Effect of acute normovolemic hemodilution on distribution of blood flow and tissue oxygenation in dog skeletal muscle

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Hutter, Jörg, Oliver Habler, Martin Kleen, Matthias Tiede, Armin Podtschaske, Gregor Kemming, Carlos Corso, Sanjay Batra, Peter Keipert, Simon Faithfull, and Konrad Messmer. Effect of acute normovolemic hemodilution on distribution of blood flow and tissue oxygenation in dog skeletal muscle. J. Appl. Physiol. 86(3): 860–866, 1999.—Acute normovolemic hemodilution (ANH) is efficient in reducing allogenic blood transfusion needs during elective surgery. Tissue oxygenation is maintained by increased cardiac output and oxygen extraction and, presumably, a more homogeneous tissue perfusion. The aim of this study was to investigate blood flow distribution and oxygenation of skeletal muscle. ANH from hematocrit of 36 ± 3 to 20 ± 1% was performed in 22 splenectomized, anesthetized beagles (17 analyzed) ventilated with room air. Normovolemia was confirmed by measurement of blood volume. Distribution of perfusion within skeletal muscle was determined by using radioactive microspheres. Tissue oxygen partial pressure was assessed with a polarographic platinum surface electrode. Cardiac index (3.69 ± 0.79 vs. 4.79 ± 0.73 l·min⁻¹·m⁻²) and muscle perfusion (4.07 ± 0.44 vs. 5.18 ± 0.36 ml·100 g⁻¹·min⁻¹) were increased at hematocrit of 20%. Oxygen delivery to skeletal muscle was reduced to 74% of baseline values (0.64 ± 0.06 vs. 0.48 ± 0.03 ml O₂·100 g⁻¹·min⁻¹). Nevertheless, tissue PO₂ was preserved (27.4 ± 1.3 vs. 29.9 ± 1.4 Torr). Heterogeneity of muscle perfusion (relative dispersion) was reduced after ANH (20.0 ± 2.2 vs. 13.9 ± 1.5%). We conclude that a more homogeneous distribution of perfusion is one mechanism for the preservation of tissue oxygenation after moderate ANH, despite reduced oxygen delivery.

heterogeneity of perfusion; oxygen transport; dog; acute normovolemic hemodilution

ACUTE NORMOVOLEMIC HEMODILUTION (ANH) is a cost-effective (24) method to reduce the need for allogenic blood transfusion during elective surgery (25). Thus potential risks such as virus transmission and immunosuppression can be diminished (1).

According to the present state of knowledge, preservation of tissue oxygenation, despite reduced oxygen-carrying capacity after ANH, is achieved through an enhancement of organ blood flow by increased cardiac output (CO) and augmented oxygen extraction ratio (20).

Based on early studies on hemodilution, it was postulated that a more homogeneous distribution of blood flow occurs in the various organs after ANH (21). So far, this has been substantiated only by in vivo microscopy studies, which found that after hemodilution the red blood cell (RBC) flux was generally maintained (23) and RBC velocity was more homogeneously distributed to individual capillaries (31). Pries et al. (27) have reported a more homogeneous RBC flow between proximal and distal regions of the capillary network. However, there are methodological concerns that the distribution of RBCs and the distribution of organ blood flow might not necessarily be correlated (6). Moreover, some of the studies did not simulate the clinical conditions of ANH, and extrapolations about the consequences to tissue oxygenation were made on the basis of the observed flow redistribution phenomena. To verify the former assumptions, it is necessary to evaluate flow heterogeneity in conjunction with direct assessment of tissue oxygenation.

In the present study, we assessed the changes of blood flow distribution in canine skeletal muscle after ANH and the ensuing changes in tissue oxygenation by using a model that closely mimicks clinical preoperative hemodilution. We tested the hypothesis that the distribution of blood flow within skeletal muscle would become more homogeneous after ANH.

METHODS

Study Design

Animals. This study was performed in 22 beagle dogs of either sex, weighing 15.6 ± 1.7 kg. All animals had been splenectomized at least 8 wk before the experiments to exclude splenic contraction-induced changes in hematocrit (Hct) during ANH (29). The study was approved by the governmental animal care and use committee. Animal care and use were in compliance with the National Research Council Guide (Guide for the Care and Use of Laboratory Animals, 7th ed., Washington, DC: Natl. Acad. Sci. Press, 1996).

Experimental design. This publication reports data obtained from a large experimental project, addressing the effects of hemodilution, hyperoxic ventilation, and administration of an artificial oxygen carrier on various hemodynamic and tissue-oxygenation parameters. The complete study protocol has been described elsewhere (8, 9). Briefly, the protocol involves ANH to Hct of 20% on room air, followed by an onset of hyperoxic ventilation (part I). The end-point of part I created the baseline conditions of part II, where animals were randomized into three subgroups, undergoing a simulated surgical blood loss to investigate the above-mentioned therapeutic interventions. This publication deals with data obtained only from part I of the protocol.

After surgical preparation and a 30-min stabilization period, baseline values were recorded ("baseline"). Subsequently, all animals were normovolemically hemodiluted within 45–60 min to an Hct of 20 ± 1% by continuous
bleeding and simultaneous infusion of 6% hydroxyethyl starch with a mean molecular mass of 200,000 and a substitution ratio of 0.45–0.55 (HAES-steril 6%, Fresenius, Bad Homburg, Germany). After another stabilization period, a second set of values was obtained (“after ANH”).

After completion of the protocol, animals were euthanized by an intracardiac injection of saturated potassium chloride, and the cleidocervicalis muscle (in addition to other organs) was removed for dissection.

Surgical preparation. After premedication with propiomazine (1.5 mg/kg body wt), general anesthesia was induced by intravenous injection of fentanyl (75 µg/kg body wt) and pancuronium bromide (0.1 mg/kg body wt) and maintained by a continuous infusion of fentanyl (0.3 µg/kg body wt) and pancuronium bromide (2 mg/h) and by inhalation of 0.8% isoflurane. Ringer solution (5 ml·kg⁻¹·h⁻¹) was infused to compensate for insensible fluid loss. Body core temperature was kept above 36.5°C by a warming pad.

All animals were intubated and mechanically ventilated on room air at 12 cycles/min with an end-expiratory pressure of 7 cmH₂O (Servo 900B, Siemens-Elema, Solna, Sweden). Tidal volume was adjusted to provide arterial normocapnia.

Arterial pressure was measured in the abdominal aorta with a manometer-tipped catheter (5-Fr, PC 370, Millar Instruments, Houston, TX) introduced via the left femoral artery. For the assessment of CO and blood temperature, a Swan-Ganz catheter was inserted into a pulmonary artery. Central venous pressure (CVP) was measured with a fluid-filled catheter placed in the superior vena cava. For the injection of radioactive microspheres and the withdrawal of the arterial reference samples, two pigtail catheters (5- and 7-Fr, Cordis, Roden, The Netherlands) were introduced into the left ventricle and abdominal aorta via the left carotid and right femoral arteries, respectively.

A median laparotomy was performed, and additional catheters were inserted but not used for obtaining the data reported in this manuscript: a balloon catheter allowing occlusion of the inferior vena cava (8/22-Fr Fogarty occlusion catheter; Baxter Healthcare, Irvine, CA), a conductance catheter in the left ventricle (7-Fr, Sentron, Roden, The Netherlands), and a venous sampling catheter in the coronary sinus (5-Fr, Cordis). The correct position of each catheter was verified by fluoroscopy and pressure readings.

The midportion of the right cleidocervicalis muscle was carefully dissected free from adjacent fat and connective tissue to expose a small area allowing the placement of the multiwire surface electrode for the measurement of the tissue PO₂ (P₀₂). Care was taken to avoid any bleeding or trauma. Between the measurements, the muscle surface was covered with a plastic membrane to prevent drying and was warmed with an infrared lamp to keep surface temperature above 36.5°C.

Measurements

Hemodynamics. CVP was recorded by using a P23 Db transducer referenced to atmospheric pressure at the level of the right atrium, with the animals in a supine position. CO was measured by means of the thermodilution technique at end expiration. Cardiac index (CI), as well as systemic vascular resistance index, were calculated by using body surface area, according to the following formula

$$\text{Body surface area} = k \times \text{body weight}^{0.7}$$

where k is 11.2 (11).

Blood samples. Arterial and mixed venous blood gases were assessed by an ABL 300 blood-gas analyzer (Radiometer, Copenhagen, Denmark). Hemoglobin concentration ([Hb]) and saturation were determined by absorbance spectrophotometry (682 CO-oximeter, Instrumentation Laboratory, Lexington, MA).

Blood volume (BV). BV was assessed by the kinetics of indocyanine green dilution (ICG; Cardio-Green, Becton Dickinson Microbiology Systems, Cockeysville, MD) by using the whole-blood method (4). Based on known dye concentrations, a three-point calibration was performed to convert measured absorbance into ICG concentrations. Thereafter, 0.25 mg/kg body wt ICG were rapidly injected via the central venous catheter. Absorbance was recorded every 30 s for at least 9 min in blood withdrawn from the abdominal aorta, which was reinfused between the recordings. By linear extrapolation of the resulting ratio of time/concentration curve to time of injection on a semilogarithmic coordinate system, the theoretical dye concentration at injection time (C₀) was obtained. BV was then calculated as $BV = D/C₀$, where D is the injected dose of ICG.

Local blood flow of skeletal muscle. Muscle blood flow (MBF) was determined by radioactive microspheres using the reference sample method (10). For each measurement, 7.2 ± 2.1 ·10⁶ microspheres (35.5 ± 0.5 µm; DuPont New England Nuclear-Trac, Wilmington, DE), labeled with a randomly selected nuclide (51Cr, 114mIn, 99mTc, 46Sc, 85Sr), were injected into the left ventricle. The activities from each microsphere were balanced with specific software to minimize methodological error (16). Before each injection, microspheres were suspended in physiological saline solution to a volume of 5 ml and vortexed for at least 3 min. The suspension was injected during continuous agitation over 50 s; no cardiorespiratory changes were observed after injection. The reference sample was withdrawn, starting with the onset of injection at a rate of 3.24 ml/min over 3 min. The volume withdrawn was simultaneously replaced by an equal amount of warm (37°C) hydroxyethyl starch. At the end of the experiments, the cleidocervicalis muscle was removed and dissected into five samples of equal size, each weighing ~4.4 g.

Reference and tissue sample activities were counted for 3 min (model 5650, Packard Instruments, Downers Grove, IL). Corrections for half-life and radiation spectra deconvolution were performed by using the software package MIC-III (7). MBF was calculated according to the following formula: MBF (ml/min) = $Q - I_s/\text{Ti}_s$, where Q is the withdrawal rate of the reference sample (ml/min) and $I_s$ and $\text{Ti}_s$ are the specific activities (in counts/min) counted in tissue and reference sample, respectively. MBF was then normalized to 1 g sample weight.

Based on MBF and arterial oxygen content (CaO₂), the estimated local oxygen delivery (Do₂) to skeletal muscle was calculated as follows: local Do₂ = mean specific flow (ml·100 g⁻¹·min⁻¹) · CaO₂ (ml O₂/ml blood).

Blood flow distribution. After injection, microspheres are distributed to the tissue along with blood flow until they are trapped in arterioles. After organ dissection and assessment of flow in each of the samples, the relative dispersion (RD) of these multiple sample flow values can be calculated for each muscle and measurement and serve as a measure of flow heterogeneity (RDobserved = SD of flow/mean flow). The calculated observed heterogeneity (RDobserved) overestimates the true heterogeneity of flow (RDrue). Scatter not attributable to blood flow results from a partly stochastic distribution of spheres (RDspheres) as well as from the assessment of radioactivity (RDdecay) and contributes to RDobserved. Both RDspheres and RDdecay increase with a decreasing number of spheres, can be predicted from a Poisson distribution, and have to be
subtracted from $RD_{\text{observed}}$ to obtain $RD_{\text{true}}$ (32). The corresponding formula is

$$RD_{\text{observed}}^2 = RD_{\text{true}}^2 + RD_{\text{sphere}}^2 + RD_{\text{decay}}^2$$

where $RD_{\text{sphere}}^2$ and $RD_{\text{decay}}^2$ are the reciprocals of the detected microspheres and the counted radioactivity, respectively.

Data were excluded if either $RD_{\text{sphere}}^2$ or $RD_{\text{decay}}^2$ exceeded 10% or if (RDspheres + RDdecay) was greater than RDobserved, which was the case in 5 of 22 animals.

As another measure of heterogeneity, the quotient $Q_s/Q_{\text{tot}}$ was calculated for each sample and measurement, where $Q_s$ is the specific flow ($\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) of the sample and $Q_{\text{tot}}$ is the mean specific flow ($\text{ml} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) of the whole muscle that the sample belongs to.

**Tissue oxygenation.** For direct assessment of tissue oxygenation of cleidocervicalis muscle, an eight-platinum-wire polarographic surface electrode (MDO, Eschweiler, Kiel, Germany) with a 0.3 mol/l KCl electrolyte was used (15). The appropriate polarization voltage as well as the temperature coefficient specific to the probe (14) have been assessed previously in our laboratory. Calibration was performed in physiological saline solution at 37°C equilibrated with calibration gas mixtures containing 0, 5, and 10% oxygen. Eight individual calibration lines were computed by linear regression and accepted if each correlation coefficient was $>0.98$. The calibration procedure was repeated after each measurement. Immediately before the measurement, the plastic membrane covering the muscle was removed, and the muscle's surface was rinsed with isotonic saline at 37°C. The electrode was placed onto the muscle surface by means of an electrode holder, thereby diminishing the pressure exerted to $\approx 1.3 \text{mmHg}$, which is far below intracapillary values. A set of eight individual values was recorded when each single signal was stable (i.e., $d(P_{O_2})/d(t) = 0$); thereafter, the electrode was subsequently rotated at least 14 times to achieve 120 independent $P_{O_2}$ values per measurement. All individual values were corrected for electrode drift and small differences in temperature between muscle surface and calibration solution.

For each set of 2,040 discrete $P_{O_2}$ values recorded before and after ANH, the distribution of relative $P_{O_2}$ frequency was calculated and depicted as a histogram.

**Statistics.**

Statistical analysis was performed with the software package SAS V6.1 (SAS Institute, Cary, NC). According to the distribution of the data sets (Shapiro Wilks test), either the paired $t$-test or Wilcoxon signed rank sum test was used for statistical testing.

$P_{O_2i}$ means (i.e., quantity of oxygenation) and $P_{O_2i}$ distributions (i.e., quality of oxygenation) were tested separately. For the latter, a $\chi^2$ test on populations was used, with the $P_{O_2i}$ distributions normalized to a common mean. In addition, values in the hypoxic range were tested by using a $\chi^2$ table test. Alpha error was set to 5%.

**RESULTS.**

**Macrohemodynamic Parameters.**

Table 1 summarizes hemodynamic and respiratory data. The Hct of 36 ± 3% at baseline was lowered to 20 ± 1% by ANH ($P < 0.001$). BV index was found unchanged. Heart rate increased by 5% ($P < 0.05$), with CI and stroke volume index increasing by 29% ($P < 0.001$) and 23% ($P < 0.001$), respectively. CVP was increased, and mean arterial pressure was unchanged after ANH. Systemic vascular resistance was reduced to 70% of the initial value ($P < 0.001$). The increase in CI did not fully compensate for the 42.5% reduction in $C_{A_0}$ ($P < 0.001$), thus total body $DO_2$ index declined to 75% of the baseline value ($P < 0.001$), but total oxygen consumption index remained unaffected. Oxygen extraction ratio was augmented to 30% ($P < 0.001$), and mixed venous $P_{O_2}$ was reduced by 11% ($P < 0.001$) after hemodilution at constant arterial $P_{O_2}$.

**MBF and Tissue Oxygenation.**

MBF as well as local $DO_2$ were altered significantly by ANH in accordance with changes in CI and $DO_2$ index (see Table 2). MBF increased by 27% ($P < 0.05$) and was accompanied by a shift to the right of the MBF histogram (see Fig. 1), whereas local $DO_2$ was diminished by 26% ($P < 0.05$). $P_{O_2i}$, however, was unchanged after hemodilution (27.4 vs. 29.9 Torr). Hence, the corresponding histogram (see Fig. 1) was modified in shape ($P < 0.05$), but $P_{O_2i}$ range and location on the abscissa remained essentially the same. The altered shape is characterized by the skewness of the histograms, which was reduced by 33% (0.423 vs. 0.282, not tested) on ANH.

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<th>Table 1. Hemodynamic and respiratory parameters</th>
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<tr>
<td>Hct, %</td>
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<td>BVI, ml/kg</td>
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<td>HR, beats/min</td>
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<td>CI, 1·min$^{-1}$·m$^{-2}$</td>
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<td>SI, ml/min/m$^2$</td>
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<td>CVP, mmHg</td>
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<td>MAP, mmHg</td>
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<td>$SVR_I$, dyn·cm$^{-5}$·s·m$^{-2}$</td>
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<td>$C_{A_0}$, ml/dl</td>
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<td>$DO_2$, ml·min$^{-1}$·m$^{-2}$</td>
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<td>$VO_2$, ml·min$^{-1}$·m$^{-2}$</td>
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<td>$Q_{O_2}$, %</td>
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<td>$P_{O_2}$, Torr</td>
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Values are means ± SD. ANH, acute normovolemic hemodilution; Hct, hematocrit; BVI, blood volume index; HR, heart rate; CI, cardiac index; SI, stroke volume index; CVP, central venous pressure; MAP, mean arterial pressure; $SVR_I$, systemic vascular resistance index; $P_{A_0}$, arterial $P_{O_2}$; $P_{O_2}$, mixed venous $P_{O_2}$; $C_{A_0}$, arterial $O_2$ content; $DO_2$, total body $O_2$ delivery index; $VO_2$, total body $O_2$ consumption index; $Q_{O_2}$, total body $O_2$ extraction. *$P < 0.05$, †$P < 0.001$ (paired $t$-test).

**Table 2. Local blood flow and tissue oxygenation of skeletal muscle**

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<th>Baseline</th>
<th>After ANH</th>
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<tr>
<td>MBF, ml·100 g$^{-1}$·min$^{-1}$</td>
<td>4.07 ± 0.44</td>
<td>5.18 ± 0.36†</td>
</tr>
<tr>
<td>Local $DO_2$, ml·$O_2$·100 g$^{-1}$·min$^{-1}$</td>
<td>0.64 ± 0.06</td>
<td>0.48 ± 0.03†</td>
</tr>
<tr>
<td>$RD$ of blood flow, %</td>
<td>20.0 ± 2.2</td>
<td>13.9 ± 1.5†</td>
</tr>
<tr>
<td>$P_{O_2i}$, Torr</td>
<td>27.4 ± 1.3</td>
<td>29.9 ± 1.4</td>
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</table>

Values are means ± SE. MBF, muscle blood flow; $DO_2$, $O_2$ delivery; $RD$, relative dispersion; $P_{O_2i}$, tissue $P_{O_2}$. Paired $t$-test or Wilcoxon signed rank sum test where appropriate. *$P < 0.05$.
The distribution of MBF was found to be more homogeneous after hemodilution. Both parameters of heterogeneity, RD as well as $Q_s/Q_{tot}$, revealed significant differences between the measurements. In 12 of 17 animals, RD declined on hemodilution, with the mean value decreasing from 20.0 to 13.9%, but in five animals RD was increased (see Table 2 and Fig. 2). This finding was consistent with significant changes in $Q_s/Q_{tot}$; the percentage of values found in the class including 1.0, which represents homogeneity of local blood flow distribution, increased from 34 to 45%. The corresponding $Q_s/Q_{tot}$ histogram (see Fig. 1) was narrowed after hemodilution.

DISCUSSION

Macrohemodynamic Parameters

In accordance with previously performed hemodilution studies, the reduction of oxygen-carrying capacity during ANH was compensated for by an increase in CI via an increase in stroke volume index and by enhanced oxygen extraction (20).

Thus, oxygen consumption remained unchanged, despite a significant reduction in $DO_2$. Balanced anesthesia was chosen, since it is a current anesthetic regime widely practiced in operative medicine. Normovolemia was maintained and confirmed by BV measurements to avoid any influence of hypo- or hypervolemia on tissue perfusion after hemodilution. We therefore conclude that our model reflects clinical conditions of ANH.

Distribution of Local Blood Flow Within Skeletal Muscle

The main finding of the present study is a more homogeneous distribution of local blood flow in skeletal muscle after ANH, resulting in preservation of tissue oxygenation despite a significantly reduced local $DO_2$.

Previous studies determining blood flow distribution in skeletal muscle with radioactive microspheres used dissection schemes that yielded numerous samples with a mass $<1$ g. In contrast, we have dissected the cleidocervicalis muscle into only five samples of larger size. Therefore, concerns might exist about the utility of the calculated RD in this study.

It might be argued that RD, calculated with data obtained from relatively large samples, primarily reflects heterogeneity of flow between arteries, which supply different portions of an organ. This would, of course, require a dissection scheme that respects the anatomic arterial domains, but in the present study the muscle was dissected geometrically into samples of equal size. With that, heterogeneity of flow in arterioles was determined, since these are the sites of microsphere entrapment. Fractal analysis revealed that blood flow heterogeneity of skeletal muscle is self-similar at different scales, with the sample weight ranging from milligram to gram. Hence, there should not be a qualitative difference in heterogeneity at low or at high resolution.
If calculated as RD, heterogeneity of flow will be averaged along with growing sample volume, due to a concomitant increase in the number of arterioles contained in the sample, and thus the degree of heterogeneity will be underestimated (13). However, the present study does not address the actual degree, but only the changes in heterogeneity after ANH.

The numbers of microspheres injected for each measurement were close to the maximum number acceptable for repeated injection without eliciting macrocirculatory disturbances (2). About 550 spheres/sample could be detected, and the mean relative error of measured flow amounted to 2.2% (5). In 6 of 170 samples, <150 microspheres were trapped. A dissection scheme yielding more, but smaller, samples would result in fewer microspheres per sample. The contribution of methodological scatter to the observed heterogeneity would thus increase disproportionately and probably outweigh \( R_{Dtrue} \).

Apparently, this problem might be resolved by either a systemic injection of an increased number of spheres or by a local injection into skeletal muscle. Beyer and Messmer (3, 19) reported a transient decrease of liver PO\(_2\), as determined with the multiwire surface electrode, after the injection of \(-1.5 \times 10^5\) microspheres/kg body wt in dogs. Although PO\(_2\), mean values were reestablished, change of the spatial pattern of tissue oxygenation could not be excluded, since it is probably the reason for recovery. Thus alteration of measured PO\(_2\), distribution can be evoked by doses of spheres three times lower than those thought to have no effect on macrocirculation (4 \times 10^6\) microspheres/kg body wt) (2). So far, there is no controlled study on the interference of microsphere embolization with PO\(_2\), measurements on skeletal muscle, and hepatic arterial blood flow, normalized to organ mass, is about six times higher than that normalized to resting skeletal muscle. However, since there are far-reaching conclusions drawn on the basis of PO\(_2\), distributions in the present study, the above-mentioned reports had to be taken into account. Thus neither injection of more spheres nor local injection of spheres was feasible. In addition, isolated or controlled perfusion, together with local injection of spheres, would have precluded monitoring of physiological flow response to ANH.

Nevertheless, the baseline values of RD are in agreement with other findings for tissue samples of comparable size from dogs (32) and other mammals (13). Relevance of RD is further confirmed by \( Q/Q_{tot} \), which was calculated by a different algorithm and also found to be significantly reduced after ANH.

Until now, only studies using in vivo microscopy are in direct support of our findings demonstrating a more homogeneous local blood flow after ANH. Tyml et al. (31) reported that heterogeneity of RBC velocity across the capillaries was reduced after hemodilution in rat skeletal muscle. Pries et al. (27) described a homogenization of RBC flow exclusively along the capillary bed of the rat mesentery. In contrast, Sarelius (28) found no homogenization of blood flow on hemodilution in hamster striated muscle.

Besides these studies, there are two reports by Kurdak et al. (17, 18) assessing blood flow distribution in exercising canine skeletal muscle with radioactive microspheres. In these studies, either [Hb] or organ blood flow was varied. RD declined from 52 to 43% concordantly to an [Hb] reduction from 14.1 to 5.7 g/dl (17), and intentional reduction of blood flow was accompanied by an increase in RD (18). Neither changes of RD were statistically significant, probably because of the small number of experiments (i.e., \( n = 6 \)) in each of these studies. In the present study, the reduction of RD after ANH reached statistical significance; it should be noted, however, that [Hb] and organ blood flow were altered simultaneously and a larger number of animals (\( n = 22 \)) was studied.

Consequences of Tissue Oxygenation

State of tissue oxygenation in skeletal muscle is determined by blood flow, metabolic needs, and oxygen diffusion capacity. All of them show a heterogeneous spatial distribution. During dilutional anemia, a decreased oxygen diffusion capacity can limit oxygen uptake during exercise when no redistribution toward a more homogeneous flow pattern is observed (17). As predicted by models neglecting diffusion limitations, a similar effect during reduction of CaO\(_2\) occurs with heterogeneous, but not with homogeneous, flow distribution (26). However, beneficial effects of a more homogeneous flow distribution are not necessarily obvious, since there is evidence that spatial patterns of metabolic needs and blood flow are not in concordance (12). The question arises, to what degree blood flow homogenization satisfies the spatially distributed metabolic demands.

Further interpretation of our data in this respect needs consideration of the limitations inherent in the microsphere method.

Tissue sample volumes, even with a mass of 1 g or less, contain numerous arterioles and capillaries, with the microspheres lodging in the arterioles. Hence, there is lack of information concerning the capillary surface area for exchange, which is the actual region of interest. In the present study, this shortcoming was compensated for by direct measurement of tissue oxygenation with the use of an eight-wire surface PO\(_2\) electrode, which is able to provide absolute values of PO\(_2\), as well as information on the local PO\(_2\), profile, as demonstrated by PO\(_2\), histograms.

With eight individual measuring wires (diameter 12 µm) spread over an area of 25 mm\(^2\), the electrode's spatial power of distinction is limited to tissue volumes supplied by whole capillary networks, rather than by single capillaries. Sweeney and Sarelius (30) showed that the heterogeneity of flow detected between the vessels feeding terminal arterioles is much larger than the heterogeneity of flow within individual capillary networks, with each of them being supplied by one of those arterioles. Hence, heterogeneity of simultaneously recorded PO\(_2\), values should be mainly due to heterogeneity of tissue oxygenation present between regions that are supplied by individual capillary net-
works. Therefore, any shape change of the PO₂ti histogram, as was observed after ANH in the present study, reflects altered tissue oxygenation in regions supplied by separate capillary networks. In the case of a constant, low muscle oxygen uptake during general anesthesia and muscle relaxation, these alterations can be attributed to changes in Do₂. In summary, a more homogeneous perfusion of skeletal muscle after ANH, determined with microspheres, is equivalent to more homogeneous Do₂ to the individual capillary networks and, therefore, should cause a redistribution of the PO₂ti values and profile, as found in the present study.

The percentage of PO₂ti values <10 Torr was reduced from 11 to 8% (P < 0.05) after ANH, indicating fewer tissue areas with below-average Do₂. Moreover, the PO₂ti histogram was less skewed after ANH (0.423 vs. 0.282), thus getting closer to normal Gaussian distribution. With respect to variabilities of microvascular architecture (e.g., differing pathway lengths), one might speculate that the latter represents the highest degree of homogeneity of tissue oxygenation measurable in case of a perfectly homogeneous inflow. Due to the reduced local Do₂, an additional right shift of the PO₂ti histogram, which has been reported previously in a model where a more pronounced increase in CI occurred after ANH (21), is not to be expected. However, a more homogeneous distribution of reduced local Do₂ in skeletal muscle per se is not a sufficient explanation for the unchanged mean PO₂ti after ANH. It would be more likely to expect a lower mean PO₂ti and the corresponding histogram, although reflecting a more homogeneous perfusion, would be shifted to the left. In the present study, mean PO₂ti was unchanged after ANH. This is only possible in the presence of an increased oxygen extraction ratio in the skeletal muscle.

The dependency of oxygen supply to tissue upon homogeneous distribution of local blood flow in the case of reduced CaO₂ was analyzed by Piiper and Haab (26) in a theoretical model. Assuming homogeneous perfusion, the authors demonstrated that the oxygen uptake-to-oxygen requirement ratio in skeletal muscle is maintained during continuous reduction of CaO₂ to 0.05 ml O₂/ml blood. During heterogeneous distribution of flow, however, this ratio was reduced continuously along with reduction in CaO₂. Furthermore, homogeneous flow was correlated with a steeper fall of venous oxygen content accompanying CaO₂ reduction, which is equivalent to enhanced oxygen extraction. Thus it may be concluded that homogenized perfusion is associated with a higher oxygen extraction, which, in turn, compensates for reduced Do₂. In the present study, CaO₂ was reduced to 0.09 ml O₂/ml blood, local blood flow became more homogeneous, and tissue oxygenation was preserved.

It is known that in chronic vascular occlusive diseases associated with failing of vasomotor adjustment hemodilution improves tissue oxygenation (22). Therefore, a redistribution of local blood flow after ANH, as observed in skeletal muscle in the present study, may be attributed to rheological changes, induced by a diluent with lower viscosity in comparison to blood. In this respect, the homogenization of local blood flow with ANH is not a phenomenon restricted to skeletal muscle, but it might occur in other organs, too.

Conclusion

In a model closely imitating clinical conditions, we have shown for the first time that the physiological heterogeneity of local blood flow in skeletal muscle is significantly reduced after ANH. We suggest that a more homogeneous perfusion is responsible for a homogenized, and therefore facilitated, oxygen supply to tissue represented by a normalized histogram. Thus blood flow homogenization may be an important mechanism for the maintenance of tissue oxygenation after ANH, especially during decreased local Do₂, despite enhanced organ blood flow.

We gratefully acknowledge the expert technical assistance of L. Kuhn and C. Csapo as well as the professional care for the experimental animals provided by O. Frisch and his team. This study was supported in part by Alliance Pharmaceutical Corp., San Diego, CA, and by the R. W. Johnson Pharmaceutical Research Institute, Raritan, NJ. Address for reprint requests: J. Hutter, Institute for Surgical Research, Klinikum Grosshadern, Univ. of Munich, Marchioninistr. 15, 81366 Munich, Germany.

Received 23 December 1997; accepted in final form 30 October 1998.

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