# General Anesthesia

## Enhanced central organ oxygenation after application of bovine cell-free hemoglobin HBOC-201

*[Amélioration de l'oxygénation des organes centraux après l'usage d'hémoglobine bovine acellulaire HBOC-201]*

Marc Freitag MD,* Thomas G. Standl MD,* André Gottschalk MD,* Marc A. Burmeister MD,* Christian Rempf MD,* Ernst P. Horn MD,* Tim Strate MD,† Jochen Schulte am Esch MD*

**Purpose:** While the effects of dilutional anemia or isovolemic hemodilution (IHD) on the oxygen extraction and tissue oxygenation in peripheral organs after application of hemoglobin-based oxygen carriers like HBOC-201 have been studied intensively, little is known about tissue oxygenation properties of hemoglobin solutions in central organs like the liver.

**Methods:** Twelve Foxhounds were anesthetized and then randomized to either a control group without hemodilution (Group 1) or underwent first step isovolemic hemodilution (pulmonary artery occlusion pressure constant) with Ringer's solution (Group 2) to a hematocrit of 25% with second step infusion of HBOC-201 until a hemoglobin concentration of +0.6 $g \cdot dL^{-1}$ was reached. Tissue oxygen tensions ($tpO_2$) were measured in the gastrocnemius muscle using a polarographic needle probe, and in the liver using a flexible polarographic electrode.

**Results:** While arterial oxygen content and oxygen delivery decreased with hemodilution in Group 2, global liver and muscle oxygen extraction ratio increased after hemodilution and additional application of HBOC-201. Hemodilution and application of HBOC-201 provided augmentation of the mean liver $tpO_2$ (baseline: 48 ± 9, 20 min: 53 ± 10, 60 min: 67 ± 11*, 100 min: 68 ± 7*; *$P < 0.05$ *vs* baseline and Group 1), while oxygen tensions in Group 1 remained unchanged. Oxygen tension in the skeletal muscle increased after hemodilution and additionally after application of HBOC-201 in comparison to baseline and to the control group ($P < 0.05$).

**Conclusion:** In the present animal model, IHD with Ringer's solution and additional application of HBOC-201 increased oxygen extraction and $tpO_2$ in the liver and skeletal muscle, in parallel and in comparison with baseline values and a control group.

***Objectif** : Les effets de l'anémie par hémodilution ou hémodilution isovolémique (HDI) sur l'extraction d'oxygène et l'oxygénation tissulaire dans les organes périphériques après l'utilisation de transporteur d'oxygène à base d'hémoglobine comme HBOC-201 ont été très étudiés, mais on connaît peu les propriétés de l'oxygénation tissulaire des solutions d'hémoglobine dans les organes centraux comme le foie.*

***Méthode** : Douze Fox-hounds ont été anesthésiés, puis assignés à un groupe témoin sans hémodilution (Groupe 1) ou soumis à la première étape de l'hémodilution isovolémique (pression artérielle pulmonaire bloquée constante) avec une solution de Ringer (Groupe 2) jusqu'à un hématocrite de 25 % et ont reçu une perfusion de seconde étape avec HBOC-201 pour obtenir une concentration d'hémoglobine de +0,6 $g \cdot dL^{-1}$ . Les tensions en oxygène tissulaire ($tpO_2$) ont été mesurées dans le muscle gastrocnémien en utilisant une sonde à injection polarographique et dans le foie au moyen d'une électrode polarographique flexible.*

***Résultats** : Tandis que le contenu en oxygène artériel et la distribution d'oxygène ont diminué avec l'hémodilution dans le Groupe 2, le ratio d'extraction globale d'oxygène hépatique et musculaire a augmenté après l'hémodilution et l'usage additionnel de HBOC-201. L'hémodilution et HBOC-201 ont fait augmenter la $tpO_2$ hépatique moyenne (mesures de base : 48 ± 9, 20 min : 53 ± 10, 60 min : 67 ± 11*, 100 min : 68 ± 7*; *$P < 0,05$ vs mesures de base et Groupe 1), tandis que les tensions en oxygène sont demeurées inchangées dans le Groupe 1. La tension en oxygène dans le muscle squelettique a augmenté après l'hémodilution et aussi après l'usage de HBOC-201 en comparaison des mesures de base et du groupe témoin. ($P < 0,05$).*

***Conclusion** : Chez le modèle animal utilisé, l'HDI avec une solution de Ringer et l'usage additionnel de HBOC-201 ont augmenté l'extraction d'oxygène et la $tpO_2$ dans le foie et le muscle squelettique, parallèlement et comparativement aux valeurs de base et au groupe témoin.*

From the Departments of Anesthesiology,* and Surgery,† University Hospital Hamburg-Eppendorf, Hamburg, Germany.

*Address correspondence to*: Dr. Marc Freitag, Department of Anaesthesiology, University Hospital Hamburg-Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany. Phone: +49 40 42803 4409; Fax: +49 40 42803 7631; E-mail: freitag@uke.uni-hamburg.de

Disclaimer: The authors are members of the University Hospital Hamburg-Eppendorf and have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials presented or discussed in the manuscript.

*Accepted for publication January 4, 2005.*

*Revision accepted May 3, 2005.*

RESTORATION of intravascular volume is the cornerstone of therapy of patients with hemorrhage. Fluid resuscitation in severe hypovolemia results in dilution of the remaining whole blood, thus causing dilutional anemia, platelet decrease and coagulation dysfunction. In contrast, during isovolemic hemodilution (IHD), blood is withdrawn from the body and simultaneously replaced by an isooncotic colloid (1:1 exchange ratio) or isotonic crystalloid solution (1:4 exchange ratio).[1] When normovolemia is strictly preserved, IHD can be extended to low hematocrit levels in healthy and younger subjects.[2,3] With further dilutional reduction of the hematocrit the compensatory mechanisms providing adequate oxygen supply to tissues, e.g., increase of cardiac output (CO) and oxygen extraction become exhausted, and finally oxygen consumption ($VO_2$) decreases.[3,4] However, little knowledge exists about changes of oxygen extraction and tissue oxygenation of central intestinal organs such as the liver during IHD, whereas several studies describe the changes of tissue oxygen tensions ($tpO_2$) in skeletal muscle.[5–7] In order to prevent tissue damage and organ failure in intestinal organs like the liver, it is necessary to preserve or restore in a timely manner oxygen delivery ($DO_2$) and tissue oxygenation, respectively. Modern cell-free hemoglobin based oxygen carriers (HBOC), e.g., ultrapurified polymerized bovine hemoglobin (HBOC-201), have been effectively used to maintain or restore oxygen delivery and tissue oxygenation after hemorrhage or during isovolemic hemodilution.[6,8]

The present prospective animal study investigated the effects of isovolemic hemodilution with Ringer's solution and additional application of HBOC-201 on oxygen delivery and tissue oxygenation of the liver as a central organ, and of the skeletal muscle representing a peripheral organ, in comparison with a non-hemodiluted control. We tested the hypothesis that the $tpO_2$ in skeletal muscle and the liver would increase significantly compared with baseline values, after hemodilution and additional application of HBOC-201.

## Methods

After approval of the Animal Care Committee, 12 Foxhounds (six male and six female, weight 31 ± 3 kg) were included in this prospective, randomized study. The animals were premedicated with *im* injection of ketamine hydrocloride (5 $mg \cdot kg^{-1}$) and xylazine (2 $mg \cdot kg^{-1}$). Anesthesia was induced with 5 $mg \cdot kg^{-1}$ thiopental *iv*, followed by endotracheal intubation and mechanical ventilation (Spiromat 650, Drager, Lübeck, Germany) with 30% oxygen in air. Minute ventilation was adjusted to maintain end-expiratory $pCO_2$ constant (36–40 mmHg, Normocap®, Datex, Helsinki, Finland). Anesthesia was maintained by continuous infusion of *iv* infusions of 0.02 $mg \cdot kg^{-1} \cdot hr^{-1}$ fentanyl, 0.4 $mg \cdot kg^{-1} \cdot hr^{-1}$ midazolam and 0.2 $mg \cdot kg^{-1} \cdot hr^{-1}$ vecuronium for muscle paralysis.

### *Measurements and data collection*

The electrocardiogram, heart rate and all hemodynamic variables were monitored continuously (Marquette, MI, USA). After surgical preparation of the right groin, an arterial catheter was placed through the femoral artery into the aorta for measurement of the mean arterial pressure (MAP) and arterial blood gas sampling. Venous access was obtained by placing an 8F introducer into the right femoral vein. A 7F pulmonary artery catheter (Oxicath®, Abbott, Germany) was inserted via the introducer for measurement of central venous pressure, mean pulmonary arterial blood pressure (MPAP), pulmonary artery occlusion pressure (PAOP) CO and temperature. CO was determined by the thermodilution method (average of three measurements) and registered by a CO computer (Oximetrix®, Abbott, Germany). Cardiac index (CI), systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated.

For measurement of the oxygen content in the vena cava ($CvO_2$ cava) the right femoral vein was used for introduction of a catheter into the inferior vena cava; this catheter was fixed directly above the junction of the liver veins and controlled under direct vision following laparotomy. A small catheter was inserted into the left popliteal vein for sampling of venous blood coming from the left M. gastrocnemius (popliteal oxygen content: $CvO_2$ popl). An electromagnetic flow probe (Cliniflow FK 701 D, Carolina Med. Electronics, King, NC, USA) was placed around the left popliteal artery which is the main vessel for the gastrocnemius muscle to measure the mean arterial blood flow to this muscle (Flow-popl).

After upper abdominal laparotomy and splenectomy to avoid blood pooling, a catheter was introduced in the remaining V. lienalis and advanced 5 cm into the V. portae for measurement of the oxygen content ($CvO_2$ port) in the V. portae. Blood flow measurement (flow-port.) in the V. portae was also performed using an electromagnetic flow probe (Cliniflow FK 701 D, Carolina Med. Electronics, King, NC, USA).

Hematocrit was determined five minutes after centrifugation of arterial blood (Biofuge 17RS, Heraeus Sepatech, Harz, Germany) using a standardized scale. Hb concentrations and oximetry were analysed from arterial and venous blood samples respectively using a six-wavelength oximeter (OSM3, Radiometer,

TABLE I Hemodynamic parameters

| | *HR* (*beats·min$^{-1}$*) | *MAP* (*mmHg*) | *MPAP* (*mmHg*) | *PAOP* (*mmHg*) | *CI* (*L·min$^{-1}$·m$^{-2}$*) | *SVR* (*dyne·sec$^{-1}$·cm$^{-5}$*) | *PVR* (*dyne·sec$^{-1}$·cm$^{-5}$*) |
|---|---|---|---|---|---|---|---|
| *Group 1 (Control)* | | | | | | | |
| Baseline | 75 ± 24 | 141 ± 23 | 21 ± 5 | 12 ± 3 | 2.8 ± 0.7 | 3808 ± 703 | 256 ± 167 |
| 20 min | 76 ± 27 | 140 ± 28 | 20 ± 4 | 12 ± 3 | 3.0 ± 0.6 | 3485 ± 568 | 227 ± 135 |
| 60 min | 75 ± 27 | 141 ± 25 | 20 ± 5 | 12 ± 4 | 3.2 ± 0.8 | 3096 ± 698 | 229 ± 146 |
| 100 min | 79 ± 30 | 139 ± 27 | 19 ± 5 | 10 ± 3 | 3.3 ± 0.8 | 3177 ± 572 | 242 ± 145 |
| *Group 2 (Hemodilution)* | | | | | | | |
| Baseline | 89 ± 21 | 133 ± 25 | 16 ± 4 | 10 ± 6 | 3.2 ± 1.0 | 2966 ± 995 | 174 ±76 |
| 20 min | 95 ± 18 | 107 ± 22 * | 16 ± 6 | 10 ± 5 | 4.0 ± 1.3 | 1934 ± 630 *# | 114 ± 50 |
| 60 min | 80 ± 25 | 140 ± 20 | 17 ± 8 | 11 ± 5 | 2.9 ± 0.9 | 3644 ± 720 | 171 ± 80 |
| 100 min | 74 ± 21 | 127 ± 19 | 19 ± 6 | 11 ± 4 | 3.0 ± 0.9 | 2995 ± 875 | 206 ± 90 |

HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary artery pressure; PAOP = pulmonary artery occlusion pressure; CI = cardiac index; SVR = systemic vascular resistance; PVR = pulmonary vascular resistence, *$P < 0.05$ compared with Group 1; #$P < 0.05$ compared with baseline; †$P < 0.05$ in comparison to hemodilution; values are expressed as mean ± SD.

TABLE II Parameters of systemic oxygen transport

| | *Hct* (*%*) | *Hb* (*g·dL$^{-1}$*) | *f-Hb* (*g·dL$^{-1}$*) | *$CaO_2$ fem* (*mL·dL$^{-1}$*) | *$CvO_2$ pulm* (*mL·dL$^{-1}$*) | *av-$DO_2$* (*mL·dL$^{-1}$*) | *$DO_2$* (*mL·min$^{-1}$*) | *$VO_2$* (*mL·min$^{-1}$*) |
|---|---|---|---|---|---|---|---|---|
| *Group 1 (Control)* | | | | | | | | |
| Baseline | 46.6 ± 4.9 | 14.5 ± 1.5 | 0.0 ± 0.0 | 21.2 ± 2.2 | 15.7 ± 2.5 | 5.5 ± 0.8 | 612 ± 172 | 155 ± 30 |
| 20 min | 45.1 ± 5.3 | 14.3 ± 1.5 | 0.0 ± 0.0 | 20.5 ± 1.9 | 15.9 ± 2.3 | 4.6 ± 0.7 | 677 ± 241 | 150 ± 46 |
| 60 min | 45.1 ± 4.9 | 13.8 ± 1.3 | 0.0 ± 0.0 | 20.1 ± 2.1 | 15.3 ± 2.4 | 4.8 ± 1.2 | 689 ± 229 | 156 ± 36 |
| 100 min | 45.7 ± 4.1 | 14.0 ± 1.1 | 0.0 ± 0.0 | 20.2 ± 1.4 | 14.8 ± 1.5 | 5.5 ± 1.0 | 728 ± 271 | 194 ± 70 |
| *Group 2 (Hemodilution)* | | | | | | | | |
| Baseline | 42.0 ± 9.2 | 12.4 ± 2.8 | 0.0 ± 0.0 | 22.0 ± 2.2 | 15.9 ± 1.3 | 6.1 ± 1.4 * | 611 ± 102 | 153 ± 45 |
| 20 min | 26.9 ± 3.6 *# | 7.4 ± 1.0 *# | 0.0 ± 0.0 | 10.7 ± 1.6 *# | 7.2 ± 1.3 *# | 3.5 ± 0.7 *# | 458 ± 112 *# | 151 ± 42 |
| 60 min | 25.4 ±4.4 *# | 7.7 ± 1.6 *# | 0.6 ± 0.2 *# | 10.4 ± 2.3 *# | 6.4 ± 2.3 *# | 4.0 ± 0.7 | 320 ± 87 *# | 123 ± 29 |
| 100 min | 24.0 ± 3.8 *# | 7.6 ± 1.2 *# | 0.6 ± 0.2 *# | 10.4 ± 2.1 *# | 5.7 ± 1.1 *# | 4.7 ± 1.3 | 332 ± 63 *# | 150 ± 6 |

Hct = hematocrit; Hb = hemoglobin concentration; f-Hb = plasma hemoglobin concentration; $CaO_2$ = arterial oxygen content; $CvO_2$ pulm = mixed-venous oxygen content; av$DO_2$ = systemic arteriovenous oxygen difference; $DO_2$ = systemic oxygen delivery; $VO_2$ = systemic oxygen consumption;*$P < 0.05$ compared to Group 1; #$P < 0.05$ compared to baseline; values are expressed as mean ± SD.

Copenhagen, Denmark). An oxygen-specific fuel cell (Lex-O2-Con, Lexington Instruments, Waltham, MA, USA) was used for measuring the arterial (A. fem.), venous (V. popl., V. portae, V. cava) and mixed-venous (A. pulm) oxygen content. Measurements were recorded and variables were calculated as outlined in the Appendix.

*Protocol (Figure 1)*

The surgical preparation and instrumentation was followed by a 20-min equilibration period. After recording all baseline variables, the animals were randomly allocated to a control group which was not hemodiluted (Group 1) or to the treatment group (Group 2) which underwent PAOP-controlled IHD to a hematocrit target value of 25% (total hemoglobin concentration of 7.4 ± 1.0 g·dL$^{-1}$) with crystalloids 1:4 (Ringer's solution). After IHD and an equilibration period of 20 min, a second measurement of all parameters was performed. After completion of the post IHD measurements, animals in Group 2 received a dose of ultrapurified, polymerized, bovine hemoglobin HBOC-201 (Hemopure™, Biopure, Cambridge, MA, USA) to ensure a plasma hemoglobin concentration of 0.6 g·dL$^{-1}$ during the experiment. The amount of HBOC-201 was calculated according to the following formula: HBOC-201 (mL) = body weight × 0.6/0.13.[9] This dose was chosen on the basis of data from a prior study,[6] which suggested that an augmentation of at least 0.6 g·dL$^{-1}$ HBOC-201 was effective in restoring tissue oxygenation to baseline values after significant reduction by profound hemodilution. HBOC-201 has a hemoglobin concentration of 13 ± 1 g·dL$^{-1}$ (methemoglobin and oxyhemoglobin = 10%)

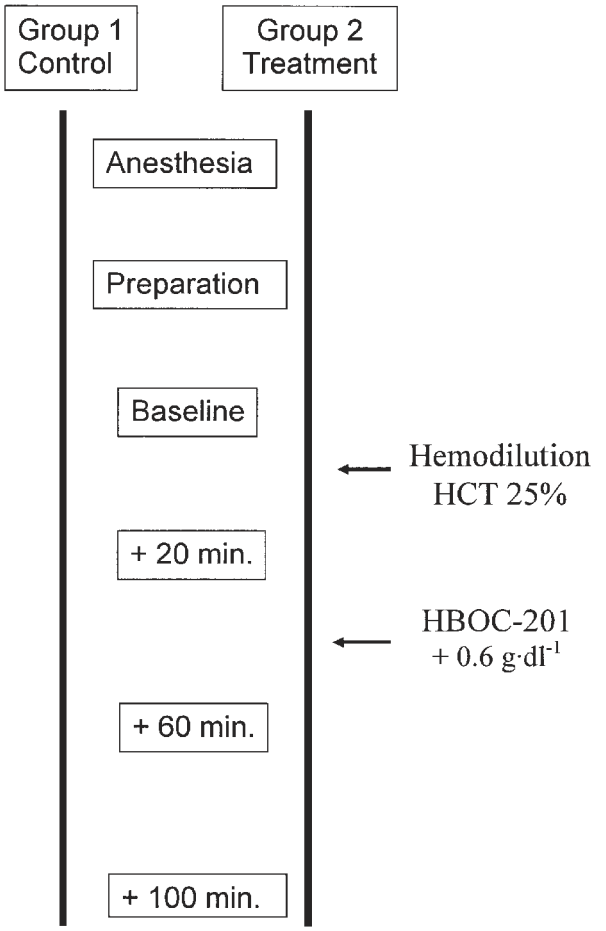


FIGURE 1 Flow diagram of the study protocol.

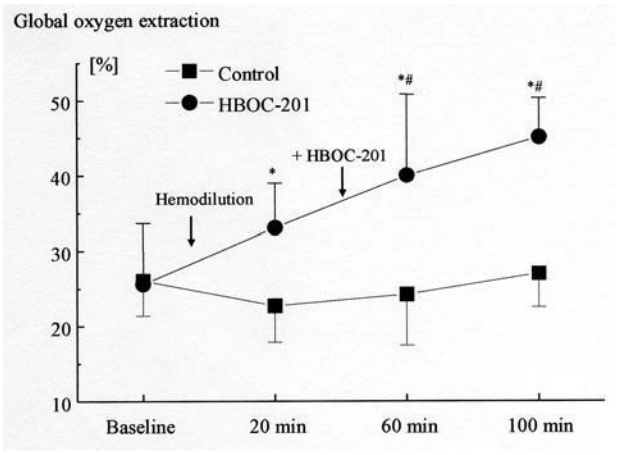


FIGURE 2 Changes of global oxygen extraction ratio without hemodilution (control) and during hemodilution with Ringer's solution and additional application of hemoglobin-based oxygen carriers (HBOC)-201 (Group 2). *$P <$ 0.05 in comparison to control; #$P < 0.05$ in comparison to baseline.

and an oncotic pressure of 17 mmHg. HBOC-201 was prepared from bovine red cells by lysis, filtration, chromatography and polymerization with glutaraldehyde (65,000 < gram molecular weight < 500,000). The sterile pyrogen free solution contains < 0.5 EU $mL^{-1}$ endotoxin and < 3 nM phospholipids and physiological concentrations of electrolytes.

The control-group (Group 1) received Ringer's solution 1000 $mL \cdot hr^{-1}$ as a fluid substitution. Until the last measurement animals of both groups received Ringer's solution as a volume substitution to provide a stable PAOP of baseline ± 1 mmHg.

The time between the respective measurements was 40 min.

*Statistics*

Analyses were performed using SPSS for windows 9.0 (SPSS Inc., Chicago, IL, USA). Animals were allocated to one of the two groups by a computerized randomization list. We tested the hypothesis that the 50% percentile (median) of the $tpO_2$ in the skeletal muscle would increase significantly compared with baseline after hemodilution and additional application of HBOC-201. Based upon a recent study[10] we expected baseline values of approximately 30 ± 5 mmHg, and an increase of 100% after hemodilution and additional application of HBOC-201, with an anticipated pooled standard deviation of 7.5 mmHg. We would permit a type I error of $\alpha = 0.05$, and with the error of ß = 0.2, this analysis achieves a power of 0.8, indicating a sample size requirement of at least six animals per group.

Descriptive analysis of parametric data is expressed as means and standard deviation. Ordinal data are expressed as medians. $TpO_2$ values were tested for statistical significance using the Whitney U test or Wilcoxon rank test. Differences between the respective hemodilution steps were tested using ANOVA for repeated measurements and Student's t test deviation. Statistical significance was assumed at $P < 0.05$.

## Results

Results of selected hemodynamic variables are presented in Table I. Mean values of heart rate, MPAP, CI and PVR did not change over time, and were similar in both groups. The PAOP also did not change during the experiment. MAP decreased during hemodilution in Group 2 in comparison to baseline values and to Group 1. After application of HBOC-201, MAP returned to baseline values in Group 2.

The variables pertaining to the systemic oxygen transport are presented in Table II. In both groups, temperature remained stable over time. Baseline hemoglobin concentrations and hematocrits were similar in both groups, and decreased only in Group 2 during hemodilution to values between 7.4 and 7.7 $g \cdot dL^{-1}$ with 1.0 $g \cdot dL^{-1}$ of plasma hemoglobin after administration of HBOC-201. Arterial oxygen content ($CaO_2$ fem), $CvO_2$ pulm and $DO_2$ decreased after hemodilution, and remained at lower levels in Group 2 in comparison to baseline to control values. Systemic arteriovenous oxygen difference ($AvDO_2$) only decreased in Group 2 after hemodilution, whereas $VO_2$ remained stable. During hemodilution the global oxygen extraction ratio increased, with a further increase after application of HBOC-201 (Figure 2).

Liver oxygen transport data are shown in Table III. Hemodilution decreased oxygen content both in the V. cava and the V. portae in Group 2 when compared to baseline levels and the control group. $AvDO_2$ in the liver decreased in Group 2 after hemodilution, whereas oxygen consumption in the liver remained stable in both groups. Oxygen delivery to the liver decreased in Group 2 after hemodilution and application of HBOC-201 in comparison to the control group at the last time of measurement. Oxygen extraction ratio in the liver, (Figure 3) increased after application of HBOC-201 in Group 2.

TABLE III Parameters of oxygen transport to the liver

| | *$CvO_2$ port ($mL \cdot dL^{-1}$)* | *$CvO_2$ cava ($mL \cdot dL^{-1}$)* | *$avDO_2$ liv ($mL \cdot dL^{-1}$)* | *Flow-port ($mL \cdot min^{-1}$)* | *$DO_2$ liv ($mL \cdot min^{-1}$)* | *$VO_2$ liv ($mL \cdot min^{-1}$)* |
|---|---|---|---|---|---|---|
| *Group 1 (Control)* | | | | | | |
| Baseline | 18.9 ± 1.6 | 17.8 ± 2.0 | 1.8 ± 1.6 | 111 ± 62 | 33.2 ± 19.4 | 3.8 ± 5.8 |
| 20 min | 18.4 ± 1.7 | 17.1 ± 2.2 | 2.0 ± 0.7 | 94 ± 49 | 27.0 ± 13.9 | 2.7 ± 1.3 |
| 60 min | 17.8 ± 2.3 | 16.5 ± 2.7 | 2.0 ± 0.8 | 96 ± 63 | 26.7 ± 17.6 | 2.7 ± 1.5 |
| 100 min | 16.7 ± 1.9 # | 15.9 ± 2.4 | 2.0 ± 1.2 | 90 ± 37 | 23.9 ± 8.6 | 3.1 ± 3.0 |
| *Group 2 (Hemodilution)* | | | | | | |
| Baseline | 16.3 ± 4.1 | 14.1 ± 4.7 | 2.9 ± 2.8 | 65 ± 22 | 16.9 ± 8.5 | 2.9 ± 3.0 |
| 20 min | 8.9 ± 1.9 *# | 8.1 ± 1.6 *# | 1.3 ± 0.8# | 99 ± 45 | 14.7 ± 7.7 | 2.0 ± 1.4 |
| 60 min | 8.6 ± 2.3 *# | 7.1 ± 2.3 *# | 2.2 ± 1.2 | 97 ± 47 | 13.7 ± 7.0 | 3.3 ± 2.9 |
| 100 min | 7.9 ± 1.4 *# | 5.7 ± 1.3 *# | 3.1 ± 0.6 | 80 ± 38 | 10.8 ± 5.7* | 3.8 ± 2.2 |

$CvO_2$ port = oxygen content in the portal vein; $CvO_2$ cava = oxygen content in the vena cava; $avDO_2$ liv = arteriovenous oxygen difference in the portal vein; Flow-port = blood flow in the portal vein; $DO_2$ liv = oxygen delivery in the portal vein; $VO_2$ = oxygen consumption in the portal vein; *$P < 0.05$ compared with Group 1; #$P < 0.05$ compared with baseline; values are expressed as mean ± SD.

TABLE IV Parameter of oxygen transport to the skeletal muscle

| | *$CvO_2$ popl ($mL \cdot dL^{-1}$)* | *$avDO_2$ mus ($mL \cdot dL–1$)* | *Flow-popl ($mL \cdot min^{-1}$)* | *$DO_2$ mus ($mL \cdot min^{-1}$)* | *$VO_2$ mus ($mL \cdot min^{-1}$)* |
|---|---|---|---|---|---|
| *Group 1 (Control)* | | | | | |
| Baseline | 14.5 ± 2.3 | 6.6 ± 2.2 | 38 ± 13 | 7.9 ± 2.6 | 2.3 ± 0.6 |
| 20 min | 15.7 ± 2.5 | 4.8 ± 1.5 | 49 ± 20 | 10.1 ± 4.9 | 2.1 ± 0.7 |
| 60 min | 14.8 ± 2.5 | 5.3 ± 1.2 | 50 ± 20 | 10.0 ± 4.4 | 2.5 ± 0.8 |
| 100 min | 14.7 ± 1.5 | 5.5 ± 1.4 | 57 ± 24 | 11.5 ± 5.2 | 3.0 ± 1.3 |
| *Group 2 (Hemodilution)* | | | | | |
| Baseline | 13.1 ± 4.2 | 5.1 ± 2.7 | 43 ± 27 | 7.2 ± 3.2 | 2.5 ± 2.6 |
| 20 min | 6.4 ± 1.2 *# | 4.3 ± 1.2 | 75 ± 66 | 7.5 ± 6.0 | 2.9 ± 1.9 |
| 60 min | 5.6 ± 2.2 *# | 4.8 ± 1.5 | 53 ± 19 | 5.2 ± 1.1 * | 2.4 ± 0.5 |
| 100 min | 5.5 ± 1.5 *# | 5.0 ± 2.0 | 60 ± 18 | 6.0 ± 0.8 * | 2.8 ± 0.9 |

$CvO_2$ popl = oxygen content in the popliteal vein; $avDO_2$ mus = arteriovenous oxygen difference in the muscle; Flow-popl = blood flow in the popliteal artery; $DO_2$ mus = oxygen delivery in the muscle; $VO_2$ mus = oxygen consumption in the muscle; *$P < 0.05$ compared with Group 1; #$P < 0.05$ compared with baseline; values are expressed as mean ± SD.

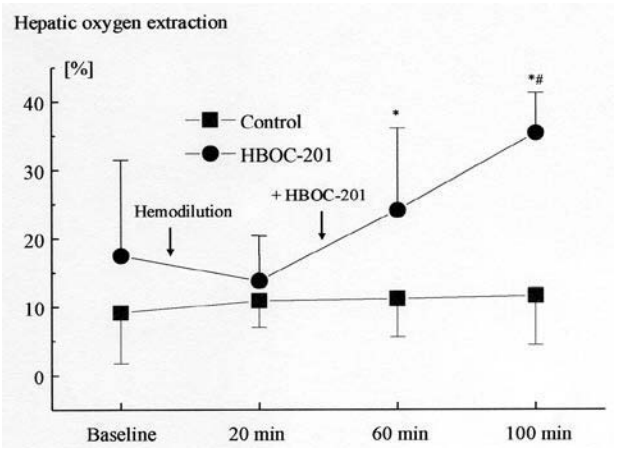


FIGURE 3 Changes of oxygen extraction ratio in the liver without hemodilution (control) and during hemodilution with Ringer's solution and additional application of hemoglobin-based oxygen carriers (HBOC)-201 (Group 2). $*P < 0.05$ in comparison to control; $\#P < 0.05$ in comparison to baseline.

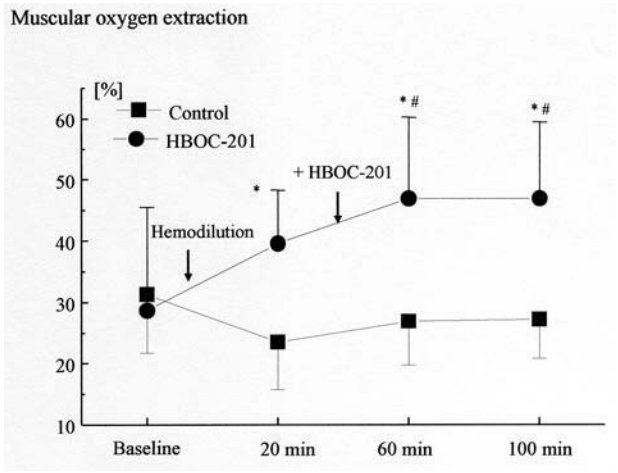


FIGURE 4 Changes of oxygen extraction ratio in the skeletal muscle without hemodilution (control) and during hemodilution with Ringer's solution and additional application of hemoglobin-based oxygen carriers (HBOC)-201 (Group 2). $*P < 0.05$ in comparison to control; $\#P < 0.05$ in comparison to baseline.

Oxygen transport variables of the skeletal muscle are presented in Table IV. In accordance with the global and liver oxygen content, the popliteal oxygen content decreased during hemodilution in Group 2 (Table IV). The oxygen extraction ratio (Figure 4) in the skeletal muscle was increased during hemodilution in comparison to Group 1 and after additional application of HBOC-201 in comparison to baseline.

Liver tissue oxygenation data are shown in Figure 5. In the control group, no changes in liver $tpO_2$ were observed. Hemodilution with Ringer's solution did not alter liver $tpO_2$ in Group 2 whereas additional application of HBOC-201 increased the hepatic $tpO_2$ by 45% in comparison to baseline and by 40% in comparison to the control group. The pooled histograms of oxygen tensions of the skeletal muscle are shown in Figure 6. The mean $tpO_2$ of the skeletal muscle increased from values around 20 mmHg to 32 mmHg after hemodilution. Application of HBOC-201 was associated with an additional increase of 32% of the muscle $tpO_2$ in comparison to post hemodilution values. The histograms of skeletal muscle $tpO_2$ shifted to the right to higher values in Group 2, while in the control group the $tpO_2$ did not change over time.

## Discussion

In the present animal study, hemodilution with Ringer's solution to a hematocrit of 25% and additional application of the hemoglobin-based oxygen carrier HBOC-201 resulted in an increase in $tpO_2$ in the liver and skeletal muscle.

The effect of maintenance or even improvement of $tpO_2$ following hemodilution is primarily the result of increased red cell velocity in the capillaries,[1] which is based mainly upon the improved rheology as a result of decreased blood viscosity and vascular tone. The basic mechanisms underlying the enhanced nutrional blood flow during IHD are caused by the improvement of blood fluidity, enhancement of shear rates, and subsequent release of endogenous nitric oxide from the vascular endothelium,[11] finally resulting in vasodilation. In addition, flow motion is enhanced during hemodilution, leading to a more homogeneous delivery of oxygen to the tissues.[12] In our study a significant effect could be demonstrated after first step moderate IHD with Ringer`s solution to a hematocrit of 25% in comparison to the non-hemodiluted control group with respect to $tpO_2$ values in the skeletal muscle. An increase of $tpO_2$ in the skeletal muscle following hemodilution has been shown before.[3,5,13] However, in our experimental setting, moderate hemodilution alone was not able to increase liver $tpO_2$. Only additional application of the cell-free HBOC-201 caused a significant increase of liver $tpO_2$ in comparison to baseline values and the control group.

This result could make HBOC-201 an important and effective instrument to improve tissue oxygen-

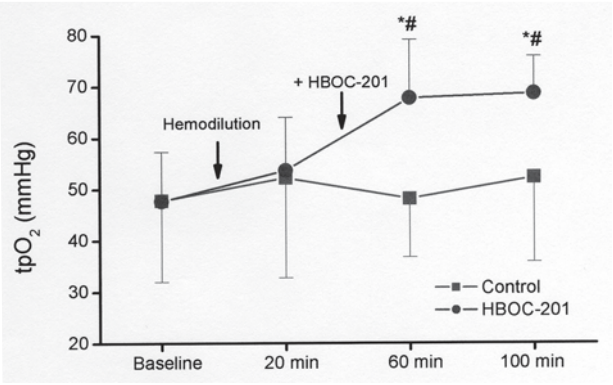


FIGURE 5 Changes of liver tissue oxygen tensions (mmHg) without hemodilution (control) and during hemodilution with Ringer's solution and additional application of hemoglobin-based oxygen carriers (HBOC)-201 (Group 2). *$P < 0.05$ in comparison to control; #$P < 0.05$ comparison to baseline.

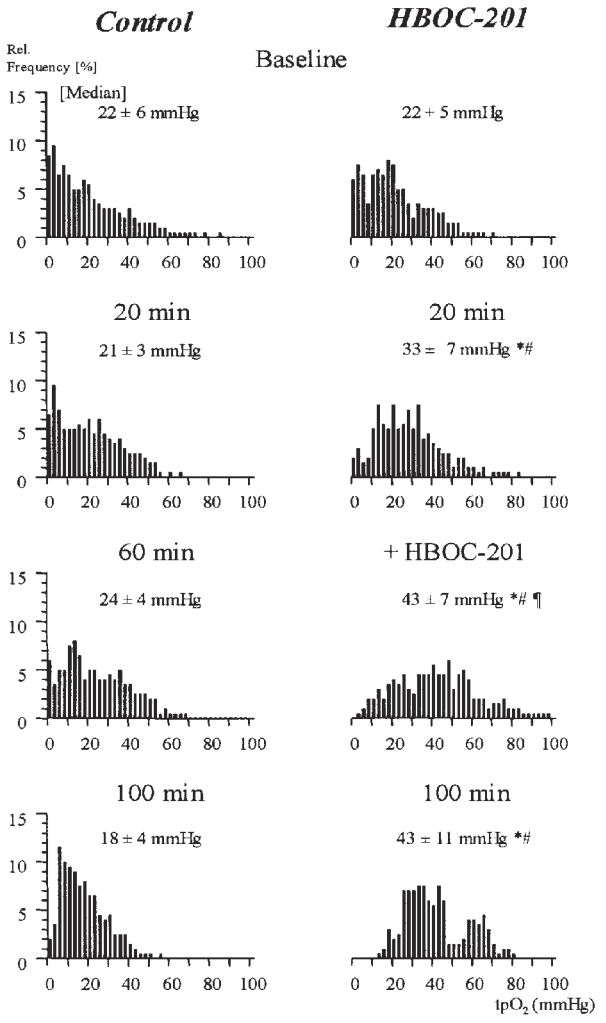


FIGURE 6 Pooled tissue oxygen tension ($tpO_2$) histograms and the median $tpO_2$ values in the skeletal muscle without hemodilution (control) and during hemodilution with Ringer's solution and additional application of hemoglobin-based oxygen carriers (HBOC)-201 (Group 2). *$P < 0.05$ in comparison to control; #$P < 0.05$ comparison to baseline; ¶$P < 0.05$ in comparison to hemodilution.

ation in the liver even in situations where rheologic therapy has reached its limitations. The precondition is, however, that HBOC-201 effectively contributes to tissue oxygenation. For the skeletal muscle this has been already shown before.[6,14–18]

With the polarographic needle probe higher $tpO_2$ values were also shown in the canine skeletal muscle, during extended IHD with an ultrapurified bovine hemoglobin solution (UBPH) in comparison with hydroxyethyl starch (HES).[18] Moreover, the skeletal muscle $tpO_2$ was even higher than baseline values at a hematocrit of only 2% in presence of UPBH, which provided a plasma hemoglobin concentration of 8.2 $g \cdot dL^{-1}$. In contrast to HES, the muscular oxygen consumption was maintained under UPBH treatment, and was paralleled by an increased systemic and muscular oxygen extraction in this animal study.

Another study from our group showed similar results which were explained by facilitated $O_2$-extraction from HBOC-201 due to its right shifted oxygen dissociation curve.[6] Dogs were hemodiluted to hematocrit 10% using 6% HES and were subsequently transfused either with autologous blood (hemoglobin 9 $g \cdot dL^{-1}$) or HBOC-201 to increase the total hemoglobin concentration by 1, 2 and 3 $g \cdot dL^{-1}$. No differences were detected between groups with respect to global tissue oxygenation parameters ($DO_2$, $VO_2$). However, because of the superior oxygen extraction, skeletal muscle $tpO_2$ values were significantly higher in the HBOC-201 group. After the final HBOC-201 infusion, contribution of the bovine hemoglobin to total $CaO_2$ was 47%. The augmented oxygen extraction in presence of HBOC, because of an increased oxygen off load, seems to be a characteristic essential of modern cell-free hemoglobin solutions which provides enhanced oxygen supply at the tissue site.[19]

In our present study, the additional application of HBOC-201 increased the hepatic $tpO_2$ by 45% in comparison to baseline, and by 40% in comparison to control. When these results are transferred to clinical settings, the application of HBOC-201 could be of potential benefit in surgeries where liver oxygenation is impaired, e.g., liver tumour resections or liver transplantations.

One point of criticism is the vasoconstrictive side effect associated with HBOC treatment caused by the well-known nitric oxide (NO) scavenging effect of HBOC.[20,21] As a consequence of the vasoconstrictor effects, increases of the MAP and, rarely, of the PAP, and decrease of the CO were often seen in experimental and clinical studies with HBOC-201.[17,22,23] It has been shown that cell-free hemoglobin molecules can be subject to uptake by vascular endothelial cells. These molecules are transferred to the media by transcytosis, where they can bind to NO, thus creating vasoconstriction, in a dose-dependent manner.[21] In this study, the decreased MAP and SVR after moderate hemodilution was only restored to baseline values after application of HBOC-201. The intensity of vasoconstriction seems to be dependent upon the degree of purification of HBOC and tetrameric stabilisation,[24,25] and is less pronounced with highly modified hemoglobin preparations like HBOC-201. There is also evidence that HBOC has an impact on organ blood flow and distributive oxygen transport in the microcirculation which is different from the vasoconstrictive effects on larger vessels.[26] Sherman *et al.* have shown that systemic vasoconstriction after HBOC administration did not reduce regional blood flow in the hamster liver.[27] After application of HBOC-201, the blood flow in the portal vein did not change significantly in our experiment. Data from Federspiel *et al.* also suggest that free hemoglobin in the plasma phase may enhance oxygen off loading to the tissues, by functionally reducing the intracapillary space between erythrocytes and endothelium, thus facilitating oxygen diffusion.[28,29] A study in dogs subjected to hemorrhage showed that HBOC-201 reconstituted splanchnic perfusion and oxidative metabolism, in spite of increased SVR and consecutively decreased CO and $DO_2$.[30] Although $DO_2$ after hemodilution in this study was decreased, the global and regional oxygen extraction in the liver and skeletal muscle increased $tpO_2$ values after application of HBOC-201 (Figures 2–4).

Another limitation of many studies is that measurements of $tpO_2$ were performed only in skeletal muscle, since skeletal muscle seems to be less important than central organs.[31] However, the skeletal muscle mass represents a major part of mammal and human tissues, is easily accessible, and can even be used for $tpO_2$ measurements in patients.[32] The effects of isovolemic hemodilution and additional application of HBOC-201 on the oxygenation of central organs and oxygen extraction like the liver remained unclear until now. With the results of the present study, we further underlined our hypothesis that changes in $tpO_2$ in the skeletal muscle can approximate the changes seen in the liver during treatment with HBOC. Knudson *et al.* were also able to show in a prehospital model of hemorrhagic shock and resuscitation that $tpO_2$ measured in the deltoid muscle using the Licox device reflects oxygen tension in the liver and can therefore be used as a monitor of splanchnic resuscitation.[33] In addition, in a recently published editorial, the author pointed out that $tpO_2$ is perhaps the most reliable quantitative index of tissue perfusion currently available.[34]

In conclusion, our data show that tissue oxygenation of the liver and skeletal muscle was increased in parallel when HBOC-201 as an oxygen delivering solution was added after moderate isovolemic hemodilution. Because of the increased oxygen off-loading capacity demonstrated by the increased oxygen extraction, HBOC-201 may help to improve liver oxygenation, even in situations where rheologic therapy has reached its limitations.

## APPENDIX

The following variables were calculated:

- Body surface:
  $BSA = \sqrt{\text{weight} \times \text{height}/3600}\ (m^2)$
- Cardiac index:
  $CI = CO/\ BSA\ (L \cdot min^{-1} \cdot m^{-2})$
- Systemic vascular resistance:
  $SVR = \frac{MAP\text{-}CVP}{CO} \times 80\ (dyne \cdot sec \cdot cm^{-5})$
- Pulmonary vascular resistance:
  $PVR = \frac{PAP\text{-}PAOP}{CO} \times 80\ (dyne \cdot sec \cdot cm^{-5})$
- Arteriovenous oxygen difference:
  $avDO_2 = CaO_2 - CvO_2\ (mL \cdot min^{-1})$
- Oxygen delivery:
  $DO_2 = CO \times CaO_2\ (mL \cdot min^{-1})$
- Oxygen consumption:
  $VO_2 = CO \times avDO_2\ (mL \cdot min^{-1})$
- Oxygen extraction:
  $ERO_2 = VO_2/DO_2 \times 100\ [\%]$

The liver blood flow was assessed by the sum of the blood flow of the liver artery (approximately 1/3) and the blood flow of portal vein (approximately 2/3), which is an estimate of liver blood flow. The parameters of hepatic oxygen delivery, oxygen consumption and arteriovenous oxygen difference were calculated as follows:

- Arteriovenous oxygen difference:
  $avDO_2\ liv = (1/3 \times CaO_2) + (2/3 \times CvO_2\ port)$
- $Cv\ O_2\ pul\ [mL \cdot dL^{-1}]$
- Liver oxygen delivery: $DO_2\ liv = [(1/3 \times CaO_2)$

+(2/3 × $CvO_2$ port)] × (1.5 × Flow port) [mL·min$^{-1}$]
- Liver oxygen consumption: $VO_2$ liv = av $DO_2$ liv × (1.5 × Flow port) [mL·min$^{-1}$]
- Liver oxygen extraction rate: $ERO_2$ = $VO_2$ liv/ $DO_2$ liv × 100 [%]

Parameters of oxygen transport in the skeletal muscle were calculated using the flow in the V. poplitea (flow-popl). Muscular arteriovenous oxygen difference av$DO_2$ mus = $CaO_2$ - $CvO_2$ pop, muscular oxygen delivery $DO_2$mus = Flow × $CaO_2$, Muscular oxygen consumption $VO_2$mus = Flow × av$DO_2$mus, Muscular oxygen extraction ratio $ERO_2$ mus = $VO_2$ mus × $DO_2$ mus × 100 [%].

Skeletal tissue oxygen tensions (peripheral $tpO_2$) were measured in the left gastrocnemius muscle by a microprocessor-controlled fast responding polarographic needle probe of 12.5 µm diameter (Eppendorf needle, Netheler-Hinz, Germany). With this device, 200 single $tpO_2$ values can be determined within a time of five minutes in a conical muscular tissue area of 2 to 3 cm$^3$ at every measurement interval. The probe was driven forward by the microprocessor through the muscle tissue in different directions in steps of 0.7 mm each followed by a reverse step of 0.3 mm every 30 sec after completion of every 20 single $tpO_2$ measurements. This technique prevented compression of muscle tissue or capillaries by the tip of the probe. After 200 single $tpO_2$ measurements, the frequency distribution of the $tpO_2$ values was calculated (Sigma-$pO_2$-Histograph KIMOC 6650, Eppendorf-Netheler-Hinz, Germany). At every measurement interval, 1,200 single $tpO_2$ values were collected. The tissue temperature was measured within the gastrocnemius muscle by a stitch probe and maintained constant by a warming lamp connected to a closed-loop system. The accuracy of $tpO_2$ measurements with polarographic needle probes has been demonstrated in several animal experiments[6,35,36] as well as in clinical investigations.[32] Due to the fact that the liver moves during the experiment as a consequence of mechanical ventilation, liver oxygen tensions (central $tpO_2$) were measured using a Licox probe ($pO_2$ microprobe, GMS, Germany). This flexible tissue oxygen probe with an electrochemical (polarographic) microcell was inserted in the liver parenchyma and remained at the same place throughout the experiment. The $pO_2$ microprobe averages the tissue oxygen tensions near the tip of the probe in a cylindric tissue layer located concentrically around the long axis of the microcatheter and at a distance of less than 1 mm from its surface. The probes used in the liver were 300 mm long, with a 5-mm sensing area 18 mm from the tip of the catheter. The probes were calibrated by the manufacturer using a special chip-card for each probe. Resulting histograms consequently do not represent a variety of values corresponding to the distribution of $tpO_2$ values in the tissue, but a variety of $tpO_2$ values in an outlined area. One hundred and twenty single $tpO_2$ values (1 every 5 sec) over a period of ten minutes were determined and displayed on the monitor of the connected computer at every time of measurement. Liver tissue temperature was also measured continuously by a thermocoupled microcatheter for temperature compensation of the $pO_2$ value. The accuracy of $tpO_2$ measurement has been demonstrated in animal experiments and in clinical studies.[5,37]

## References


1 *Kreimeier U, Messmer K.* Hemodilution in clinical surgery: state of the art 1996. World J Surg 1996; 20: 1208–17.

2 *Fontana JL, Welborn L, Mongan PD, Sturm P, Martin G, Bunger R.* Oxygen consumption and cardiovascular function in children during profound intraoperative normovolemic hemodilution. Anesth Analg 1995; 80: 219–25.

3 *Freitag M, Standl T, Horn EP, Wilhelm S, Schulte am Esch J.* Acute normovolaemic haemodilution beyond a haematocrit of 25%: ratio of skeletal muscle tissue oxygen tension and cardiac index is not maintained in the healthy dog. Eur J Anaesth 2002; 19: 487–94.

4 *van Bommel J, Trouwborst A, Schwarte L, Siegemund M, Ince C, Henny CP.* Intestinal and cerebral oxygenation during severe isovolemic hemodilution and subsequent hyperoxic ventilation in a pig model. Anesthesiology 2002; 97: 660–70.

5 *Boekstegers P, Riessen R, Seyde W.* Oxygen partial pressure distribution within skeletal muscle: indicator of whole body oxygen delivery in patients? Adv Exp Med Biol 1990; 277: 507–14.

6 *Standl T, Horn P, Wilhelm S, et al.* Bovine haemoglobin is more potent than autologous red blood cells in restoring muscular tissue oxygenation after profound isovolaemic haemodilution in dogs. Can J Anaesth 1996; 43: 714–23.

7 *Horn EP, Sputtek A, Standl T, Rudolf B, Kuhnl P, Schulte am Esch J.* Transfusion of autologous, hydroxyethyl starch-cryopreserved red blood cells. Anesth Analg 1997; 85: 739–45.

8 *Standl T, Lipfert B, Reeker W, Schulte am Esch J, Lorke DE.* Acute effects of a complete blood exchange with ultra-purified haemoglobin solution or hydroxyethyl starch, on liver and kidney in an animal model (German). Anasthesiol Intensivmed Notfallmed Schmerzther 1996; 31: 354–61.

9 *Liard JF, Kunert MP.* Hemodynamic changes induced

by low blood oxygen affinity in dogs. Am J Physiol 1993; 264: R396–401.
10 *Standl T, Freitag M, Burmeister MA, Horn EP, Wilhelm S, Schulte am Esch J.* Hemoglobin-based oxygen carrier HBOC-201 provides higher and faster increase in oxygen tension in skeletal muscle of anemic dogs than do stored red blood cells. J Vasc Surg 2003; 37: 859–65.
11 *Buga GM, Gold ME, Fukuto JM, Ignarro LJ.* Shear stress-induced release of nitric oxide from endothelial cells grown on beads. Hypertension 1991; 17: 187–93.
12 *Mirhashemi S, Ertefai S, Messmer K, Intaglietta M.* Model analysis of the enhancement of tissue oxygenation by hemodilution due to increased microvascular flow velocity. Microvasc Res 1987; 34: 290–301.
13 *Holbeck S, Grande PO.* Effects on capillary fluid permeability and fluid exchange of albumin, dextran, gelatin, and hydroxyethyl starch in cat skeletal muscle. Crit Care Med 2000; 28: 1089–95.
14 *Horn EP, Standl T, Wilhelm S, Jacobs EE, Freitag U, Freitag M, Schulte am Esch J.* Bovine hemoglobin increases skeletal muscle oxygenation during 95% artificial arterial stenosis. Surgery 1997; 121: 411–8.
15 *Nolte D, Botzlar A, Pickelmann S, Bouskela E, Messmer K.* Effects of diaspirin-cross-linked hemoglobin (DCLHb™) on the microcirculation of striated skin muscle in the hamster: a study on safety and toxicity. J Lab Clin Med 1997; 130: 314–27.
16 *Sielenkamper AW, Eichelbronner O, Martin CM, Madorin SW, Chin-Yee IH, Sibbald WJ.* Diaspirin cross-linked hemoglobin improves mucosal perfusion in the ileum of septic rats. Crit Care Med 2000; 28: 782–7.
17 *Slanetz PJ, Lee R, Page R, Jacobs EE Jr, LaRaia PJ, Vlahakes GJ.* Hemoglobin blood substitutes in extended preoperative autologous blood donation: an experimental study. Surgery 1994; 115: 246–54.
18 *Standl TG, Reeker W, Redmann G, Kochs E, Werner C, Schulte am Esch J.* Haemodynamic changes and skeletal muscle oxygen tension during complete blood exchange with ultrapurified polymerized bovine haemoglobin. Intensive Care Med 1997; 23: 865–72.
19 *Page TC, Light WR, Hellums JD.* Prediction of microcirculatory oxygen transport by erythrocyte/hemoglobin solution mixtures. Microvasc Res 1998; 56: 113–26.
20 *Hughes GS Jr, Antal EJ, Locker PK, Francom SF, Adams WJ, Jacobs EE Jr.* Physiology and pharmacokinetics of a novel hemoglobin-based oxygen carrier in humans. Crit Care Med 1996; 24: 756–64.
21 *Faivre-Fiorina B, Caron A, Fassot C, et al.* Presence of hemoglobin inside aortic endothelial cells after cell-free hemoglobin administration in guinea pigs. Am J Physiol 1999; 276: H766–70.
22 *LaMuraglia GM, O'Hara PJ, Baker WH, et al.* The reduction of the allogenic transfusion requirement in aortic surgery with a hemoglobin-based solution. J Vasc Surg 2000; 31: 299–308.
23 *Standl T, Wilhelm S, Horn EP, Burmeister M, Gundlach M, Schulte am Esch J.* Preoperative hemodilution with bovine hemoglobin. Acute hemodynamic effects in liver surgery patients (German). Anaesthesist 1997; 46: 763–70.
24 *Biro GP, Taichman GC, Lada B, Keon WJ, Rosen AL, Sehgal LR.* Coronary vascular actions of stroma-free hemoglobin preparations. Artif Organs 1988; 12: 40–50.
25 *Gilroy D, Shaw C, Parry E, Olding-Smee W.* Detection of a vasocontrictor factor in stroma-free haemoglobin solutions. J Trauma 1988; 28: 1312–6.
26 *Gulati A, Sharma AC, Burhop KE.* Effect of stroma-free hemoglobin and diaspirin cross-linked hemoglobin on the regional circulation and systemic hemodynamics. Life Sci 1994; 55: 827–37.
27 *Sherman IA, Dlugosz JA, Perelman V, Hsia CJ, Wong LT, Condie RM.* Systemic hemodynamic and hepatic microvascular responses to a 33% blood volume exchange with whole blood, stroma-free hemoglobin, and oxypolyhemoglobin solutions. Biomater Artif Cells Immobilization Biotechnol 1993; 21: 537–51.
28 *Federspiel WJ.* Pulmonary diffusing capacity: implications of two-phase blood flow in capillaries. Respir Physiol 1989; 77: 119–34.
29 *Page TC, Light WR, McKay CB, Hellums JD.* Oxygen transport by erythrocyte/hemoglobin solution mixtures in an in vitro capillary as a model of hemoglobin-based oxygen carrier performance. Microvasc Res 1998; 55: 54–64.
30 *Driessen B, Jahr JS, Lurie F, Griffey SM, Gunther RA.* Effects of haemoglobin-based oxygen carrier hemoglobin glutamer-200 (bovine) on intestinal perfusion and oxygenation in a canine hypovolaemia model. Br J Anaesth 2001; 86: 683–92.
31 *Zander R.* Failure to prove the effectiveness of hemoglobin solutions as oxygen carriers in the paper of Horn et al. Anaesthesist (1998) 47: 116-123. Anaesthetist 1998; 47: 998–1003.
32 *Standl T, Burmeister MA, Schroeder F, et al.* Hydroxyethyl starch (HES) 130/0.4 provides larger and faster increases in tissue oxygen tension in comparison with prehemodilution values than HES 70/0.5 or HES 200/0.5 in volunteers undergoing acute normovolemic hemodilution. Anesth Analg 2003; 96: 936–43.
33 *Knudson MM, Lee S, Erickson V, Morabito D, Derugin N, Manley GT.* Tissue oxygen monitoring during hemorrhagic shock and resuscitation: a comparison of lactated Ringer's solution, hypertonic saline dextran, and

HBOC-201. J Trauma 2003; 54: 242–52.

34 *Ragheb J, Buggy DJ*. Tissue oxygen tension ($PTO_2$) in anaesthesia and perioperative medicine (Editorial). Br J Anaesth 2004; 92: 464–8.

35 *Fleckenstein W, Schaffler A, Heinrich R, Petersen C, Gunderoth-Palmowski M, Nollert G*. On the differences between muscle $pO_2$ measurements obtained with hypodermic needle probes and with multiwire surface probes. Part 2: Systemic diffusion error of the multiwire surface $pO_2$ probe when applied on the tissue. *In*: Ehrly AM, Fleckenstein W, Hauss J, Huch R (Eds). Clinical Oxygen Pressure Measurement. Berlin: Blackwell Ueberreuter Wissenschaft; 1990; 268–78.

36 *Fleckenstein W, Weiss C*. A comparison of $pO_2$ histograms from rabbit hind-limb muscles obtained by simultaneous measurements with hypodermic needle electrodes and with surface electrodes. Adv Exp Med Biol 1984; 169: 447–55.

37 *Noldge GF, Priebe HJ, Geiger K*. Splanchnic hemodynamics and oxygen supply during acute normovolemic hemodilution alone and with isoflurane-induced hypotension in the anesthetized pig. Anesth Analg 1992; 75: 660–74.