{O_2}}$ returned to 0.21.

Effect of hypoxia on inspired and arterial blood-gas levels. When $F_{I_{O_2}}$ was dropped from 0.21 to 0.07, the change at the inlet to the tracheal catheter, as determined with the Ocean Optics FOXY spectrophotometer, was 95% complete in 2.6 ± 0.12 s (time constant 0.61 ± 0.05 s) (n = 5). The return to 0.21 was 95% complete in 3.2 ± 0.18 s (time constant = 0.82 ±

(17 s) (n = 5). Arterial blood-gas determinations during hypoxia are shown in Table 1. The mean time interval between control and measurements during hypoxia was 26 min and between hypoxia and recovery measurements was 19 min. Arterial P_{O_2} averaged 98.0 ± 1.9 Torr in the control period and fell to 31.7 ± 0.9 Torr after 1 min. Almost all of the P_{O_2} change (93%) occurred in the first 10 s. The values at 10, 30, and 60 s of hypoxia were all significantly different from the control ($P < 0.001$) but not from each other. Recovery values were not significantly different from control.

The arterial P_{CO_2} level averaged 39.2 ± 0.9 Torr during the control period and fell to 27.2 ± 1.2 Torr after 1 min. The arterial P_{CO_2} levels at 10, 30, and 60 s were significantly less than control ($P < 0.001$) but not significantly different from each other. Note that most of the fall in arterial P_{CO_2} occurred in the first 10 s.

Arterial blood pH rose from 7.41 ± 0.01 in the control period to7.51 ± 0.02 after 1 min and returned to control levels in the recovery period. The 10-s, 30-s, and 1-min values were significantly different from control ($P < 0.001$) but not from each other.

Effect of hypoxia on microcirculatory and tissue P_{O_2}. The time course of change in microcirculatory oxygen levels during hypoxia is shown in Fig. 1. Under control conditions, P_{O_2} values were stable. The mean P_{O_2} during the control period was 52.2 ± 2.8 Torr in 29.0 ± 2.7-μm arterioles (n = 12), 35.1 ± 2.6 Torr in 29.7 ± 1.9-μm venules (n = 10), and 26.8 ± 1.7 Torr in the tissue interstitium near venous capillaries (n = 11). All of these values were significantly different from each other ($P < 0.05$).

When the hypoxic gas mixture was introduced, arteriolar P_{O_2} dropped rapidly with 68% of the change to the final value of 15.8 ± 1.2 Torr occurring in the first 10 s. All hypoxia values were significantly less than control ($P < 0.001$). The 30- and 60-s values in arterioles were significantly lower than the 10-s value ($P < 0.001$) but not from each other. Venular P_{O_2} reached a final value of 11.4 ± 1.0 Torr with 38% of the change occurring in the first 10 s. P_{O_2} values in venules at all time points during hypoxia were significantly lower than control ($P < 0.001$). P_{O_2} levels in venules at 30 and 60 s were significantly different from that at 10 s ($P < 0.001$) but not from each other. Tissue P_{O_2} near the venous capillaries fell to 9.6 ± 1.0 Torr at 60 s with 47% of the change occurring in the first 10 s. All hypoxia values in tissue were significantly less than control ($P < 0.001$). The 30- and 60-s values in tissue were significantly lower than the 10-s value ($P < 0.001$) but not from each other. When $F_{I_{O_2}}$ was returned to 0.21, the P_{O_2} at arteriolar and tissue sites returned to a stable value within 30 s, whereas the mean venular P_{O_2} appeared to lag somewhat; it was significantly different from the subsequent recovery values ($P < 0.01$). To summarize, during hypoxia, P_{O_2} in the microcirculation and tissue fell significantly from control to 10 s and from 10 to 30 s but not between 30 and 60 s. We note, however, that during the latter period the mean P_{O_2} in all three groups dropped (Fig. 1) and P_{O_2} decreased at 29 of the 33 measurement sites.

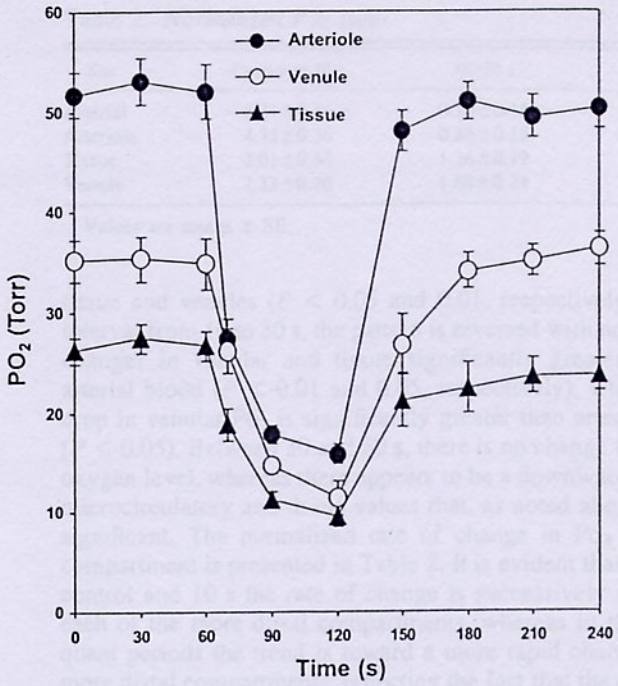

Fig. 1. P_{O_2} levels in arterioles, venules, and tissue sites in the venous capillary network during a 1-min control period, 1-min reduction of inspired oxygen fraction ($F_{I_{O_2}}$) from 0.21 to 0.07, and 2-min recovery period at 0.21. Data points are means ± SE.

Table 1. *Arterial blood gas and pH during hypoxia and recovery*

Time	P_{O_2}, Torr	P_{CO_2}, Torr	pH	n
Control	98.0±1.9	39.2±0.9	7.41±0.01	16
10 s	36.5±1.4	24.7±2.2	7.54±0.02	6
30 s	32.7±1.7	21.7±1.0	7.57±0.01	5
60 s	31.7±0.9	27.2±1.0	7.51±0.02	5
Recovery	95.4±2.3	37.3±1.9	7.42±0.20	15

Values are means ± SE; n, no. of animals.

Heterogeneity of P_{O_2} in the microcirculation and tissue increased during hypoxia. The mean CV rose significantly from 22% in the control period to 29% during hypoxia but was not significantly different among microcirculatory vessels and tissue. The increased CV during hypoxia was not due to variation in arterial P_{O_2} among animals as the latter was 9% in the control period and 8% during hypoxia. As noted in METHODS, the CV of the measurement procedures was much lower than the in vivo values, namely 1.3% for arterial blood gas and 2.1% for the phosphorescence technique.

Comparison of arterial, microcirculatory, and tissue oxygen tension changes during hypoxia. P_{O_2} levels in microvascular and tissue compartments are compared with arterial P_{O_2} in Fig. 2. The control value of arterial P_{O_2} was significantly greater than microcirculatory and tissue values and remained significantly above those levels throughout hypoxic exposure as shown in Fig. 2*A*. Similarly, microcirculatory and tissue values were significantly different from each other except for arterioles vs. venules at 10 s and venules vs. tissue at 60 s. In the first 10 s, the absolute change in arterial and arteriolar P_{O_2} was significantly greater than in venules and tissue, and the fall in arterial P_{O_2} was significantly more than arteriolar P_{O_2}. Between 10 and 30 s and between 30 and 60 s there were no significant differences in the change in P_{O_2} among compartments, although, as noted above, microcirculatory and tissue P_{O_2} did fall

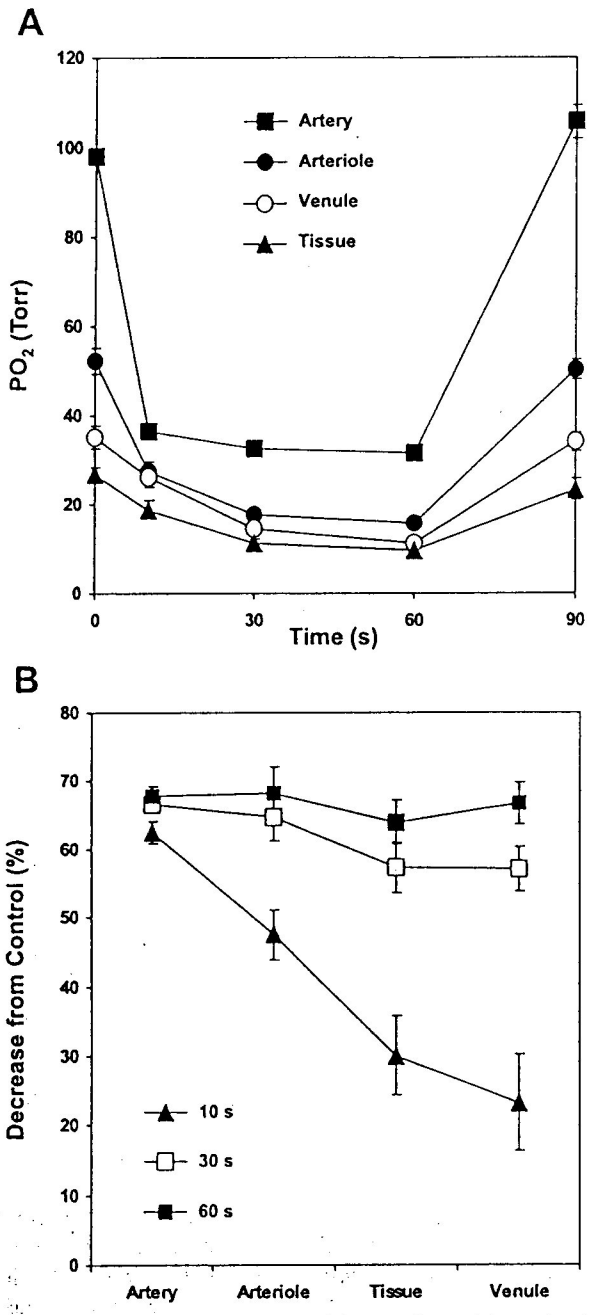

Fig. 2. *A*: Po_2 levels in arterial blood, arterioles, venules, and tissue sites in the venous capillary network during a 1-min reduction of Fi_{O_2} from 0.21 to 0.07 and a subsequent return to 0.21. *B*: percent changes in the Po_2 levels shown in *A*. Data points are means ± SE.

significantly between 10 and 30 s, whereas arterial Po_2 did not. The normalized changes for all compartments are shown in Fig. 2*B*. After 60 s of hypoxia, the percent changes are very similar in all compartments, ranging from 64 to 68%, and there are no significant differences among sites. However, the changes during earlier time periods are quite different. Po_2 in the arterial blood fell 63% below the control value in the first 10 s, which is not significantly different from the 47% fall in the arterioles but is significantly different from that in the tissue spaces (30%; $P < 0.01$) and the venules (23%; $P < 0.001$). The change in arteriolar Po_2 is also significantly greater than in

Table 2. *Normalized Po_2 rate*

Site	Control to 10 s	10–30 s	30–60 s
Arterial	6.24±0.16	0.20±0.14	0.03±0.03
Arteriole	4.75±0.36	0.86±0.16	0.12±0.06
Tissue	3.01±0.56	1.36±0.19	0.22±0.09
Venule	2.32±0.70	1.69±0.34	0.32±0.07

Values are means ± SE.

tissue and venules ($P < 0.05$ and 0.01, respectively). In the interval from 10 to 30 s, the pattern is reversed with normalized changes in venular and tissue significantly greater than in arterial blood ($P < 0.01$ and 0.05, respectively), whereas the drop in venular Po_2 is significantly greater than arteriolar Po_2 ($P < 0.05$). Between 30 and 60 s, there is no change in arterial oxygen level, whereas there appears to be a downward trend in microcirculatory and tissue values that, as noted above, is not significant. The normalized rate of change in Po_2 for each compartment is presented in Table 2. It is evident that between control and 10 s the rate of change is successively slower in each of the more distal compartments, whereas in the subsequent periods the trend is toward a more rapid change in the more distal compartments, reflecting the fact that the change is not complete in those areas.

Effect of hypoxia on longitudinal Po_2 differences between compartments. The sequential changes in oxygen levels as blood transits the circulatory system are shown in Fig. 3. In this graph, it is apparent that, under control conditions, most of the Po_2 drop occurs between the arterial and arteriolar sites, i.e., 45.9 compared with 25.4 Torr between arterioles and tissue adjacent to venous capillaries. There is a rise of 8.3 Torr from tissue to the venules, indicating an overall drop of 17.1 Torr from 29-μm arterioles to venules of similar size. After 10 s of hypoxia, the drop from the arterioles to tissue is virtually

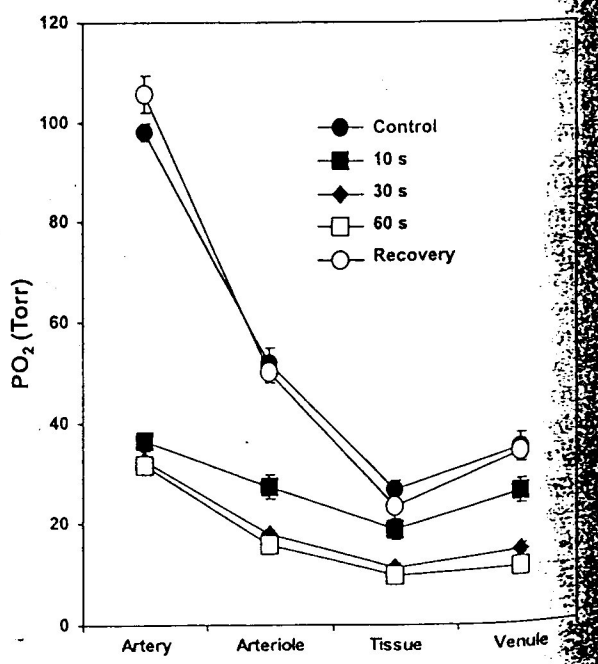

Fig. 3. Longitudinal profile of Po_2 levels in arterial, arteriolar, and venular blood and tissue sites in the venous capillary network during reduction of Fi_{O_2} from 0.21 to 0.07 for 1 min and return to 0.21. Data points are means

identical to that from arteries to arterioles (8.6 and 9.2 Torr). However, the increase from tissue to venules is 7.4 Torr, indicating that the principal oxygen loss at this time is upstream from these arterioles. At 30 and 60 s, the fall in mean P_{O_2} again is greater from artery to arteriole (14.9 and 16.0 Torr) than from arteriole to tissue near venous capillaries (6.6 and 6.2 Torr), whereas the rise in P_{O_2} from tissue to venules decreases to 3.4 Torr at 30 s and 1.8 Torr at 60 s. The difference in mean P_{O_2} between arterial and arteriolar compartments remained significant ($P < 0.05$) at all time periods. The difference between arteriolar and tissue P_{O_2} was significant ($P < 0.05$) at all time periods. The difference between tissue and venular P_{O_2} was significant at 10 and 30 s ($P < 0.05$) but not at 60 s.

Changes in HbO_2 saturation during hypoxia. The estimated HbO_2 saturation in the large artery and microcirculatory vessels during hypoxia is shown in Fig. 4 and was obtained from the P_{O_2} data shown above and the P_{50} and n_{50} values given in METHODS using Hill's equation. Corrections for pH are as described in METHODS.

Venous capillary HbO_2 saturation was estimated using the tissue P_{O_2} value (Fig. 2) obtained in the venous capillary region. To test the assumption that this tissue P_{O_2} can be used as an estimate of capillary P_{O_2}, we measured the difference in P_{O_2} between postcapillary venules (inner diameter of 10.4 ± 0.62 μm) and adjacent tissue ($n = 5$) during control, 1-min exposure to 10% oxygen in the inspired air, and recovery. The mean P_{O_2} in the postcapillary venules was 0.46 ± 0.56 Torr higher than in adjacent tissue, but the two values were not significantly different. Therefore, it was concluded that the tissue P_{O_2} values from Fig. 2 could be used as an estimate of P_{O_2} in the adjacent venous capillaries.

Under control conditions, the arterial blood is ~95% saturated, and arteriolar blood is 69% saturated. A somewhat larger difference (39%) is found between arterioles and venous capillaries with an increase of ~16% from that site to the venules.

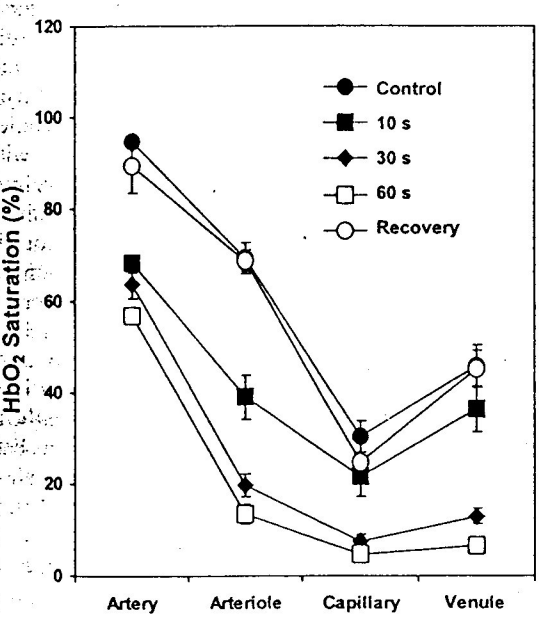

Fig. 4. Longitudinal profile of calculated oxyhemoglobin (HbO_2) saturation in arterial, arteriolar, venous capillary, and venular blood during reduction of F_{IO_2} from 0.21 to 0.07 for 1 min and return to 0.21. Data points are means ± SE.

Overall, there is a difference of 50% in HbO_2 saturation from artery to 30-μm venules. All HbO_2 saturation values during the control period were significantly different from each other. At 10 s into the hypoxic period, the HbO_2 saturation in the arterioles has fallen by about the same amount as in arterial blood, but the drop in the capillaries and venules is considerably less. As a consequence, the overall difference in HbO_2 saturation from artery to venule is now 32%. At 10 s, all HbO_2 saturation values are significantly different from each other except for arterioles vs. venules.

At 30 and 60 s of hypoxia, the overall arteriovenous difference in HbO_2 saturation returns to the control level (50%), but almost all of the oxygen loss now occurs in the arterial to arteriolar section. Arterial saturation at 30 and 60 s is significantly greater than microcirculatory levels. Arteriolar HbO_2 saturation is significantly greater than capillary and venular levels at 30 and 60 s, but the latter two compartments are not significantly different. It is also notable that at 60 s there is little oxygen left in the blood arriving at the venous capillaries and venules; the estimated HbO_2 saturation in these compartments is 5 and 6%, respectively.

The rate of change of HbO_2 saturation in the first 10 s (% saturation/s) was significantly greater in arterial blood than in venules and in arterioles compared with both capillaries and venules, but there were no significant differences in the subsequent periods. In contrast, the normalized rate was not significantly different among the compartments in the first 10 s of hypoxia, whereas in the 10- to 30-s period, the rate of desaturation was significantly greater in capillary and venular blood compared with arterial blood and in venular compared with arteriolar blood but not between arterial and arteriolar blood. The normalized rate of change was not significantly different among compartments in the 30- to 60-s period.

DISCUSSION

Systemic effects of hypoxia. Exposure to 7% oxygen for 1 min led to a substantial (40%) decrease in arterial pressure in this study. A similar finding has been reported previously in rats during hypoxia (9, 28) and has been attributed to a dominance of local vasodilator mechanisms in the peripheral circulation over centrally mediated vasoconstrictor mechanisms (9). However, we found in a separate study (unpublished results) using our hypoxic regimen that blood flow in the cremaster muscle falls to a greater degree than arterial pressure, suggesting that vasoconstriction may predominate in this muscle.

Causal factors in arterial blood desaturation. In this study, exposure to 7% oxygen in nitrogen led to a rapid drop in P_{O_2} at the entrance to the tracheal cannula that was 95% complete in 2.6 ± 0.12 s. The subsequent fall in arterial P_{O_2} was 93% complete at the time of our first measurement in hypoxia (10 s). Mean arterial P_{O_2} values at 30 and 60 s are slightly lower than at 10 s, but the differences were not statistically significant. Studies of the dynamics of arterial desaturation during apnea have led to the suggestion that the process occurs in two stages, the first being due to depletion of the oxygen store in the lung and the second due to depletion of oxygen in the blood (39). It was also shown that the oxygen level in the venous blood was an important determinant of the magnitude of the initial change. In the present study, depletion of the oxygen store in the lung would have occurred very rapidly with the shift in

inspired oxygen level. That may primarily account for the fall in the arterial blood P_{O_2} at 10 s. The venous oxygen level, as reflected in venular P_{O_2}, continued to fall throughout the hypoxic period, but depletion of this source apparently was not sufficient to cause a significant fall in the oxygen level of the arterial blood.

Oxygen levels in the circulation in the control state. Under control conditions, the P_{O_2} in arterial blood was 98 Torr and 52 Torr in 29-μm arterioles, as shown in Fig. 2. A substantial P_{O_2} gradient along the arteriolar network has been reported previously by a number of investigators (4, 8, 17, 34). Studies in this laboratory have shown, in addition, a large P_{O_2} gradient across the arteriolar wall, leading to the suggestion that the longitudinal gradient is mainly due to a high rate of oxygen consumption of the arteriolar wall (34). The further fall to 27 Torr at venous capillary tissue sites presumably reflects additional loss from the more distal arterioles and the capillary network. The small rise in P_{O_2} of 8 Torr from tissue sites to 30-μm venules is consistent with the findings of others (16, 29, 31) and may be due to mixing of well-oxygenated blood from high flow pathways with poorly oxygenated blood from low flow pathways (31). It may also reflect diffusive shunting of oxygen from arterioles to adjacent venules as seen in this muscle between paired small arteries and veins (19).

P_{O_2} changes in the circulation with acute hypoxia. In support of our first hypothesis, this study demonstrates a progressively slower fall in P_{O_2} in the more distal regions of the circulation compared with arterial blood during acute hypoxia. In the first 10 s, the change in arterial P_{O_2} was significantly greater than microcirculatory and tissue P_{O_2}, whereas arteriolar P_{O_2} fell more than venular and tissue P_{O_2}. P_{O_2} in arterial blood reached 93% of its final value, whereas the P_{O_2} change was 68% complete in 29-μm arterioles, 47% at venous capillary tissue sites, and 38% in 30-μm venules. In the remaining time periods, there were no significant differences among compartments in absolute changes in P_{O_2}, although between 10 and 30 s P_{O_2} did fall significantly in the microcirculation and tissue but not in arterial blood. Between 30 and 60 s, there was a downward trend in mean P_{O_2} of the microcirculation and tissue, but it was not statistically significant.

The slower time course of change in P_{O_2} in tissue and microcirculatory vessels compared with arterial blood is also evident in Fig. 2*B*, in which normalized (percent) changes are plotted. During the first 10 s, the normalized change of arterial P_{O_2} was significantly greater than that in venules and tissue but not compared with arterioles. Between 10 and 30 s, the trend reversed with the normalized change being significantly greater in tissue and venules compared with arterial blood and in venules compared with arterioles. The rate of change in P_{O_2} for the four compartments during the three time periods presented in Table 2 shows a slower change in the more distal compartments during the first 10 s and a subsequent reversal of that trend since the P_{O_2} in the more distal compartments continues to fall proportionately more than in the arterial compartment. This reflects the overall time course of fall in P_{O_2} during hypoxia, which was significantly longer in venules and tissue compared with arterial blood.

The slower changes in the microcirculation and tissue may reflect the time required for the deoxygenated blood to reach the microcirculation as well as the oxygen storage capacity of the tissue. The mean transit time of labeled red blood cells and plasma in the rat cremaster muscle has been reported to be ~5 s from artery to 28-μm arterioles and ~10 s from artery to 32-μm venules (3). These values are highly dependent on the state of vascular tone and would increase with a higher degree of tone, for example. The oxygen storage of the tissue can be estimated from the solubility of oxygen in muscle tissue (0.047 ml oxygen·ml tissue^{-1}·atm^{-1} at 35°C) (14). If the average tissue P_{O_2} falls by the same amount as in our measurement site (8.0 Torr in the first 10 s), the tissue would supply 0.00049 ml oxygen/ml tissue or 35% of the oxygen consumption of 0.79 ml·min^{-1}·100 g^{-1} (0.85 ml·min^{-1}·100 ml^{-1} assuming a specific gravity of 1.08) reported in another rodent muscle (hamster retractor) (10). An alternative calculation of oxygen consumption is based on the maximal rate of fall in tissue P_{O_2} of 1.44 Torr/s obtained at a tissue site 30 μm from a 20-μm venule when blood flow to the rat spinotrapezius muscle was occluded (25). This estimate is based on the maximal slope shown in Fig. 2 of that study. This fall is presumably due to oxygen consumption, and an 8-Torr drop would provide 52% of the oxygen consumption of the tissue. Between 10 and 30 s of hypoxia, the contribution of tissue oxygen stores to oxygen consumption would be half as great and in the last 30 s would be <5%. Because the P_{O_2} values used in these calculations were from the tissue area where P_{O_2} is lowest, these values likely underestimate the actual contribution. From these observations, it appears that both the blood transit time and the oxygen content of the tissues contribute significantly to the slower fall in microcirculatory and tissue P_{O_2} compared with arterial blood during brief hypoxia.

Changes in microvascular P_{O_2} at 1 min of hypoxia. As shown in Fig. 2*B*, at 1 min of hypoxia, the fall in P_{O_2} in arterial blood, the microcirculation and tissue are all very nearly in proportion to the drop in $F_{I_{O_2}}$ (67%). Similarly, Shah et al. (28) found that, after a 1-min exposure to 10% $F_{I_{O_2}}$, the mean P_{O_2} of a 1-mm-diameter region of the rat cremaster muscle determined with a similar technique fell to the same degree as the inspired gas. Figures 1 and 2 show that, in our studies, the absolute drop in P_{O_2} during hypoxia was much greater in the arterioles than in the other microvascular compartments, and as a consequence the difference between the arterioles and the tissue and venules decreased. In addition, at 1 min of hypoxia the rise in P_{O_2} from the tissue capillary site to the venules was much smaller than in the control state. In agreement with this finding, Stein et al. (30) found higher oxygen levels in the larger venules of the hamster retractor muscle compared with the small venules during normoxic breathing but not in hypoxia. This finding could be explained if the higher oxygen levels in the larger venules were due to uneven oxygen extraction at the capillary level in the control state and the site of major oxygen extraction shifted upstream to the arteriolar network in hypoxia. Alternatively, because the P_{O_2} difference between arterioles and venules also decreased greatly in our study, there would be less countercurrent exchange between such vessels lying adjacent to each other, as has been shown in this muscle from small arteries adjacent to small veins (19).

The CV of P_{O_2} in the microcirculation and tissue under control conditions was substantially higher than in the arterial blood, as would be expected due to heterogeneity of microcirculatory flow. With hypoxia, the CV of microcirculatory and tissue P_{O_2}, but not of arterial blood P_{O_2}, rose significantly. This observation does not support the hypothesis that the P_{O_2}

gradient in the venular network in the control state is due to flow heterogeneity. The increased CV in hypoxia may not be transitory because a similar finding was reported in myocardial venous oxygen saturation during 20-min hypoxia (40).

HbO_2 saturation with hypoxia. In this study, we found that the mean Po_2 in postcapillary venules (10.4 ± 0.6-μm inner diameter) is 0.46 ± 0.56 Torr higher than that in adjacent tissue, but the difference is not statistically significant. Based on this finding, we made the assumption that the Po_2 in capillary blood can be considered equivalent to that of adjacent tissue for purposes of calculating HbO_2 saturation.

The changes in HbO_2 saturation in the blood during hypoxia may be understood by reference to Figs. 3 and 4. During the control period, HbO_2 saturation drops by a little more than 60% from the artery to venous capillaries, with a small increase from these vessels to the 30-μm venules. After 10 s of hypoxia, arterial and arteriolar HbO_2 saturation have fallen by similar amounts, although the fall in Po_2 in the arterioles is much less than in the arteries (32 vs. 62 Torr), reflecting the steeper slope of the HbO_2 dissociation curve at lower Po_2. The degree of desaturation is also influenced by the leftward shift of the dissociation curve with a shift in arterial pH, which is already apparent with 10 s of hypoxia. This change is obviously secondary to hyperventilation since Pco_2 falls (Table 1) and tends to maintain a higher HbO_2 saturation than if ventilation were constant. At the capillary and venule level, the estimated drop in HbO_2 is small due to the very small change in Po_2 (9 Torr). At 30 s of hypoxia, there is little further change in arterial HbO_2 saturation but large changes in HbO_2 in arterioles and venules due to the greater changes in Po_2 and the steep slope of the dissociation curve at these Po_2 levels. Between 30 and 60 s, there is no further change in arterial Po_2 and HbO_2 and minor changes in estimated HbO_2 in the microvasculature with the small additional drop in Po_2.

HbO_2 saturation data also support our first hypothesis. During the first 10 s, the rate of change in HbO_2 saturation was significantly greater in the arterial compartment than in venules and arterioles compared with both capillaries and venules. When the data were normalized, differences were seen only in the 10- to 30-s period, where a significant downward trend was seen in capillaries and venules compared with arterial blood and in venules compared with arterioles. Compared with the Po_2 data, there were fewer instances of significant differences in the change in HbO_2 saturation due to the steeper slope of the dissociation curve in the microcirculatory vessels offsetting, in part, the smaller change in Po_2.

The fall in arterial HbO_2 of 40% at 1 min of hypoxia should lead to similar shifts in the microcirculatory compartments if oxygen delivery along that pathway remained constant. In fact, the venular HbO_2 saturation also fell 40% at 1 min, but the change was much greater in the arterioles, indicating that most of the oxygen loss at this time occurred upstream from these 29-μm vessels. It is possible that the loss upstream reflects slower blood flow in the arterioles, which would lead to a larger fraction of the total oxygen loss in these vessels. As noted above, we have observed that microcirculatory flow decreases more than the fall in arterial pressure during this hypoxic protocol (unpublished results). On the basis of this observation, it would be expected that the overall difference in HbO_2 saturation would increase during hypoxia. This was not seen for reasons that are not clear. In estimating the change in HbO_2 saturation in the microcirculation during hypoxia, we have assumed that the pH shift is equivalent to that in the arterial blood. If the shift is less, HbO_2 saturation in the microvascular vessels during hypoxia would be lower than we have calculated.

Changes in tissue Po_2 during hypoxia The tissue Po_2 values we obtained in the control state (26.8 ± 1.7 Torr) are in the upper part of the range found previously in this muscle; with the use of oxygen microelectrodes, mean values of 19 and 30 Torr were reported in two studies of tissue Po_2 (21, 24). A value of 22.8 ± 3.3 Torr was found in the vicinity of venous capillaries of cat sartorius muscle using oxygen microelectrodes (4). In rat spinotrapezius muscle, the Po_2 obtained with the phosphorescence technique at tissue sites 30 μm from a 20-μm venule was 17.1 ± 0.5 Torr (25).

In the rat spinotrapezius muscle, tissue levels of NADH began to rise when interstitial Po_2 measured by the phosphorescence technique fell to 2.4–2.9 Torr (25). In the present study, the mean interstitial Po_2 in the vicinity of venous capillaries was 9.6 ± 1.0 Torr at the end of the 60-s hypoxic exposure and the two lowest individual values were 5.4 and 5.5 Torr. Based on an earlier study (23), the venous capillary network is the region where a shift to anaerobic metabolism would first occur. Therefore, the findings of this study do not support our hypothesis that during this hypoxic regimen tissue Po_2 would fall to a level at which a shift to anaerobic metabolism is known to occur. We note, however, that the variability in Po_2 (CV) in the microcirculation and tissue was somewhat greater during hypoxia than in the control state, increasing the likelihood that some tissue areas would become hypoxic, although none were seen in this study. It should also be noted that our control tissue Po_2 values were near the high end of the range previously reported and may reflect a lower level of arteriolar vascular tone (36), although mean red cell velocity in the arterioles (8.0 ± 0.99 mm/s) in our study appears to be about one-third less than that found in another study on vessels of similar size in the same muscle (21). Under conditions of higher vascular tone or any other condition that would decrease the ratio of oxygen delivery to oxygen consumption, the critical Po_2 could be reached. Intracellular acidosis as determined with ^{31}P-NMR developed in the rat brain when arterial Po_2 was reduced to ~41 Torr (26).

Possible relation to sleep apnea. Our findings may provide some insight to the changes in oxygen tension in microcirculatory vessels and tissue during sleep apnea. In that disorder, the mean duration of apnea is 21–24 s, according to clinical reports (5, 27), and thus would fall within the time frame where the greatest change occurred in our study. A study by Fletcher et al. (13) showed that arterial oxygen saturation fell 25.5% over a 30-s period during obstructive apnea in baboons, whereas in our study estimated arterial saturation decreased 31% in this same time period. This suggests that with obstructive apnea of 30-s duration the fall in Po_2 levels in the microcirculation and tissues would be slightly less than those observed in our study, at least in vascular beds with similar ratios of blood flow to oxygen consumption. Although the tissue interstitial Po_2 with 30-s hypoxia is considerably lower than seen under control conditions, it is still well above the threshold for a shift to anaerobic metabolism. It appears, therefore, that episodes of sleep apnea are not likely to induce anaerobic metabolism in resting muscle, although the increased

heterogeneity of microcirculatory P_{O_2} with hypoxia makes it difficult to rule this out completely. Our finding that the change in P_{O_2} in the microcirculation and tissue at equilibrium is very nearly proportional to the change in inspired gas may be useful in interpreting changes in sleep apnea. In the latter case, the rate of change in the alveolar gas and arterial blood is slower than in this study, and as a consequence it is likely that the time lag between P_{O_2} changes in the arterial blood and the microcirculation in sleep apnea is much less than with a step change.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Kristina Flores, Scott Dunning, Yan Zheng, and Patricia Nance.

GRANTS

This study was supported by National Heart, Lung, and Blood Institute Grants HL-66318, HL-62354, HL-64395, and HL-62318.

REFERENCES

1. **Baez S.** An open cremaster muscle preparation for the study of blood vessels by in vivo microscopy. *Microvasc Res* 5: 384–394, 1973.
2. **Bakehe M, Miramand JL, Chambille B, Gaultier C, and Escourrou P.** Cardiovascular changes during acute episodic repetitive hypoxic and hypercapnic breathing in rats. *Eur Respir J* 8: 1675–1680, 1995.
3. **Baker CH, Wilmoth FR, and Sutton ET.** Red blood cell and plasma distribution in SHR cremaster muscle microvessels. *Am J Physiol Heart Circ Physiol* 242: H381–H391, 1982.
4. **Boegehold MA and Johnson PC.** Periarteriolar and tissue P_{O_2} during sympathetic escape in skeletal muscle. *Am J Physiol Heart Circ Physiol* 254: H929–H936, 1988.
5. **Bradley TD, Martinez D, Rutherford R, Lue F, Grossman RF, Moldofsky H, Zamel N, and Phillipson EA.** Physiological determinants of nocturnal arterial oxygenation in patients with obstructive sleep apnea. *J Appl Physiol* 59: 1364–1368, 1985.
6. **Bryan PT and Marshall JM.** Adenosine receptor subtypes and vasodilatation in rat skeletal muscle during systemic hypoxia: a role for A1 receptors. *J Physiol* 514: 151–162,1999.
7. **Cabel M, Smiesko V, and Johnson PC.** Attenuation of blood flow-induced dilation in arterioles after muscle contraction. *Am J Physiol Heart Circ Physiol* 266: H2114–H2121, 1994.
8. **Duling BR and Berne RM.** Longitudinal gradients in perivascular oxygen tension. A possible mechanism for the participation of oxygen in the local regulation of blood flow. *Circ Res* 27: 669–678, 1970.
9. **Edmunds NJ and Marshall JM.** Oxygen delivery and oxygen consumption in rat hindlimb during systemic hypoxia: role of adenosine. *J Physiol* 536: 927–935, 2001.
10. **Ellsworth ML and Pittman RN.** Heterogeneity of oxygen diffusion through hamster striated muscles. *Am J Physiol Heart Circ Physiol* 246: H161–H167, 1984.
11. **Findley LJ, Ries AL, Tisi GM, and Wagner PD.** Hypoxemia during apnea in normal subjects: mechanisms and impact of lung volume. *J Appl Physiol* 55: 1777–1783, 1983.
12. **Fletcher EC.** Effect of episodic hypoxia on sympathetic activity and blood pressure. *Respir Physiol* 119: 189–197, 2000.
13. **Fletcher EC, Goodnight-White S, Munafo D, Miller 3rd CC, Luckett R, and Qian W.** Rate of oxyhemoglobin desaturation in obstructive vs. nonobstructive apnea. *Am Rev Respir Dis* 143: 657–660, 1991.
14. **Homer LD, Shelton JB, Dorsey CH, and Williams TJ.** Anisotropic diffusion of oxygen in slices of rat muscle. *Am J Physiol Regul Integr Comp Physiol* 246: R107–R113, 1984.
15. **Imadojemu VA, Gleeson K, Gray KS, Sinoway LI, and Leuenberger UA.** Obstructive apnea during sleep is associated with peripheral vasoconstriction. *Am J Respir Crit Care Med* 165: 61–66, 2002.
16. **Intaglietta M, Johnson PC, and Winslow RM.** Microvascular and tissue oxygen distribution. *Cardiovasc Res* 32: 632–643, 1996.
17. **Kerger H, Torres Filho IP, Rivas M, Winslow RM, and Intaglietta M.** Systemic and subcutaneous microvascular oxygen tension in conscious syrian golden hamsters. *Am J Physiol Heart Circ Physiol* 268: H802–H810, 1995.
18. **Kobayashi H and Takizawa N.** Oxygen saturation and pH changes in cremaster microvessels of the rat. *Am J Physiol Heart Circ Physiol* 270: H1453–H1461, 1996.
19. **Kobayashi H and Takizawa N.** Imaging of oxygen transfer among microvessels of rat cremaster muscle. *Circulation* 105: 1713–1719, 2002.
20. **Lesske J, Fletcher EC, Bao G, and Unger T.** Hypertension caused by chronic intermittent hypoxia-influence of chemoreceptors and sympathetic nervous system. *J Hypertens* 15: 1593–1603, 1997.
21. **Lombard JH, Frisbee JC, Greene AS, Hudetz AG, Roman RJ, and Tonellato PJ.** Microvascular flow and tissue P_{O_2} in skeletal muscle of chronic and reduced renal mass hypertensive rats. *Am J Physiol Heart Circ Physiol* 279: H2295–H2302, 2000.
22. **Mian R and Marshall JM.** Responses observed in individual arterioles and venules of rat skeletal muscle during systemic hypoxia. *J Physiol* 436: 485–497, 1991.
23. **Pal M, Toth A, Ping PP, and Johnson PC.** Capillary blood flow and tissue metabolism in skeletal muscle during sympathetic trunk stimulation. *Am J Physiol Heart Circ Physiol* 274: H430–H440, 1998.
24. **Prewitt RL and Johnson PC.** The effect of oxygen on arteriolar red cell velocity and capillary density in the rat cremaster muscle. *Microvasc Res* 12: 59–70, 1976.
25. **Richmond KN, Shonat RD, Lynch RM, and Johnson PC.** Critical P_{O_2} of skeletal muscle in vivo. *Am J Physiol Heart Circ Physiol* 277: H1831–H1840, 1999.
26. **Rolett EL, Azzawi A, Liu KJ, Yongbi MN, Swartz HM, and Dunn JF.** Critical oxygen tension in rat brain: a combined ^{31}P-NMR and EPR oximetry study. *Am J Physiol Regul Integr Comp Physiol* 279: R9–R16, 2000.
27. **Sforza E, Krieger J, Weitzenblum E, Apprill M, Lampert E, and Ratamaharo J.** Long-term effects of treatment with nasal continuous positive airway pressure on daytime lung function and pulmonary hemodynamics in patients with obstructive sleep apnea. *Am Rev Respir Dis* 141: 866–870, 1990.
28. **Shah S, Allen J, Wood JG, and Gonzalez NC.** Dissociation between skeletal muscle microvascular P_{O_2} and hypoxia-induced microvascular inflammation. *J Appl Physiol* 94: 2323–2329, 2003.
29. **Shonat RD and Johnson PC.** Oxygen tension gradients and heterogeneity in venous microcirculation: a phosphorescence quenching study. *Am J Physiol Heart Circ Physiol* 272: H2233–H2240, 1997.
30. **Stein JC, Ellis CG, and Ellsworth ML.** Relationship between capillary and systemic venous P_{O_2} during nonhypoxic and hypoxic ventilation. *Am J Physiol Heart Circ Physiol* 265: H537–H542, 1993.
31. **Swain DP and Pittman RN.** Oxygen exchange in the microcirculation of hamster cremaster muscle. *Am J Physiol Heart Circ Physiol* 256: H247–H255, 1989.
32. **Tahawi Z, Orolinova N, Joshua IG, Bader M, and Fletcher EC.** Altered vascular reactivity in arterioles of chronic intermittent hypoxic rats. *J Appl Physiol* 90: 2007–2013; 2001.
33. **Teisseire BP, Soulard CD, Herigault RA, Leclerc LF, and Laver MB.** Effects of chronic changes in hemoglobin-O_2 affinity in rats. *J Appl Physiol* 46: 816–822, 1979.
34. **Tsai AG, Friesenecker B, Mazzoni MC, Kerger H, Buerk DG, Johnson PC, and Intaglietta M.** Microvascular and tissue oxygen gradients in the mesentery. *Proc Natl Acad Sci USA* 95: 6590–6595, 1998.
35. **Tsai AG, Friesenecker B, McCarthy M, Sakai H, and Intaglietta M.** Plasma viscosity regulates capillary perfusion during extreme hemodilution in hamster skinfold model. *Am J Physiol Heart Circ Physiol* 273: H2170–H2180, 1998.
36. **Tsai AG, Johnson PC, and Intaglietta M.** Oxygen gradients in the microcirculation. *Physiol Rev* 83: 933–963, 2003.
37. **Vandegriff KD, Rohlfs RJ, Magde MD Jr, and Winslow RM.** Hemoglobin-oxygen equilibrium curves measured during enzymatic oxygen consumption. *Anal Biochem* 256: 107–116 1998.
38. **Vanderkooi JM, Maniara G, Green TJ, and Wilson DF.** An optical method for measurement of dioxygen concentration based upon quenching of phosphorescence. *J Biol Chem* 262: 5476–5482, 1987.
39. **Wilkinson MH, Berger PJ, Blanch N, and Brodecky V.** Effect of venous oxygenation on arterial desaturation rate during repetitive apnea in lamb. *Respir Physiol* 101: 321–331, 1995.
40. **Zhu N and Weiss HR.** Myocardial venous O_2 saturation becomes more heterogeneous during hypoxic and carbon monoxide hypoxia. *Microvasc Res* 49: 253–267, 1995.