Microvascular oxygen distribution in awake hamster window chamber model during hyperoxia

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Hyperoxic ventilation has been proposed as a means to augment tissue oxygenation through the increase of arterial oxygen content in conditions of anemia by increasing the amount of oxygen dissolved in plasma (9). This procedure is used clinically to increase oxygen delivery to tissues jeopardized by ischemia, such as in conditions of unstable angina, myocardial infarction, and stroke (2). It has also been advocated as a means for bringing oxygen to the tissues using fluorocarbon-based oxygen carriers (10), a blood substitute that carries oxygen as a function of its solubility. This material, when used in physiological concentrations and conditions, carries about three to four times the amount of oxygen dissolved by plasma; therefore, it provides additional transport capacity only at high oxygen partial pressures.

Although there is some acceptance that oxygen supplementation may be beneficial, there is no overwhelming evidence that it produces a positive clinical outcome. This may be due to the fact that the increased tissue PO2 seen with increased oxygen inhalation is accompanied by lowered tissue perfusion as a consequence of vasoconstriction, which has been demonstrated in many vascular beds, such as the brain, heart, and limbs.

A different form of arteriolar vessel wall hyperoxia may be induced by using molecular hemoglobin as an oxygen carrier in the formulation of blood substitutes that are presently used in clinical trials related to oxygen-facilitated diffusion by oxyhemoglobin (27). In this context, Winslow (38) proposed that the detrimental vasoconstrictor effect found with these materials is due to autoregulatory blood flow restriction aimed at reducing oxygen oversupply to arteriolar wall from the augmented oxygen flux from oxyhemoglobin diffusion. This additional oxygen flux was found to significantly increase the oxygen transfer from the oxygenated red blood cell (RBC) mass to the arteriolar blood vessel wall even when modest concentrations of molecular hemoglobin were introduced in the circulation.

Hyperoxia causes vasoconstriction (1, 6, 22, 24, 29). In the study of Lindbom and Arfors (17), exposure of muscle to high oxygen content superfusion solution caused a decrease in functional capillary density (FCD, number of capillaries with RBC transit per unit volume of tissue). Thus the final outcome in terms of tissue oxygenation cannot be predicted because the reduction of capillary perfusion may lead to a decrease in the tissue oxygen supply. The study of Thorborg et al. (30) shows that the distribution of tissue Po2 on the surface of skeletal muscle increases mean tissue Po2 when inspired oxygen (FiO2) is progressively increased from normal (0.21) to 0.70. However, the dispersion of tissue values increases in such a fashion that although the average tissue Po2 is higher, there is a greater number of tissue regions with low and high Po2. In other words, the distribution presents a standard deviation that is increased relative to the absolute in-
crease of the average of the distribution. In contrast, Whalen and Nair (36) showed a fall in deep tissue Po2 of cat skeletal muscle when the surface of the tissue was exposed to elevated oxygen levels. The same effect was also observed by Duling (6), who noted reduced perivascular Po2 in the arterioles of the hamster cheek pouch when elevating the Po2 of the suffusion solution. These findings suggest that hyperoxia may not provide a uniform benefit for tissue oxygenation. Thus the outcome in terms of tissue oxygenation cannot be predicted because the reduction of capillary perfusion may lead to a decrease in the tissue oxygen supply.

Recent measurements of oxygen tension distribution in the microcirculation of intact tissue, isolated from the environment, in awake animals (11) show a significant amount of oxygen is released by the arteriolar circulation a priori to blood arriving at the capillaries, a process that establishes a longitudinal oxygen gradient along the arterial vascular tree. There is evidence that this distribution is sensed and that the process of metabolic autoregulation attempts to maintain it at near-normal conditions by regulating oxygen delivery through vasoconstriction and vasodilatation, which modulate blood flow, a process that ultimately controls the level of tissue oxygenation (8). In this context vasoconstriction makes hyperoxic ventilation self-limiting as a means of increasing tissue oxygen delivery, whereas the corresponding increase in peripheral vascular resistance may impair microvascular function.

Whereas increased FiO2 has been proposed to increase tissue Po2, direct measurement of O2 transfer in the microcirculation has not previously been made. The present study was carried out to determine the functional effects of normobaric hyperoxic ventilation (100% O2, FiO2 = 1.00) at the single microscopic blood vessel level. The objective was to determine the extent by which vasoconstriction interferes with oxygen delivery when blood oxygen content and Po2 are increased in the tissue of the intact and unanesthetized model of the hamster window preparation and to determine the related systemic hemodynamic responses.

MATERIALS AND METHODS
Animal Preparation

Investigations were performed in Golden Syrian hamsters (Simonsen; Gilroy, CA) of 55–65 g body wt size. Animal handling and care was provided in accordance with the procedures outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was approved by the local Animal Subjects committee. The hamster window chamber model is widely used for microvascular studies in the unanesthetized state, and the surgical technique is described in detail elsewhere (7). Briefly, the animal was prepared for chamber implantation with a 50-mg/kg ip injection of pentobarbital sodium anesthesia. After hair removal, sutures were used to lift the dorsal skin of the back away from the animal, and one frame of the chamber was positioned on the animal’s dorsum. The chamber consists of two identical titanium frames with a 15-mm circular window. One side of the skinfold was removed following the outline of the window with the aid of backlighting and a stereomicroscope. Subcutaneous and retractor muscle was removed until only a thin layer of retractor muscle and the intact subcutaneous skin of the opposing side remained. The exposed tissue was sealed with a glass coverslip incorporated into the other frame of the chamber. The intact skin on the other side of the skinfold remained exposed to the ambient environment. The animal was allowed at least 2 days for recovery, at which time the chamber was assessed under the microscope (×650) for signs of edema, bleeding, or unusual neovascularization. Barring these complications, the animal was reanesthetized, and arterial and venous catheters were implanted into the carotid artery (polyethylene-50) and jugular vein (polyethylene-10), respectively. Catheters were filled with a heparinized saline solution (30 IU/ml) to ensure their patency during the experiments. The catheters were tunneled under the skin and exteriorized at the dorsal side of the neck where they were attached to the chamber frame with tape away from the grasp of the animal. Experiments were performed after at least 24 h but within 48 h of catheter implantation.

Inclusion criteria. Animals were included in the study if their systemic parameters were 1) heart rate (HR) > 320 beats/min; 2) mean systemic blood pressure (MAP) > 80 mmHg; 3) systemic hematocrit (Hct) > 45%; and 4) systemic arterial Po2 > 50 mmHg. The microvasculature of the chamber tissue was examined under low (×150) and high (×650) magnification for edema and bleeding. Animals were excluded from the study when these signs of trauma were observed. These inclusion criteria represent the minimum acceptable parameters rather than the range of normal values used in our previous studies. This approach provides for lesser rejection in the number of animals to be included in the study, whereas the variability ensuing from the greater disparity in systemic data is compensated by analyzing results in terms of effects relative to controls.

Systemic Parameters

MAP was tracked continuously during the experimental period, and the HR was determined from the pressure trace (Spectrum Pressure Transducer, Biopac; Santa Barbara, CA). Systemic Hct was measured from centrifuged arterial blood sample taken in heparinized capillary tubes (Readacrit Centrifuge, Clay Adams, Division of Becton and Dickinson; Parsippany, NJ).

Blood Chemistry

Arterial and venous blood was sampled from the carotid artery and jugular vein catheters into heparinized capillary tubes and immediately analyzed for Po2, Pco2, and pH at 37°C (pH/blood gas analyzer, model 248, Bayer). Hemoglobin concentration was measured using a hand-held photometer (B-Hemoglobin; Hemocue, Sweden).

Cardiac Output Measurements

Cardiac output was measured by a modified thermodilution technique (4a). A saline indicator (bolus of 0.15 ml at 25°C) was injected via the jugular vein catheter, and the change in temperature was detected at the aortic arch using a thermocouple (0.15 diameter, IT18T75, Physitemp Instruments) placed at the tip of the carotid artery catheter. Normally with the standard thermodilution method, the indicator is injected directly into the right atrium. In the present configuration, the indicator also passes through the lungs, increasing the heat exchange area. This procedure was selected because in these small awake animals it is not feasible
to inject the indicator directly into the right atrium. The conversion of the area under the indicator dilution temperature curve into flow was done with a fluid circuit that reproduced flow rates, volumes, and heat exchange properties of the hamster circulation between the locations of saline injection and temperature measurements, which included the superior and inferior vena cava, the right side of the heart, the pulmonary artery, the lungs, and the left side of the heart and the aortic arch. Ventricles and the atrium volumes were dimensioned assuming that heart and lung volumes were 10 and 7% of blood volume, respectively. The model was made with rubber tubing, of which the size simulated the different compartments and arteries and veins of our small animals, and subjected to different flow rates.

Thermodilution curves peaked rapidly and decayed exponentially. The signal due to recirculation was eliminated by truncating the curve 30–50% after the maximum change in temperature. Calculation of the area under the thermodilution curve was approximated using a gamma fit (20). This modified thermodilution technique was compared with the carboxyfluorescein-labeled (DF labeled) erythrocytes method in both paired and unpaired measurements and in three animals of which the weights were 85, 92, and 107 g. Cardiac index (means ± SD) was 273.2 ± 14.7 ml·min⁻¹·kg⁻¹ using the thermodilution method and 261.6 ± 44.7 ml·min⁻¹·kg⁻¹ using labeled erythrocytes.

Measurements were done under normoxic conditions and in hyperoxic conditions after an adaptation period of 30 min. The area under the curve of temperature over time, recorded by the thermocouple, was estimated using a computer algorithm previously calibrated in the in vitro circuit. This area is proportional to cardiac output when the corresponding calibration is applied. Cardiac index is calculated from the cardiac output divided by the animal weight.

**Microhemodynamic Parameters**

Detailed mappings were made of the chamber vasculature so that the same vessels were studied throughout the experiment.

*Functional capillary density.* Functional capillaries, defined as capillary segments, i.e., capillary conduits between bifurcations that have RBC transit in a 30-s period, were assessed in a region of ~0.5 mm², recording all capillaries in the field of view. Observation of this region was done system- atically by displacing the microscopic field of view by a field width across the window chamber in 10 to 15 successive steps. The first field was chosen by some distinctive anatomic landmark (i.e., large bifurcation) to easily and quickly rees- tablish the same fields, and vessels after the oxygen content of the inspired air was changed. Each field at this magnification has two to five capillaries. FCD (cm⁻¹), defined as the total length of RBC-perfused capillaries divided by the area of the microscopic field of view, was evaluated by measuring and adding the length of capillaries that had RBC transit. The relative change in FCD from baseline levels after the intervention is an indicator of capillary perfusion.

*Arterioles and venules.* Microvessels were classified according to their position in the microvascular network (16). Arterioles were categorized into large feeding arterioles (A1), small arcading arterioles (A2), transverse arterioles (A3), and terminal arterioles (A4). Venules were classified as large venules (V1) and small collecting venules (Vc). Articolar and venular diameters were measured by the video image shear- ing method (13). When arterioles exhibited vasomotion, the diameter was measured for 1 min and averaged over this interval. Articolar and venular RBC velocity were measured on-line by using the photodiode/cross-correlator system of Intaglietta et al. (12) using a fiber optic photo diode pickup and a Velocity Tracker (model 102B of Vista Electronics; San Diego, CA). The measured centerline velocity was corrected according to vessel size to obtain the mean RBC velocity (V) (18). Blood flow was calculated from measured parameters as Q = V · πD²/4 where D is diameter.

**Microvascular PO₂ Distribution**

O₂ measurements were made using the Pd-phosphorescence quenching method developed by Wilson (37), which was later adapted for microcirculatory studies by Torres Filho and Intaglietta (31). This noninvasive method of measuring oxygen levels is based on the oxygen-dependent quenching of phosphorescence emitted by albumin-bound metalloporphyrin complex after pulsed light excitation. The decay rate of the light-excited phosphorescence is inversely proportional to the partial pressure of oxygen according to the Stern-Volmer equation (31). The Pd-phosphorescence quenching method has been used in this preparation for both intravascular and extravascular oxygen tension measurements because albumin exchange between plasma and tissue allows for suffi- cient concentrations of albumin-bound dye within the interstitium to achieve an adequate signal-to-noise ratio (4, 32). Animals received a slow intravenous injection of 15 mg/kg body wt at a concentration of 10.1 mg/ml of a palladium- meso-tetra(4-carboxyphenyl)porphyrin (Porphyin Products; Logan, UT). The dye was allow to circulate for 10 min before oxygen measurements.

In our system, intravascular measurements are made by placing an optical rectangular window ~5 μm × 40 μm longitudinally within the vessel of interest, with the longest side of the rectangle being positioned parallel to the vessel wall. Tissue PO₂ measurements were performed within intercapillary spaces in regions void of large vessels with an optical window size of 6 μm × 6 μm. The decay curves were analyzed off-line, using a standard single exponential least squares numerical fitting technique, and the resultant time constants were applied to the Stern-Volmer equation (35) to calculate PO₂ using parameters corrected for this animal model. The phosphorescence decay due to quenching at a specific PO₂ yields a single decay constant, and in vitro calibration has been demonstrated to be valid for in vivo measurements (35).

**Tissue Oxygen Consumption from Microvascular Data**

The microvascular methodology used in our studies allows a detailed analysis of oxygen consumption in the tissue as presented in the DISCUSSION, which includes data from the present study and data from other control studies published from this laboratory, in the same animal species and prepa- ration and using the same protocol. Calculations of oxygen consumption are made using the equation

\[
O_2 \text{ delivery} = \left[ (Hb_{RBC} \times \gamma \times \Delta_{AV} \% \times (1 - Hct) \times \alpha \times \Delta_{AV}PO_2) \times Q \right] 
\]

where Hb_{RBC} is the hemoglobin in RBCs expressed as grams per deciliter of plasma, γ is the oxygen-carrying capacity of hemoglobin at 100% saturation or 1.38 ml O₂/g Hb, Δ_{AV} is the difference in arteriolar-venular oxygen saturation of RBCs, 1 − Hct is the fractional plasma volume and converts the equation from per deciliter of plasma to per deciliter of blood, α is the solubility of oxygen in plasma, 2.14 × 10⁻⁹ ml O₂/ml plasma mmHg, Δ_{AV}PO_2 is the arteriolar-venular diff- erence in partial pressure of oxygen, and Q is the averaged
microvascular flow for all microvessels as percentage of baseline. The oxygen dissociation curve for hamster RBCs was determined from freshly collected blood (34).

Experimental Design

The unanesthetized animal was placed in a restraining tube. A plastic tent with an inlet valve connected to a gas tank was placed in the front of the restraining tube. The gas flow rate into the tent was diffused by a cotton filter barrier so that the hamster was not subjected to a direct stream of gas flow. The restraining tube was attached to the stage of an inverted microscope (IMT-2 Olympus; New Hyde Park, NY) equipped with a water immersion ×40 objective (Olympus Wplan, numerical aperture = 0.7). The tissue image was projected onto a CCD camera (COHU, San Diego 4815-2006) connected to a video cassette recorder (AG-7355, Panasonic) and viewed on a Sony monitor (PVM-1271Q).

Sites of investigation were chosen based on their visual acuity. At least six to eight arterioles and six to eight venules in each animal were chosen for investigation using visual acuity as the first criterion and second in terms of vessel type within the network. The same sites of study were followed throughout the experiment so that comparisons could be made directly to baseline levels.

Fields of interest were chosen while the animal was subjected to a flow of room air into the tent. Baseline systemic parameters, blood gas analysis, vascular diameter, RBC velocity, and FCD measurements commenced at least 30 min after the animal had become accustomed to the restraining tube and to the tent air-flow system. After microvascular and systemic baseline characterization, the inlet gas to the plastic tent was switched to 100% O2 to induce hyperoxia. After 30 min of exposure, systemic parameters and blood gas analysis were evaluated, as well as FCD. The Pd-porphyrin dye was injected over 1 min intravenously and allowed 10–15 min of exposure. Intravascular oxygen tension in the vessels chosen for study was measured along with vessel diameter and RBC velocity.

Microvascular characterization and intravascular PO2 measurements were followed by interstitial tissue PO2 measurements. Finally, blood gas analysis was repeated to ensure that the induced oxygen level had been maintained during the observation period. Skin temperature was continuously monitored throughout the experiment via a temperature probe placed on the back of the skinfold.

Control data for microvascular PO2 were obtained from our database, which includes over 50 independent experiments carried out over a period of 10 years using the same animal model (11, 16, 33). This procedure is in part justified because the use of the Pd-porphyrin complex for PO2 measurement is considered for study was measured along with vessel diameter and RBC velocity.

All measurements were compared with corresponding values at baseline when the animal is exposed to room air: except for the microvascular oxygen tensions, which PO2 levels were compared between two groups of animals (control group). Comparisons were made with the Mann-Whitney rank sum test (Statistica Windows version 2.03; SPSS). Changes are considered statistically significant if P ≤ 0.05. Trends in the data, independent of the scatter, are visualized by presenting the data in terms of means ± SE. Data are presented as absolute values and ratios relative to baseline values. A ratio of 1.0 would signify no change from baseline, whereas lower and higher numbers are indicative of changes, respectively, higher and lower than baseline (i.e., 1.5 would mean a 50% increase from baseline levels and 0.5 would mean a 50% decrease in baseline levels). Calculations of global oxygen transport parameters and peripheral vascular resistance that are not directly measurable in our model are based on mean values of measured parameters. These calculations can be identified as those presented without standard deviations to focus on their tendencies rather than on the variability of the measurement.

RESULTS

The microvascular study was performed on six animals. Cardiac output measurements were carried out on another group of five animals. All animals entered into the study completed the experimental protocol.

Table 1 summarizes the changes in systemic parameters in response to hyperoxia. Alteration in the inspired gas (FiO2 = 1.00) did not statistically modify blood pressure and HR in these animals; however, it significantly changed cardiac index; cardiac index was reduced to 0.75 ± 0.07 of baseline. 100% oxygen inspira
tion caused a significant increase in arteriolar and venular PO2 (P < 0.05); 2) an increase in arterial and venous PCO2; and 3) a decreased arterial and venous pH. These changes are expected and should be the consequence of hypoventilation, a normal response to hyperoxia.

The microvascular diameter responses to FiO2 = 1.00 are shown in Fig. 1. FiO2 resulted in a statistically significant diameter decrease in A1 vessels (0.90 ± 0.03), A2 (0.77 ± 0.03), and A3 (0.81 ± 0.05), respectively. Changes in venular diameter were not significantly different from baseline. FCD was reduced to 0.74 ± 0.16 (P < 0.05) of baseline. Figure 2 shows the change of the RBC flow velocity in arterioles and venules following FiO2 = 1.00. Inhalation of high oxygen levels tended to increase flow velocity, which was statistically significant in the small arterioles A2 (1.22 ± 0.08) relative to baseline.

Table 1. Systemic parameters and blood gas analysis during baseline and 100% O2 inspiration

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>100% O2</th>
</tr>
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<tbody>
<tr>
<td>Blood pressure, mmHg</td>
<td>88.3 ± 6.4</td>
<td>87.7 ± 7.1</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>420 ± 28.3</td>
<td>392 ± 26.8</td>
</tr>
<tr>
<td>Arterial PO2, mmHg</td>
<td>60.0 ± 1.2</td>
<td>477.9 ± 19.9*</td>
</tr>
<tr>
<td>Arterial PCO2, mmHg</td>
<td>59.8 ± 4.1</td>
<td>64.3 ± 3.1*</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.36 ± 0.02</td>
<td>7.33 ± 0.01*</td>
</tr>
<tr>
<td>Venous PO2, mmHg</td>
<td>21.7 ± 7.1</td>
<td>26.4 ± 3.3*</td>
</tr>
<tr>
<td>Venous PCO2, mmHg</td>
<td>70.4 ± 2.8</td>
<td>82.9 ± 8.2*</td>
</tr>
<tr>
<td>Venous pH</td>
<td>7.33 ± 0.01</td>
<td>7.27 ± 0.03*</td>
</tr>
<tr>
<td>Cardiac index, ml·min⁻¹·kg⁻¹ (normalized to baseline)</td>
<td>196 ± 13</td>
<td>144 ± 31*</td>
</tr>
<tr>
<td>Vascular resistance, mmHg·min·kg·ml⁻¹ (normalized to baseline control)</td>
<td>0.46 (1.00)</td>
<td>0.61 (1.33)</td>
</tr>
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</table>

Values are means ± SE. *P < 0.05 (statistically significantly different from baseline). Numbers in parentheses refer to results normalized to control.
The diameter and RBC velocity data were used to compute microvascular blood flow in each vessel studied. Figure 3 shows the average for each vessel order resulting from this calculation. Inhalation of 100% oxygen caused a decrease of blood flow in the feeding arterioles (A1 0.79 ± 0.09) and small arterioles (A2 0.72 ± 0.05) relative to baseline. Although there were no other statistically significant changes relative to baseline in the remainder of the vascular network, the overall trend is indicative of a decrease in microvascular perfusion in consonance with the decrease in cardiac index. The overall change in blood flow, determined by averaging the mean flow change in each vessel order in the network relative to baseline, is 0.83 after exposure to hyperoxia.

Figure 4 shows the distribution of P O\(_2\) in the microvascular network and in the interstitial space for hyperoxic and normal conditions (control group) (14, 33). These oxygen tension data are presented as means ± SD. There is a steady decrease in oxygen tension in the arteriolar network during both hyperoxia and normoxia. The oxygen tension was a minimum in the tissue under both conditions and was progressively higher in the network during hyperoxia. The same trend was apparent in normoxia, although there was no statistically significant difference between V\(_c\) and V\(_l\). Oxygen tension in the major arterioles (A1) in hyperoxic and normal conditions were 96.9 ± 8.5 and 35.9 ± 7.3 mm Hg, respectively. A decrease was also observed in the collecting venules (V\(_c\)) and large venules (V\(_l\)).

**Fig. 1.** Changes of vessel diameter in arteriolar and venular microvessels in response to inspiring 100% O\(_2\) versus baseline conditions with animals breathing atmospheric oxygen. Data are presented as means ± SE. High oxygen level results in a statistically significant decrease relative to baseline in the A1 (0.90 ± 0.03), A2 (0.77 ± 0.03), and A3 (0.84 ± 0.05) arterioles and a nonsignificant decrease in the A4 arterioles (0.95 ± 0.05). Baseline diameter (number of vessels) are the following: A1, 59.1 ± 5.4 µm (18); A2, 25.3 ± 1.8 µm (15); A3, 9.7 ± 0.8 µm (15); A4, 6.4 ± 0.5 µm (15); collecting venule (V\(_c\)), 32.0 ± 3.3 (15); large venule (V\(_l\)), 65.0 ± 5.4 (15). Dotted line represents baseline level. *Significantly different relative to baseline (P < 0.05).

**Fig. 2.** Changes in red blood cell (RBC) velocity in response to inspiring 100% normobaric oxygen relative to baseline. High oxygen levels tend to increase RBC velocity in the microcirculation; however, only the changes in the A2 arterioles were significant from baseline (*P < 0.05). Data are presented as means ± SE. Baseline velocity (number of vessels) are the following: A1, 5.2 ± 0.7 mm/s (18); A2, 3.2 ± 0.3 mm/s (15); A3, 1.3 ± 0.2 mm/s (15); A4, 1.0 ± 0.3 mm/s (15); V\(_c\), 0.6 ± 0.1 (15); V\(_l\), 1.4 ± 0.3 (15). Dotted line represents baseline level.

**Fig. 3.** Changes in blood flow as a result of 100% normobaric oxygen inspiration. Data are presented as means ± SE. The calculation of blood flow was made for each vessel using the corresponding diameter and RBC velocity. Dotted line represents baseline level. *Significantly different relative to baseline (P < 0.05); #significantly different relative to A1 arterioles. A1 (0.79 ± 0.09); A2 (0.72 ± 0.05); A3 (0.89 ± 0.10); A4 (0.69 ± 0.15); V\(_c\), (0.86 ± 0.12); V\(_l\) (1.00 ± 0.17).

**Fig. 4.** Distribution of oxygen tension (P O\(_2\)) in the hamster window chamber model during normoxia and normobaric hyperoxia. All values at 100% O\(_2\) inspiration are statistically significantly different from control (P < 0.05). Data are presented in terms of means ± SD. Cap, capillary; Tis, tissue. *No statistical significance between V\(_c\) and V\(_l\) during normoxia.
56.8 ± 8.1 mmHg, respectively. The interstitial oxygen tension in hyperoxic and normal conditions was 31.9 ± 0.9 and 21.7 ± 3.5 mmHg, respectively. Blood PO2 of the large draining venules in hyperoxic and normal conditions was 42.5 ± 5.3 and 32.5 ± 9.1 mmHg, respectively. Oxygen tension levels in each order of the microvasculature during hyperoxia were statistically different from the corresponding order in normoxia.

DISCUSSION

The principal findings of this study are that inspiration of 100% oxygen has a limited effect on microvascular PO2 and causes the decrease of FCD to 0.74 of control. This later finding may substantiate the perception that hyperoxia may not be uniformly beneficial to the tissue, because functional capillaries are needed not only for the delivery of oxygen, but also for the extraction of metabolic by-products. A previous study from our laboratory using the same preparation and protocol but focusing specifically on the microcirculation of the muscle tissue, identified by parallel running capillaries, showed a reduction in FCD to 0.52 ± 0.10 of baseline with inspiration of 100% O2 (1). Decrease in FCD to 0.74 of baseline (or even 0.52) is probably inconsequential in a healthy organism; it may pose significant risks if superposed to conditions that cause a decrease in FCD per se. As an example, the study of hemorrhagic shock by Kerger et al. (15) showed that subjects whose FCD remained at 40% of baseline or above survived, whereas those with FCD lower than this threshold succumbed, independent of the tissue oxygenation level. The decrease in FCD found in this study evidences a maldistribution of flow that does not affect tissue oxygenation, because direct measurements of tissue PO2 show that this is more than adequate. A problem directly related to the decrease of FCD is the impairment of local extraction of the slowly diffusible by-products of metabolism, of which the accumulation is potentially toxic and may be an additional significantly negative factor in an organism jeopardized by disease and injury. Therefore, using hyperoxia as a treatment for hemorrhagic shock, although in principle may increase tissue oxygenation, it may pose significant risks if superimposed to the intrinsic vasoconstrictor response to blood losses, which is centrally mediated by a neurogenic mechanism. This process may be enhanced during hyperoxia, because the latter is regulated peripherally and therefore independently. From our experience with this experimental model, the transition of capillaries from functional to nonfunctional tends to be irreversible within the time frame of these studies.

Arteriolar constriction did not result in an increased blood pressure due to the compensatory decrease in cardiac output, suggesting that vasoconstriction occurs systemically and is not solely present in the tissues included in the window chamber model, composed mainly of skeletal muscle, adipose, and subcutaneous connective tissue. This contention is supported by the findings of Sjöberg et al. (28), who found that hyperoxia reduced capillary flow in the pig brain. Our previous report on the effects of hyperoxia on vessel diameter by Bertuglia et al. (1) was restricted to a specific branching order starting at the capillaries and extending to the terminal arteriolar network. These vessels, of which their largest diameter was 13.8 ± 2.5 μm, had an average (number weighted) reduction in diameter of 0.88 of baseline, which is comparable to the reduction of 0.94 of baseline observed in the A4 arterioles of the present study, although this change was not statistically significant in the present study.

The decreased cardiac output during hyperoxia is well documented and has been found to be 0.81 of baseline in conscious dogs (28–30 kg) (19). In our present study, the cardiac index decreased to 0.75 of baseline. Peripheral vascular resistance can be calculated from the cardiac output and blood pressure. Compared with normal conditions (1.00), hyperoxia increases vascular resistance to 1.42 of baseline. This result corresponds to the increase in peripheral vascular resistance manifested by the decrease in arteriolar diameter shown in Fig. 2. The lowered FCD is probably a consequence of vasoconstriction because of the decrease of capillary pressure occurring downstream from constricted arterioles, an effect that is probably widespread throughout an organism exposed to inspired 100% O2. Furthermore, when the lowered cardiac index (~25%) is related to the decrease in heart rate (~7%), it becomes apparent that stroke volume is decreased in hyperoxia, which suggests a redistribution of blood volume, following the reduction of cardiac filling. Similar results were found in the heart by Bourdeau-Martini et al. (3) and Lund et al. (21) in normal healthy individuals.

The flow responses in the different orders of branching of this preparation are heterogenous because of the presence of interconnecting (arcading) arterioles within a given vessel order. Mass balance of arteriolar and venular flow changes should in principle be obtained if all the vessels of a branching order could be measured in a large tissue region, which cannot be done due to the limits determined by the chamber window extension. A compromise, which increases significantly the number of vessels included in the analysis and thus the accuracy of the data, is to average the blood flow changes of all the vessels measured. This overall average yields a flow change value that is closely reflected by the change in cardiac index (0.75 for cardiac index vs. 0.83 microvascular flow), and thus it should represent the actual microvascular flow changes in this tissue.

The determination of oxygen saturation requires information on the Bohr effect because the 100% FIO2 condition results in hypoventilation, increased PCO2, and decreased pH. The extent of the Bohr effect is not available for hamster blood. Therefore, as a first approximation, we assumed the effect of pH and PCO2 on hamster and human hemoglobin is the same, and calculations were made based on Winslow's human blood model (39). In this calculation, the pH and PCO2 measured in arterial and venous blood are also assumed to
be the same as in arteriolar and venular blood of the tissue in the window chamber.

Table 2 shows the importance of taking into account the changes in the oxygen saturation curve of hemoglobin because of the prevailing pH and PO2 as a consequence of the Bohr effect. If this effect is not taken into account, oxygen delivery to the tissue in relative values would be 0.84 and 0.88 for control and hyperoxia, respectively, whereas oxygen consumption (extraction) would be, respectively, 0.39 and 0.26, supporting findings that hyperoxia lowers oxygen consumption in the unanesthetized dog (19) and the isolated limb of dogs (5). Conversely, when the Bohr effect is taken into account, oxygen delivery is 0.76 and 0.83, respectively, and oxygen extraction is 0.35 and 0.36, respectively, showing that tissue oxygen consumption in this tissue remains unchanged from baseline in hyperoxia. The maintenance of tissue oxygen extraction during hyperoxia should be considered relative to the increase in oxygen consumption associated with vasoconstriction in different organs (40).

The higher tissue PO2 found in hyperoxia does not by itself limit the transfer of oxygen to the tissue because the blood-tissue oxygen gradients for oxygen transfer from arterioles to tissue, which is a major source of oxygen to the tissue in this preparation as shown by Intaglia et al. (11), are generally higher in hyperoxia. The finding that arteriolar RBC velocity is higher than control indicates that less oxygen is delivered by the arterioles because high flow velocities lower the rate of oxygen exit from the arterioles (25). This may lead to a greater amount of oxygen being shunted from the arterioles to the venules at a PO2 in which the oxygen dissociation curve for hemoglobin is shallow, resulting in a reduced net amount of oxygen delivered to the capillaries.

The rate of oxygen delivery to each microvascular arteriolar order is calculated by using the PO2 distribution as shown in Fig. 5. Hyperoxia changes the distribution of oxygen delivery from an essentially uniform configuration in normoxia to one where oxygen delivery is prevalently shifted to the smaller arterioles at the expense of the larger vessels. Therefore, hyperoxia changes the distribution of PO2 and also the rate at which oxygen is delivered to the different arteriolar orders. This process should be viewed vis a vis the theory of metabolic autoregulation of blood perfusion whereby excess, or different oxygen distribution and availability, is a signal that modulates blood flow to maintain the oxygen supply to the tissue in balance with oxygen demand. In this context Fig. 5 shows that in the hamster model the largest change (increase) in oxygen delivery to the tissue occurs in the A3 and A4 arterioles, a group of vessels that is related to the finding by Saltzman et al. (26), in rat skeletal muscle, where A2 and A3 arterioles were found to possess the highest density of adrenergic innervation, an anatomic configuration that may be related to the sensing-controlling mechanism for this form of autoregulation. As a practical issue, these findings are consistent with the hypothesis that vasoconstriction and increased resistance associated with hyperoxia, seen with the use of cell-free hemoglobins, may be explained by the over delivery of oxygen to vessel walls (23).

In conclusion, temporary hyperoxic ventilation causes vasoconstriction, reduction of cardiac output, decreased microvascular blood flow, and FCD in the hamster window chamber model. The changes in hemodynamic parameters appear to be widespread throughout the organism because they are present in both the systemic and microvascular settings. This level of functional decrease should be tolerable and within the range of autoregulatory adjustments of the intact, healthy organism; however, it may lead to adverse and potentially dangerous conditions in situations where the limits of autoregulatory capacity have been exceeded, such as in hemorrhagic shock and
trauma. They are the consequence of the response to microvascular compensatory mechanisms that strive to maintain oxygen delivery in balance with oxygen demand. These findings also indicate that the use of hemoglobin-based blood substitutes of which their molecular characteristics cause facilitated diffusion of oxygen to the arteriolar wall, a form of hyperoxia, should be avoided because they cause vasoconstriction, impairing microvascular perfusion, a potentially deleterious outcome if superposed to vasoconstrictor stimuli of central, neurogenic origin.

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DISCLOSURES

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