

Original Paper

The Limit of Anemia Tolerance during Hyperoxic Ventilation with Pure Oxygen in Anesthetized Domestic Pigs

A. Pape^a M. Steche^a M. Laout^a M. Wedel^a F. Schwerdel^a
C.F. Weber^a B. Zwissler^c O. Habler^b

^aDepartment of Anesthesia, Intensive Care Medicine and Pain Therapy, University Hospital Frankfurt, and ^bClinic of Anesthesiology, Surgical Intensive Care Medicine and Pain Management, Krankenhaus Nordwest, Frankfurt/Main, and ^cClinic of Anesthesiology, Ludwig Maximilians University Hospital, Munich, Germany

Key Words

Acute normovolemic anemia · Critical hemoglobin concentration · Hemodilution · Hyperoxic ventilation · Oxygen transport

Abstract

Background: During acellular replacement of an acute blood loss, hyperoxic ventilation (HV) increases the amount of O₂ physically dissolved in the plasma and thereby improves O₂ supply to the tissues. While this effect could be demonstrated for HV with inspiratory O₂ fraction (FiO₂) 0.6, it was unclear whether HV with pure oxygen (FiO₂ 1.0) would have an additional effect on the physiological limit of acute normovolemic anemia. **Methods:** Seven anesthetized domestic pigs were ventilated with FiO₂ 1.0 and subjected to an isovolemic hemodilution protocol. Blood was drawn and replaced by a 6% hydroxyethyl starch (HES) solution (130/0.4) until a sudden decrease of total body O₂ consumption (VO₂) indicated the onset of O₂ supply dependency (primary endpoint). The corresponding hemoglobin (Hb) concentration was defined as 'critical Hb' (Hb_{crit}). Secondary endpoints were parameters of myocardial function, central hemodynamics, O₂ transport and tissue oxygenation. **Results:** HV with FiO₂ 1.0 enabled a large blood-for-HES exchange (156 ± 28% of the circulating blood volume) until Hb_{crit} was met at 1.3 ± 0.3 g/dl. After termination of the hemodilution protocol, the contribution of O₂ physically dissolved in the plasma to O₂ delivery and VO₂ had significantly increased from 11.7 ± 2 to 44.2 ± 9.7% and from 29.1 ± 4.2 to 66.2 ± 11.7%, respectively. However, at Hb_{crit} cardiovascular performance was found to have severely deteriorated. **Conclusion:** HV with FiO₂ 1.0 maintains O₂ supply to tissues during extensive blood-for-HES exchange. In acute situations, where profound anemia must be tolerated (e.g. bridging an acute blood loss until red blood cells become available for transfusion), O₂ physically dissolved in the plasma becomes an essential source of oxygen. However, compromised cardiovascular performance might require additional treatment.

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Andreas Pape, MD
Clinic of Anesthesia, Intensive Care Medicine and Pain Therapy
University Hospital Frankfurt
Theodor-Stern-Kai 7, DE-60590 Frankfurt/Main (Germany)
E-Mail a.pape@em.uni-frankfurt.de

Introduction

The initial treatment of an acute blood loss usually consists in the infusion of crystalloid and/or colloidal fluids. However, the restoration of normovolemia with acellular fluids leads to a dilution of the red cell mass remaining in the vasculature (acute normovolemic anemia). In this context, the term ‘anemia tolerance’ refers both to the patient’s physiological condition to tolerate acute anemia and the physician’s readiness to accept low hemoglobin (Hb) values (e.g. during restrictive transfusion regimes) [1].

Although O_2 transport capacity decreases progressively with hemodilution, tissue oxygenation can be sustained over a wide range of decreasing Hb concentrations [2]. First, acute normovolemic anemia is compensated by increases in cardiac output and arteriovenous O_2 extraction (O_2ER) [3] so that O_2 delivery (DO_2) to the tissues and O_2 consumption (VO_2) remain constant. Second, at physiologic Hb concentrations, DO_2 exceeds VO_2 by the factor 3–4 [4]. Therefore, O_2 supply to the tissues is still sufficient to meet their O_2 demand even when DO_2 begins to decrease at lower Hb concentrations (supply independency of VO_2 ; fig. 1).

When DO_2 falls below the value of VO_2 , O_2 metabolism becomes dependent on DO_2 and VO_2 starts to decline (supply dependency of VO_2 [5, 6]). The Hb concentration corresponding to the sudden decrease in VO_2 is called ‘critical Hb’ (Hb_{crit}) and reflects the individual limit of anemia tolerance [1].

Hyperoxic ventilation (HV) provides a simple but effective possibility to improve O_2 supply to the tissues, when the number of circulating red blood cells becomes insufficient to meet total-body O_2 demand. In a previous study, we could already demonstrate that the institution of HV with inspiratory O_2 fraction (FiO_2) 0.6 prior to induction of acute normovolemic anemia allowed for hemodilution to lower values of Hb_{crit} than did ventilation with ambient air [7]. However, the question remained open whether using pure oxygen could additionally increase anemia tolerance. We therefore hypothesized that HV with FiO_2 1.0 allows for a more extensive hemodilution, even resulting in a lower value of Hb_{crit} than HV with FiO_2 0.6.

Materials and Methods

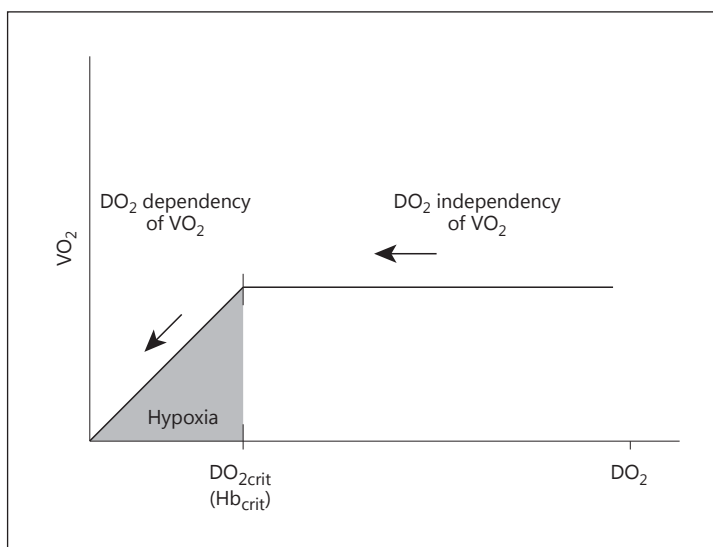
With the approval of the governmental review board (approval code V54-19c 20/15-91/30), experiments were performed in 7 healthy farm-bred pigs of either sex (body weight 29.8 ± 3.7 kg). All animals received good care in compliance with the *Guide for the Care and Use of Laboratory Animals*.

Anesthesia and Ventilation

Twelve hours before the experiments, the animals were denied food but had free access to water. After i.m. premedication with 10 mg/kg ketamine and 1 mg/kg midazolam, anesthesia was induced by i.v. injection of fentanyl (20 μ g/kg) and propofol (1.5 mg/kg) and maintained by continuous infusion of midazolam (0.01 mg/kg/min), propofol (0.16 mg/kg/min) and fentanyl (0.8 μ g/kg/min). To facilitate intubation, animals were paralyzed by a bolus injection of pancuronium (0.2 mg/kg). For adequate assessment of oxygen tension (tpO_2) on the surface of a skeletal muscle (see below), neuromuscular blockade (NMB) was maintained by continuous infusion of pancuronium (0.12 mg/min). Estimated fluid losses (particularly including insensible perspiration owing to the open-chest model, see below) were replaced by a balanced electrolyte solution (0.25 ml/kg/min). All trade names of anesthetics and infusion fluids used are listed in the Appendix.

After orotracheal intubation, animals were ventilated using the CMV mode (controlled mandatory ventilation; 12 cycles/min, PEEP 5 cm H_2O ; Vela Ventilator, Viasys Healthcare, Höchberg, Germany). Based on repeated blood gas analyses, tidal volume was adjusted to provide arterial normocapnia and was then maintained throughout the entire protocol. Until the end of surgical preparation, all animals were ventilated with ambient air.

Fig. 1. Relationship between VO_2 and DO_2 . Physiologically, DO_2 amounts to 3- to 4-fold of VO_2 . Over a long period, VO_2 remains independent of DO_2 despite the anemia-related decrease of DO_2 . When Hb_{crit} is reached, DO_2 falls short of the actual O_2 demand and VO_2 begins to decrease (onset of O_2 supply dependency of VO_2).



Instrumentation and Monitoring

All animals were placed in supine position. A 5-lead electrocardiogram (II, V5) was installed for detection of arrhythmias and ST segment changes. Animals were instrumented with 2 electronic tip manometer catheters (PC 370, Millar Instruments, Houston, Tex., USA) placed in the abdominal part of the aorta and in the left ventricle. A double-lumen catheter (Arrow, Reading, Pa., USA) was inserted into the superior vena cava, and a Swan-Ganz catheter (right ventricular ejection fraction/volumetric catheter, Baxter, Irvine, Calif., USA) was floated into a branch of the pulmonary artery. After midline sternotomy and opening of the pericardium, an ultrasonic flow probe (diameter 14 mm; Transonic Systems, Ithaca, N.Y., USA) was placed around the root of the aorta for continuous measurement of cardiac output. The left anterior descending coronary artery (LAD) was dissected free from surrounding tissue, and an additional ultrasonic flow probe (diameter 2 mm; Transonic Systems) was implanted for assessment of coronary blood flow. For withdrawal of coronary venous blood samples, the anterior interventricular vein was cannulated with an 18-gauge Teflon catheter (Leader Cath, Vygon, Ecouen, France). A large-bore hemodialysis catheter and a 7-French introducer sheath (both by Arrow) were inserted into the left femoral vein and into the right femoral artery for withdrawal of blood and isovolemic infusion of hydroxyethyl starch (HES), respectively. Via a mini-laparotomy, a Foley catheter (Rüsch, Kern, Germany) was inserted into the urinary bladder. An area of 3 × 5 cm of the anterior rectus abdominis muscle was dissected free from surrounding tissue for measurement of tpO_2 . Body temperature was kept constant using a warming pad and a warming lamp.

Experimental Protocol

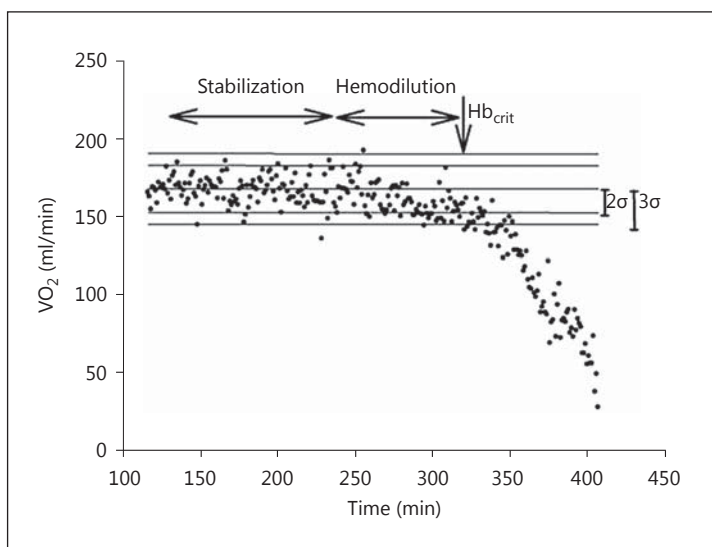
After completion of surgical preparation and installation of the different measuring devices, FiO_2 was increased to 1.0 and a new calibration of the Oxycon Pro™ device (Viasys Healthcare) was performed with pure oxygen. Subsequently, a 60-min stabilization period was allowed to elapse to achieve stable baseline conditions.

The baseline data set was recorded, and subsequently, acute normovolemic anemia was induced by simultaneous exchange of blood for 6% tetrastarch (HES 130/0.4, exchange rate 1 ml/kg/min). The target parameter was the animal's individual Hb_{crit} . When this target was met, a second set of data was collected. Subsequently, animals were killed by intracardial injection of saturated potassium solution.

Determination of Hb_{crit}

Hb_{crit} is the correlate of the critical limitation of DO_2 and marks the onset of total body O_2 supply dependency (fig. 1). The corresponding decrease of VO_2 was detected in an automated and investigator-independent manner: VO_2 was measured with an ergospirometer (Oxycon Pro). VO_2 values were recorded and computed with a specific software (DeltaCrit System, DCS) [8]. During the stabilization period, DCS includes VO_2 values into an online regression analysis and calculates mean and third standard deviation (SD). During

Fig. 2. Typical example of VO_2 recording in the course of the experimental protocol. A linear regression analysis including the calculation of SD was performed with VO_2 values collected during the 60-min stabilization period. During the subsequent hemodilution protocol, a critical limitation of DO_2 was assumed when 3 consecutive VO_2 values fell below the lower 3σ range.



the subsequent hemodilution period, every VO_2 value is compared to the mean value predicted by DCS. When 3 subsequent VO_2 values are outside the predefined range ($3 \times \text{SD}$ of regression line), a significant decrease of VO_2 is assumed and indicated by a visual and acoustic computer alert [8]. A typical VO_2 recording is depicted in figure 2.

Measurements

Blood volume was determined at baseline and at the individual Hb_{crit} ($\text{Hb}_{\text{crit}}-1.0$) using the 'whole-blood' method of the indocyanine green indicator dilution technique, which has already been described in detail elsewhere [9]. The pressure transducers and flowmeters of the hemodynamic measurement devices were connected with a multichannel recorder (Hugo Sachs, March-Hugstetten, Germany) and measurement readings were recorded with a personal computer. The indices of cardiac output (cardiac index, CI) and systemic vascular resistance index (SVRI) as well as pulmonary vascular resistance index (PVRI) were calculated relative to body surface area (see Appendix).

Arterial, mixed venous and coronary venous blood samples were drawn at baseline and at $\text{Hb}_{\text{crit}}-1.0$ for blood gas analysis (ABL 300, Radiometer, Copenhagen, Denmark) and assessment of Hb concentration (682 CO-Oximeter, Instrumentation Laboratory, Lexington, Mass., USA).

During the hemodilution period, arterial and mixed venous blood samples were drawn after every 200 ml of blood exchange. Hemodynamic parameters were registered continuously with the laboratory setup.

For comparison with the results of our previous study, 2 additional data sets corresponding to $\text{Hb} \sim 2.4$ g/dl ($\text{Hb}_{\text{crit}}-0.21$, i.e. Hb_{crit} in animals ventilated with FiO_2 0.21) and $\text{Hb} \sim 1.6$ g/dl ($\text{Hb}_{\text{crit}}-0.6$, i.e. Hb_{crit} in animals ventilated with FiO_2 0.6) were retrospectively read out from the continuous recordings.

tpO_2 on the surface of the skeletal muscle was determined at baseline and at $\text{Hb}_{\text{crit}}-1.0$ using 2 multiwire platinum surface electrodes (MDO-Electrode, Eschweiler, Kiel, Germany) connected with a microprocessor system (Ingenieurbüro Mussler, Aachen, Germany). After equilibration with the tissue surface, this method enables exact assessment of tpO_2 [10]. With each measurement, 240 individual tpO_2 values were recorded; the location of the electrodes on the muscle surface was changed 3–5 times each.

Statistics

Statistical analysis was performed using the SAS 9.2 software package (SAS Institute, Cary, N.C., USA). All data are presented as means \pm SD. Data distribution was assessed using Shapiro-Wilk test.

In case of normal distribution, time effects on the different variables were tested by repeated analysis of variance (ANOVA). Post hoc analysis of differences detected with ANOVA was performed with the Student-Newman-Keuls test. In case of nonnormal distribution, time effects on the parameters were tested by analysis of variance on ranks (rANOVA). Post hoc analysis of differences detected with rANOVA was performed with Tukey's test.

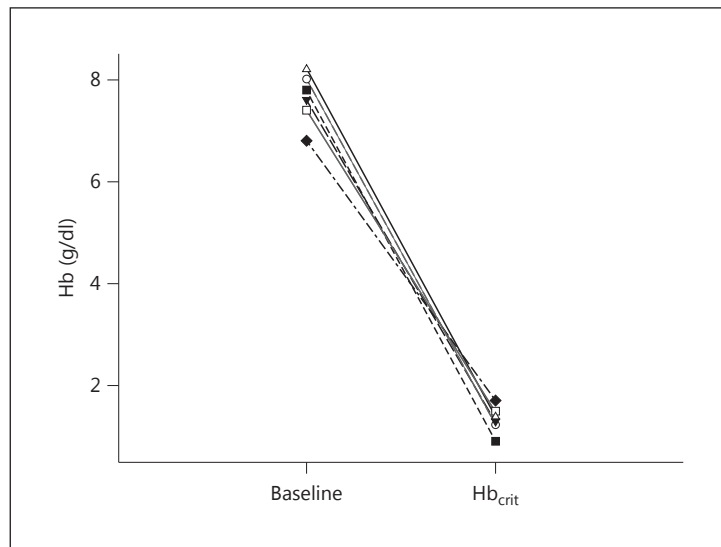


Fig. 3. Hb concentrations at baseline and at Hb_{crit}. Beginning with a Hb concentration of 7.7 ± 0.5 g/dl at baseline, Hb_{crit} was met at 1.3 ± 0.3 g/dl.

Statistical analysis of the parameter 'tpO₂' was performed by a paired t test analysis of the medians of the single tpO₂ values obtained per observation (see above).

For all parameters, statistical significance was accepted at $p < 0.05$.

Results

Primary Endpoint: Hb_{crit}

Hemodilution to Hb_{crit}-1.0 required the exchange of $3,749 \pm 640$ ml of blood for HES, corresponding with an exchange of $156 \pm 28\%$ of the circulating blood volume. Beginning with a Hb concentration of 7.7 ± 0.5 g/dl at baseline, Hb_{crit}-1.0 was met at 1.3 ± 0.3 g/dl (fig. 3).

Secondary Endpoints: Hemodynamic and O₂-Derived Parameters

Table 1 displays secondary endpoint parameters at baseline, after hemodilution to Hb 2.4 g/dl (i.e. Hb_{crit}-0.21) and Hb 1.6 g/dl (i.e. Hb_{crit}-0.6), and finally at the critical Hb concentration (Hb_{crit}-1.0).

Hemodynamics and Myocardial Function

During hemodilution to Hb 2.4 g/dl, heart rate (HR) increased by 27% and increased by a further 36% during hemodilution to Hb 1.5 g/dl. At Hb_{crit}-1.0, HR decreased to baseline level. While mean pulmonary arterial pressure remained almost unchanged during the hemodilution protocol, mean arterial pressure decreased continuously and was significantly beyond baseline level at Hb_{crit}. Consistently, CI increased initially, while SVRI decreased by 51% during hemodilution to Hb 2.4 g/dl, by 36% during hemodilution to Hb 1.5 g/dl and by a further 37% during the final hemodilution step.

Left ventricular (LV) systolic contractility reflected by the maximal slope of LV pressure increase (dp/dt_{max}) increased at the beginning of the hemodilution protocol, but decreased under baseline level at Hb_{crit}-1.0. Likewise, LV diastolic function represented by the maximum LV pressure decrease (dp/dt_{min}) had severely deteriorated at Hb_{crit}-1.0.

Table 1. Hemodynamic and O₂-derived parameters assessed in animals ventilated with FiO₂ 1.0

Parameter	Baseline	Hb _{crit} -0.21	Hb _{crit} -0.6	Hb _{crit} -1.0
Hb, g/dl	7.7±0.5	2.4±0.5*	1.6±0.4*,\$	1.3±0.3*,\$
BVI, m/m ²	81±8	n.d.	n.d.	79±38
HR, l/min	74±9	94±17*	101±20*	85±16
MAP, mm Hg	91±18	78±31	57±28*,\$	34±6*,\$,#
MPAP, mm Hg	23±3	25±4	23±3	21±4\$
CI, l/min/m ²	3.3±0.3	4.7±0.4*	4.6±1.0*	3.7±1.2
SVI, ml/m ²	45±5	51±9	46±9	44±9
SVRI, dyn•s/cm ⁵ /m ²	2,352±556	1,172±464	755±345*	482±110*,\$
PVRI, dyn•s/cm ⁵ /m ²	304±111	163±158	159±211	164±224
LVP, mm Hg	107±20	98±29	78±24*	59±6*,\$
LVEDP, mm Hg	10±2	16±5	15±9	14±5
dp/dt _{max} , mm Hg/s	2,258±796	2,850±2,471	2,638±2,607	1,178±462
dp/dt _{min} , mm Hg/s	-3,372±835	-3,003±1,821	-2,265±2,587	-863±350*
VO ₂ I, ml/m ² /min	154±16	135±16	131±18	140±15
DO ₂ I, ml/m ² /min	374±43	233±28*	148±27*,\$	110±32*,\$,#
CaO ₂ , ml/dl	11.4±0.7	4.9±0.5*	3.2±0.2*,\$	3.0±0.2*,\$
O ₂ ER, %	36±3	45±4*	55±1*,\$	60±4*,\$,#
paO ₂ , mm Hg	432±78	397±41	389±50	418±64
pvO ₂ , mm Hg	44±2	42±4	40±3	38±3*
pH	7.45±0.02	7.41±0.07	7.37±0.07	7.35±0.07*
paCO ₂ , mm Hg	39±4	40±5	38±4	38±5
HCO ₃ , mm Hg	26±2	24±2*	21±2*,\$	21±1*,\$
BE, mmol/l	3.3±1.5	0.3±3.0	2.9±2.7*	3.9±2.5*,\$
Lactate, mmol/l	0.9±0.2	0.8±0.2	1.7±0.8*,\$	2.2±0.9*,\$
CPP, mm Hg	59±21	41±29*	23±24*,\$	9±7*,\$
LAD flow, ml/min	44±13	123±44*	120±49*	78±27*,\$,#
mDO ₂ , ml/min	509±164	626±267	382±150\$	230±76*,\$
mVO ₂ , ml/min	367±119	n.d.	n.d.	153±64*
mO ₂ ER, %	72±6	n.d.	n.d.	66±16
cv lactate, mmol/l	0.6±0.2	n.d.	n.d.	2.2±0.8

BVI = Blood volume indexed to body surface area; MAP = mean aortic pressure; MPAP = mean pulmonary arterial pressure; SVI = stroke volume index; LVP = left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; VO₂I and DO₂I = indices of VO₂ and DO₂; paO₂ and pvO₂ = arterial and mixed venous O₂ partial pressures; paCO₂ = arterial carbon dioxide partial pressure; HCO₃ = arterial bicarbonate concentration; BE = base excess; lactate = lactate concentration; mDO₂ and mVO₂ = DO₂ and VO₂ of LAD-supplied myocardium; mO₂ER = myocardial O₂ER; cv lactate = coronary venous lactate concentration.

* p < 0.05 vs. baseline; \$ p < 0.05 vs. Hb_{crit}-0.21; # p < 0.05 vs. Hb_{crit}-0.6.

O₂ Transport and Tissue Oxygenation

Proportionally with the decrease in Hb concentration and arterial O₂ content (CaO₂), DO₂ index decreased by 38% (hemodilution to Hb 2.4 g/dl), 37% (Hb 1.5 g/dl) and 36% (Hb_{crit}-1.0). As a compensation of acute anemia, arteriovenous O₂ER increased with every hemodilution step, which was accompanied by a slight but constant decrease of mixed venous O₂ partial pressure (pvO₂).

Hemodilution markedly decreased the contribution of Hb-transported O₂ to DO₂ and VO₂. Simultaneously, the contribution of physically dissolved O₂ to DO₂ increased from 11.7 ± 2% (baseline) to 44.2 ± 9.7% (Hb_{crit}; p < 0.05) and its contribution to VO₂ increased from 29.1 ± 4.3 to 66.2 ± 11.7% (p < 0.05; fig. 4a, b).

Despite the utilization of physically dissolved O₂, lactate concentration was elevated above baseline level at Hb 1.5 g/dl (p < 0.05) and further increased after continuation of

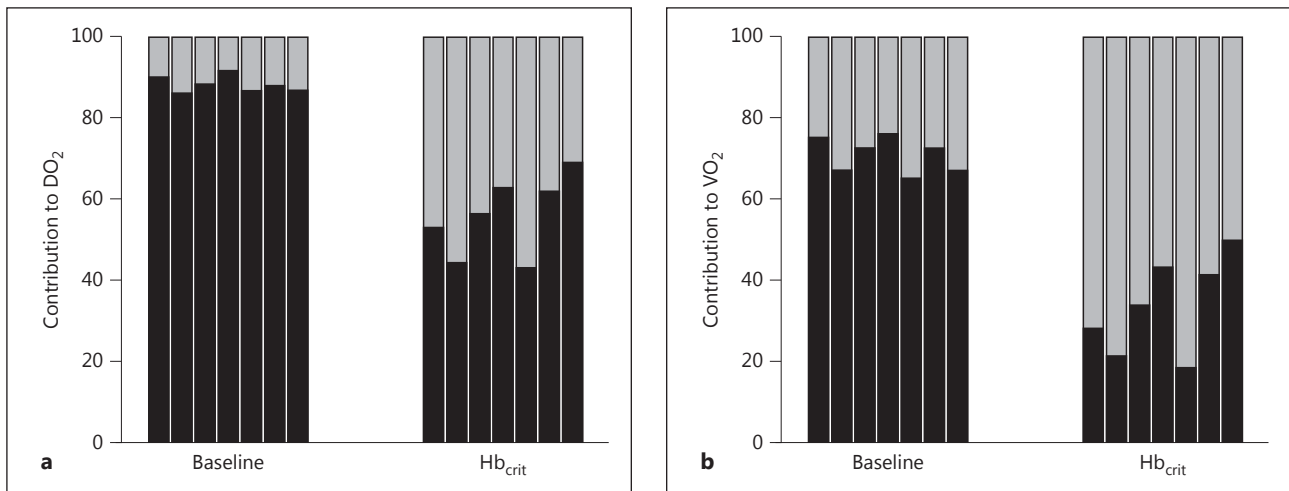


Fig. 4. a Contribution of Hb-transported O_2 (black) and physically dissolved O_2 (gray) to DO_2 at baseline and at Hb_{crit} . Each bar represents 1 animal. During hemodilution, the contribution of physically dissolved O_2 increased from 11.7 ± 2 to $44.2 \pm 9.7\%$ ($p < 0.05$). **b** Contribution of Hb-transported O_2 (black) and physically dissolved O_2 (gray) to VO_2 at baseline and at Hb_{crit} . Each bar represents 1 animal. During hemodilution, the contribution of physically dissolved O_2 to VO_2 increased from 29.1 ± 4.3 to $66.2 \pm 11.7\%$ ($p < 0.05$).

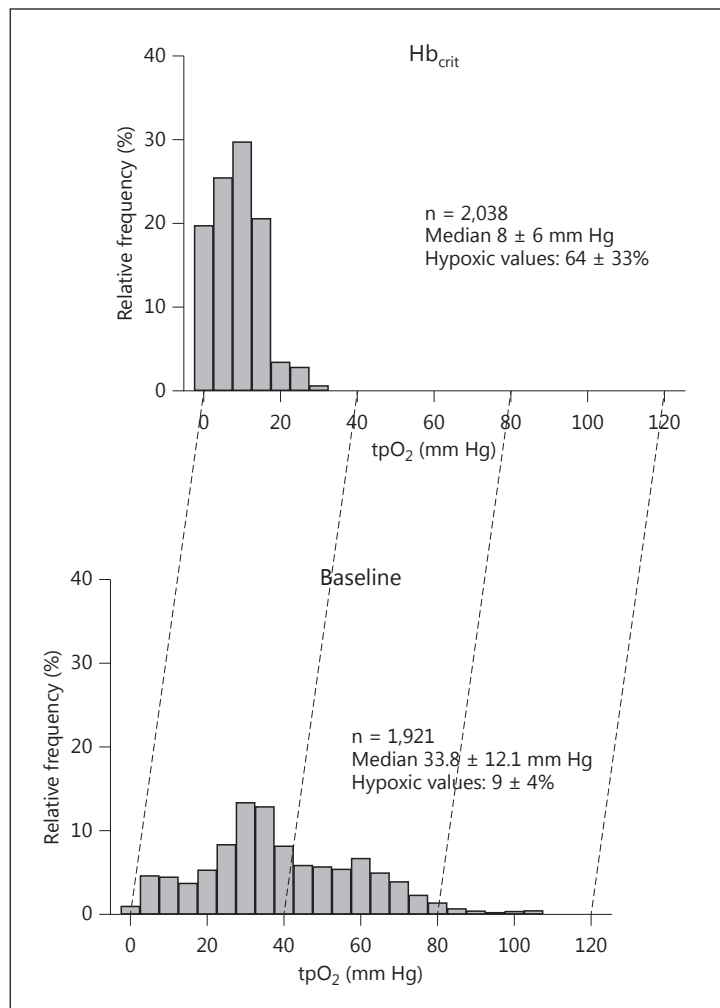


Fig. 5. Sum histograms of all tpO_2 values obtained on the surface of a skeletal muscle at baseline (bottom) and at Hb_{crit} (top). x-axis: tpO_2 values are displayed in classes of 5 mm Hg. y-axis: relative frequency of tpO_2 values. At Hb_{crit} the histogram is left shifted, indicating that the relative frequency of low tpO_2 values had increased: the median of the tpO_2 values decreased from 33.8 ± 12.1 to 8 ± 6 mm Hg ($p < 0.05$) and the rate of hypoxic values increased from 9 ± 4 to $64 \pm 33\%$ ($p < 0.05$).

hemodilution to Hb_{crit} -1.0. Consistently, base excess decreased by 90% during the first 2 hemodilution steps and by a further 34% during hemodilution from 1.5 g/dl to Hb_{crit} .

Manifest tissue hypoxia on the surface of the skeletal muscle was indicated by the typical left shift of tpO_2 sum histograms (fig. 5). The median decreased from 33.8 ± 12.1 to 8 ± 6 mm Hg ($p < 0.05$) and the fraction of hypoxic values increased from 9 ± 4 to $64 \pm 33\%$ ($p < 0.05$).

Myocardial Perfusion and Oxygenation

To compensate for increased myocardial O_2 demand, coronary blood flow in the LAD increased initially by 279% (hemodilution to Hb 2.4 g/dl) and had a tendency to decrease during hemodilution from Hb 1.5 g/dl to Hb_{crit} -1.0.

However, during the entire hemodilution protocol, LAD flow remained increased. Consistently with the decrease of SVRI, coronary perfusion pressure (CPP) decreased continuously beyond baseline level. While DO_2 to the LAD-supplied myocardium (mDO_2) decreased continuously, myocardial O_2ER and VO_2 remained almost unaltered. However, coronary venous lactate concentration was significantly increased at Hb_{crit} -1.0, indicating compromised myocardial lactate extraction and aerobic capacity.

Discussion

The main result of the present study is that HV with FiO_2 1.0 only provided a marginal increase of anemia tolerance as compared to animals ventilated with FiO_2 0.6 in our previous study [7]. This is indicated by a slightly lower value of Hb_{crit} (1.3 ± 0.3 vs. 1.6 ± 0.4 g/dl) and a higher volume of blood exchanged for HES ($3,749 \pm 640$ vs. $2,634 \pm 407$ ml).

Regarding secondary endpoints (table 2), animals ventilated with pure oxygen appeared slightly superior to animals ventilated with FiO_2 0.6 regarding the following criteria: first, they presented less tachycardiac and less hypotensive at Hb_{crit} -0.21. However, at their individual Hb_{crit} , cardiovascular performance was equally impaired. Second, the increase of lactate appeared attenuated and pVO_2 tended to be higher at any time point. Third, at Hb_{crit} -0.6, myocardial perfusion appeared less compromised.

Proportionally with FiO_2 , HV increases the amount of O_2 physically dissolved in the plasma. This effect gains importance in profound anemia, as the contribution of red blood cells to O_2 transport is declining and the significance of the plasma compartment as an O_2 reservoir is increasing. At the site of microcirculation, physically dissolved O_2 is utilized more readily than Hb-bound O_2 , since plasma and red blood cells are separated in such a manner that the inner vascular wall is covered with a plasma layer. During HV, the utilization of physically dissolved O_2 is additionally favored due to the elevated gradient of O_2 partial pressure to the interstitium [11, 12]. The actual utilization of physically dissolved O_2 is reflected by our data, as HV with pure O_2 significantly increased the contribution of physically dissolved O_2 to DO_2 and VO_2 (fig. 4a, b).

HV with FiO_2 1.0 did not result in a substantially lower Hb_{crit} when compared to FiO_2 0.6 (1.3 vs. 1.5 g/dl). However, the absolutely lowest values of Hb_{crit} were observed in 2 animals ventilated with FiO_2 1.0, which met Hb_{crit} at 0.8 g/dl. Moreover, a more extensive blood-for-HES exchange was necessary for institution of Hb_{crit} than in animals ventilated with FiO_2 0.6.

Hb_{crit} marks the ultimate limit of anemia tolerance [5, 13]; without further treatment (e.g. elevation of FiO_2 , transfusion of red blood cells or infusion of artificial O_2 carriers), hemodilution to Hb_{crit} is associated with 100% mortality [14–16]. Hb_{crit} therefore reflects the limit of anemia tolerance at the level of the whole organism by averaging VO_2 of all organs. However, this approach disregards the cellular and organ-specific level of anemia tolerance. A recent study investigated the limits of anemia tolerance of the heart, the brain, the kidneys, the liver,

Table 2. Hemodynamic and O₂-derived parameters obtained in animals ventilated with ambient air (group 0.21) or with FiO₂ 0.6 (group 0.6)

Parameter	Group	Baseline	Hb _{crit} -0.21	Hb _{crit} -0.6
Hb, g/dl	0.21	7.6±0.8	2.4±0.5*	
	0.6	8.2±0.7	2.7±0.4*	1.6±0.4*
HR, l/min	0.21	102±20	138±27	
	0.6	100±13	129±18	110±35
MAP, mm Hg	0.21	98±18	75±23	
	0.6	107±14	92±23	35±12*
MPAP, mm Hg	0.21	26±6	22±4	
	0.6	16±5 [§]	15±7	17±9
CI, l/min/m ²	0.21	3.3±0.3	5.4±1.1*	
	0.6	3.5±0.6	5.2±0.6*	3.7±1.7
SVI, ml/m ²	0.21	33±5	39±5	
	0.6	35±7	41±5	34±12
SVRI, dyn•s/cm ⁵ /m ²	0.21	2,195±520	929±235*	
	0.6	2,452±380	1,419±477*, [§]	933±263
PVRI, dyn•s/cm ⁵ /m ²	0.21	246±229	11±165	
	0.6	262±117	157±96	113±146
LVP, mm Hg	0.21	98±26	83±29*	
	0.6	121±24	110±19	60±15*
LVEDP, mm Hg	0.21	11±7	21±8	
	0.6	4±2 [§]	5±3*, [§]	12±5*
dp/dt _{max} , mm Hg/s	0.21	2,011±1,132	1,896±3,786 [§]	
	0.6	2,698±1,554	3,119±1,815	1,790±741
dp/dt _{min} , mm Hg/s	0.21	-2,817±1,176	-2,568±1,299	
	0.6	-3,152±1,076	-2,838±1,395	-1,383±1,001
VO ₂ I, ml/m ² /min	0.21	190±40	162±34	
	0.6	224±21	215±21 [§]	150±46*
DO ₂ I, ml/m ² /min	0.21	331±43	191±60*	
	0.6	400±69	231±44*	108±43*
paO ₂ , mm Hg	0.21	97±6	116±16	
	0.6	272±21 [§]	279±26 [§]	279±46
CaO ₂ , ml/dl	0.21	10±1	3.5±0.6*	
	0.6	12±0.9 [§]	4.4±0.5*, [§]	3.0±0.4*
pvO ₂ , mm Hg	0.21	32±4	25±2*	
	0.6	43±12 [§]	33±5 [§]	31±7
O ₂ ER, %	0.21	47±13	50±13	
	0.6	40±11	49±11	52±14
pH	0.21	7.48±0.03	7.43±0.05	
	0.6	7.35±0.03	7.44±0.04	7.38±0.08
BE, mmol/l	0.21	3.8±1.4	0.0±2.4	
	0.6	4.0±1.3	2.4±1.5	-2.1±2.7
Lactate, mmol/l	0.21	2.2±1.1	2.8±1.3	
	0.6	1.2±0.4	1.1±0.4 [§]	3.2±1.4*
CPP, mm Hg	0.21	69±15	39±23	
	0.6	81±13	56±15 [§]	10±10*
LAD flow, ml/min	0.21	54±15	181±65*	
	0.6	47±20	138±100*	99±44
mDO ₂ , ml/min	0.21	540±155	634±259	
	0.6	541±209	622±478	309±149
cv lactate, mmol/l	0.21	1.8±0.8	2.8±1.7*	
	0.6	0.7±0.2		4.2±1.9*

Data excerpted from our previous study [7]. For abbreviations, see text and table 1. * p < 0.05 vs. baseline; [§] p < 0.05 vs. group 0.21.

the small intestine and the skeletal muscle. Especially in the kidneys and in the skeletal muscle, signs of tissue hypoxia were observed before the critical limit of systemic O₂ supply was reached [17].

Although neglecting the organ-specific level of anemia tolerance may be judged as a limitation of the present experimental model, the assessment of total-body Hb_{crit} has been used in several experimental hemodilution studies. In particular, a reduction of Hb_{crit} could be achieved by hypothermia (moderate reduction of body core temperature reduces total body O₂ demand [18]), infusion of norepinephrine (stabilization of CPP during hemodilution [19]), infusion of artificial O₂ carriers (maintenance of CaO₂ despite reduced hematocrit [20, 21]) and continuous NMB (lowering skeletal muscular O₂ demand [22]).

Notably, in paralyzed animals, Hb_{crit} was found about 1 g/dl lower than in animals without NMB. NMB was used in the present study as well as in our previous study [7] to eliminate muscular activity and thereby to enable exact performance of tpO₂ measurement.

Moreover, the choice of the infusion fluid used for hemodilution has an impact on Hb_{crit}. While the use of HES in the clinical setting is presently a matter of controversy, mid-molecular HES preparations have at least in the experimental setting proven effective regarding the maintenance of tissue oxygenation in profound anemia and were in this respect superior to gelatin or to crystalloids [23]. The advantages of HES 130/0.4 were explained with a lower degree of extravasation resulting in a more sustained volume effect and reduced tissue edema formation.

Hemodilution and hyperoxia have antipodal effects on microvascular resistance. Accelerated blood flow increases shear stress at the endothelial vascular layer, resulting in the release of nitrous oxide and vasodilation [24, 25]. These effects are, however, counteracted by hyperoxic vasoconstriction, which has been observed in large vessels as well as in microvessels (arterioles, venules) [26, 27]. While these effects are balanced at moderate degrees of anemia, stages of profound anemia are characterized by hypotension. When blood viscosity and, thus, intracapillary blood pressure fall below the hydrostatic pressure of the capillary-surrounding tissue, microvascular collapse is imminent [28, 29].

In the present study, microvascular collapse is indicated by the extreme left shift of the tpO₂ histograms, which occurred despite the significant portion of physically dissolved O₂ to O₂ supply. Impaired microvascular perfusion is also reflected by global indicators of tissue hypoxia (decreasing base excess and pH, increasing lactate concentration). As single organs have not been investigated in the present study, it is neither possible to localize potential origins of lactate production, nor to distinguish the degree of tissue hypoxia within particular organs.

Moreover, at their individual Hb_{crit}, animals subjected to HV were more hypotensive than animals ventilated with ambient air. At extremely low Hb concentrations, myocardial oxygenation became compromised by anemic hypoxia as well as by decreasing CPP. As a consequence, cardiac output declines, resulting in a decrease of coronary blood flow and a further limitation of myocardial perfusion.

The onset of this vicious circle not only accelerates the breakdown of hemodynamic compensation, thereby directly decreasing VO₂. This implies that at extremely low Hb levels, the primary endpoint of the experimental protocol is influenced by effects beyond limited O₂ transport capacity, which should be judged as a weakness of our experimental model. However, at Hb_{crit}-0.21, VO₂ was obviously not affected by circulatory failure, so that our main conclusion remains applicable, namely that HV with either FiO₂ increased anemia tolerance and therefore enabled continuation of hemodilution to Hb values below 2.4 g/dl (i.e. Hb_{crit}-0.21).

The present study was motivated by the fact that in our previous study, FiO₂ had to be restricted to 0.6 for methodological reasons; the endpoint of the hemodilution protocol (i.e.

the onset of O₂ supply dependency of VO₂) was assessed with a Deltatrac™ II metabolic monitor (Datex-Engström, Helsinki, Finland) [7]. This device calculates VO₂ as the quotient of CO₂ production (VCO₂) and the respiratory quotient (RQ), the latter being calculated with the Haldane transformation (see Appendix). As this algorithm yields implausible results when FiO₂ exceeds a maximum of 0.65, the institution of FiO₂ 1.0 is impossible using the Deltatrac II metabolic monitor [30]. Therefore, the question remained open whether a further increase of FiO₂ from 0.6 to 1.0 would additionally increase the tolerance of acute normovolemic anemia.

At a later time point, the Oxycon Pro device became available. The algorithm of this device is based on the measurement of expiratory O₂ and CO₂ fractions with a paramagnetic and an infrared absorption sensor, respectively. According to the manual, the accuracy of measurement is 3% or 0.05 l/min, respectively. However, the Oxycon Pro device exerts a modified Haldane transformation, which allows assessment of VO₂ even during HV with pure oxygen, providing that the system has been calibrated with FiO₂ 1.0. Although this algorithm underwent an in-house validation by the manufacturer, it has neither been published in detail so far, nor has it been validated against other techniques (e.g. reverse Fick principle). While the validity and reliability of VO₂ assessment with the Oxycon Pro device has already been investigated under normoxic conditions [31], this device was used in a recently published study investigating the effects of HV (FiO₂ 1.0) on VO₂ and RQ in healthy spontaneously breathing volunteers [32].

In the setting of experimental hemodilution, the Oxycon Pro device enabled us for the first time to assess VO₂ even during HV with pure oxygen. With respect to the number of experimental animals and for ethical reasons, we decided to only perform the present 7 experiments and to use the results of the previous study in terms of 2 historical reference groups.

Our data demonstrate that HV with pure O₂ only provides a marginal gain of anemia tolerance as compared with FiO₂ 0.6. Overall, the extensive blood-for-HES exchange until the onset of O₂ supply dependency implies that HV allows to delay the transfusion of red blood cells, e.g. in case of bridging an acute blood loss until blood products become available. However, while the lack of O₂ carriers can be compensated by physically dissolved O₂ in extreme anemia (i.e. Hb concentrations <2 g/dl), O₂ transport becomes additionally impaired by cardiovascular collapse and microvascular perfusion disorders. Despite the excellent bioavailability of physically dissolved O₂, the management of extreme normovolemic anemia might require a multimodal approach including the choice of the optimal infusion fluid, deep NMB and circulatory support with catecholamines [1]. HV with FiO₂ 1.0 might temporarily be useful; however, HV with FiO₂ 0.6 has equipotent effects on maintaining O₂ supply to the tissues.

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Appendix

Body surface area (BSA) was calculated according to Holt et al. [33]:

$$BSA = k \cdot BW^{2/3} \text{ (m}^2\text{)},$$

where BW = body weight (in kg) and k = 9 (constant for the species pig).

CI was calculated as:

$$CI = \frac{CO}{BSA} \left(\frac{l}{m^2} \right),$$

where CO = cardiac output.

Stroke volume index (SVI) was calculated as:

$$SVI = \frac{CI}{HR} \left(\frac{l}{m^2} \right),$$

where HR = heart rate.

SVRI and PVRI were calculated as:

$$SVRI = \frac{(MAP - CVP) \cdot 79.9}{CI} \left(\frac{\text{dyn} \cdot \text{s}}{\text{cm}^5 \cdot \text{m}^2} \right) \text{ and } PVRI = \frac{(MPAP - LVEDP) \cdot 79.9}{CI} \left(\frac{\text{dyn} \cdot \text{s}}{\text{cm}^5 \cdot \text{m}^2} \right),$$

respectively, where MAP = mean aortic pressure; CVP = central venous pressure; MPAP = mean pulmonary arterial pressure, and LVEDP = left ventricular end-diastolic pressure.

O₂ER was calculated as follows:

$$O_2ER = \frac{CaO_2 - CvO_2}{CaO_2} \cdot 100(\%),$$

where CaO₂ and CvO₂ are arterial and mixed venous O₂ content.

The contribution of physically dissolved O₂ (physO₂) to DO₂ and to VO₂ was calculated as:

$$\frac{physO_2}{DO_2} = \frac{0.0031 \cdot paO_2}{CaO_2} \cdot 100(\%) \text{ and } \frac{physO_2}{VO_2} = \frac{(0.0031 \cdot paO_2 - 0.0031 \cdot pvO_2) \cdot 10}{CaO_2 - CvO_2} \cdot 100(\%),$$

respectively, where paO₂ = arterial oxygen partial pressure.

CPP was calculated as:

$$CPP = DAP - LVEDP,$$

where DAP = diastolic aortic pressure.

Myocardial DO₂ (mDO₂) and myocardial VO₂ (mVO₂) were calculated for the LAD-supplied area:

$$mDO_2 = LAD \cdot CaO_2$$

$$mVO_2 = LAD \cdot (CaO_2 - CcvO_2),$$

where LAD = LAD blood flow.

For the same myocardial area, myocardial O₂ER (mO₂ER) ratio was calculated as:

$$mO_2ER = \frac{CaO_2 - CcvO_2}{CaO_2} \cdot 100(\%).$$

For calculation of VO₂, CO₂ production (VCO₂) is divided by the RQ, which is calculated by the Haldane transformation:

$$(1) VO_2 = \frac{VCO_2}{RQ}$$

$$(2) RQ = \frac{1 - FiO_2}{\frac{FiO_2 - FeO_2}{FeCO_2} - FiO_2},$$

where FeO₂ = expiratory O₂ fraction and FeCO₂ = expiratory CO₂ fraction.

For induction and maintenance of anesthesia, the following drugs were used: ketamine (Ketavet™, Parke-Davis, Berlin, Germany); midazolam (Midazolam™, Ratiopharm, Ulm, Germany); propofol (Brevi-myta™, Braun, Bad Melsungen, Germany); fentanyl (Fentanyl™, Janssen, Neuss, Germany); pancuronium (Pancuronium™, Curamed, Karlsruhe, Germany); balanced electrolyte solution (Tutofusin™, Pharmacia, Erlangen, Germany), and HES 130/0.4 (Volumen™, Fresenius Kabi, Bad Homburg, Germany).

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