Laboratory Investigations

Bovine haemoglobin is more potent than autologous red blood cells in restoring muscular tissue oxygenation after profound isovolaemic haemodilution in dogs

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Purpose: *This study compares the effects of stored red cells, freshly donated blood and ultrapurified polymerized bovine haemoglobin (HBOC) on haemodynamic variables, oxygen transport capacity and muscular tissue oxygenation after acute and almost complete isovolaemic haemodilution in a canine model.*

Methods: *Following randomization to one of three groups, 24 anaesthetized Foxhounds underwent isovolaemic haemodilution with 6% hetastarch to haematocrit levels of 20%, 15% and 10% before they received isovolaemic stepwise augmentation of 1 $g \cdot dl^{-1}$ haemoglobin. In Group 1, animals were given autologous stored red cells which they had donated three weeks before. In Group 2, animals received freshly donated blood harvested during haemodilution. In Group 3, animals were infused with HBOC. Skeletal muscle tissue oxygen tension was measured with a polarographic 12 μ needle probe.*

Results: *In all groups, heart rate and cardiac index were increased with decreasing vascular resistance during haemodilution ($P < 0.05$). Haemodynamic variables showed a reversed trend during transfusion when compared to haemodilution but remained below baseline ($P < 0.05$). Arterial and venous oxygen content were changed in parallel to changes of haematocrit and haemoglobin concentrations but were lower in Group 3 than in Groups 1 and 2 ($P < 0.05$) during transfusion. In contrast, the oxygen extraction ratio was higher in Group 3 (59 ± 8%, $P < 0.01$) at the end of transfusion than in Group 1 (37 ± 13%) and 2 (32 ± 5%). In Group 3, mean tissue oxygen tension increased from 16 ± 5 mmHg after haemodilution to 56 ± 11 mmHg after transfusion ($P < 0.01$) and was higher than in Group 1 (41 ± 9, $P < 0.01$) and Group 2 (29 ± 11, $P < 0.01$). While in Group 3 an augmentation of 0.7 $g \cdot dl^{-1}$ haemoglobin resulted in restoring baseline tissue oxygenation, higher doses of 2.7 $g \cdot dl^{-1}$ and 2.1 $g \cdot dl^{-1}$ were needed in Groups 1 and 2 to reach this level ($P < 0.01$).*

Conclusion: *The results show a higher oxygenation potential of HBOC than with autologous stored red cells because of a more pronounced oxygen extraction.*

Objectif: *Cette étude compare les effets des hématies conservées, du sang fraîchement prélevé et de l'hémoglobine bovine polymérisée ultrapurifiée (HBOC) sur les variables*

Key words
BLOOD: haemodilution;
OXYGEN: transport;
TRANSFUSION: autologous, bovine haemoglobin.

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hémodynamiques, la capacité de transport en oxygène et l'oxygénation du tissu musculaire après hémodilution isovolémique aiguë et presque complète sur un modèle canin.

***Méthodes**: Après randomisation en trois groupes, 24 foxhounds ont subi une hémodilution isovolémique en paliers avec de l'hétastarch à 6% pour réaliser des hématocrites de 20%, 15% et 10% avant de recevoir une augmentation isovolémique en paliers de 1 g·dl^{-1} d'hémoglobine. Dans le groupe 1, les chiens ont reçu les hématies autologues conservées prélevées trois semaines auparavant. Dans le groupe 2, les animaux ont reçu de sang frais recueilli au moment de l'hémodilution. Dans le groupe 3, les animaux ont été perfusés avec HBCO. La tension en oxygène du tissus musculaire a été mesurée avec une sonde polarographique.*

***Résultats**: Dans tous les groupes, la fréquence et l'index cardiaques ont augmenté avec la baisse de la résistance vasculaire pendant l'hémodilution ($P < 0,05$). Pendant la transfusion, les variables hémodynamiques ont révélé une tendance inverse de celle de l'hémodilution mais sont demeurées sous la ligne de base ($P < 0,05$). Pendant la transfusion, les contenus artériels et veineux en oxygène ont changé parallèlement aux changements de l'hématocrite et de la concentration de l'hémoglobine mais étaient plus bas dans le groupe 3 que dans les groupes 1 et 2 ($P < 0,05$). Par contre, à la fin de la transfusion, l'extraction de l'oxygène a été plus grande dans le groupe 3 (59 ± 8%, $P < 0,01$) que dans les groupes 1 (37 ± 13%) et 2 (31 ± 5%). Dans le groupe 3, après la transfusion, la tension tissulaire moyenne en oxygène a augmenté de 16 ± 5 mmHg à 56 ± 11 mmHg ($P < 0,01$) et était plus élevée que dans les groupes 1 (41 ± 9, $P < 0,01$) et 2 (29 ± 11, $P < 0,01$). Alors que dans le groupe 3, une augmentation de 0,7 g·dl^{-1} a permis de ramener l'oxygénation tissulaire à la ligne de base, des quantités plus grandes (2,7 g·dl^{-1} et de 2,2 g·dl^{-1}) ont été requises pour atteindre ce niveau dans les groupes 1 et 2.*

***Conclusion**: Ces résultats montrent un potentiel d'oxygénation plus élevé avec HBCO qu'avec des hématies autologues conservées en raison d'une extraction plus prononcée de l'oxygène.*

Recent concerns about the safety of homologous blood transfusions have intensified the search for alternative blood replacement. Besides increased application of pre- and perioperative autologous blood donation and improved storage of packed red cells, many investigations have been performed in stroma-free haemoglobin solutions during the last decade.[1-3] Technical progress in purification and engineering of haemoglobin resulted in a new generation of stroma-free haemoglobins without toxic side effects on liver, kidney and coagulation.[4,5] Bovine haemoglobin shows low oxygen affinity which is regulated by chloride ions rather than by 2,3-diphosphoglycerate.[6] Previous studies have shown excellent biocompatibility and long-term survival after nearly complete blood exchange with polymerized bovine haemoglobin.[7,8] Tissue oxygenation is one of the most important issues in experimental blood substitution. However, there are few studies which investigated tissue oxygen tension during blood replacement with haemoglobin solutions.[9-11] There is some theoretical basis to expect that free haemoglobin in plasma may have a higher potency for oxygen release to the tissues than red cells.[12,13] To investigate this possibility the present prospective study was designed to examine skeletal muscle tissue oxygen tension after severe acute anaemia and isovolaemic resuscitation with equivalent doses of stored red cells, freshly donated blood and polymerized bovine haemoglobin in a dog model.

Methods

Following approval of the Animal Care Committee, 24 Foxhounds (15 male and 9 female, mean age 2 ± 0.5 yr, mean weight 30 ± 14 kg) were included in the study.

Preparation and measurements

Anaesthesia was induced with 5 mg·kg^{-1} ketamine hydrochloride (Parke-Davis, FRG) and 2 mg·kg^{-1} *im* xylazine (Bayer, FRG), and maintained by continuous infusion of 0.025 mg·kg^{-1}·hr^{-1} fentanyl (Janssen, FRG), 0.4 mg·kg^{-1}·hr^{-1} midazolam (Roche, FRG) and 0.2 mg·kg^{-1}·hr^{-1} vecuronium (Organon, FRG). Mechanical ventilation was performed with 30% oxygen in air (Spiromat 650, Drager, FRG) after endotracheal intubation. Temperature, ECG and heart rate (HR) were monitored continuously (Marquette, USA). Catheters were inserted into both right femoral arteries and veins for measurements of mean arterial pressure (MAP), mean pulmonary artery pressure (PAP), central venous pressure (CVP) and pulmonary capillary wedge pressure (PCWP) as well as for arterial and mixed-venous blood gas sampling (ABL 505, Radiometer, Danmark). Cardiac output (CO) was determined by the thermodilution method (average of four measures) via a 7-F pulmonary artery catheter (Opticath, Abbott, FRG) connected with a cardiac output computer (Oximetrix 3, Abbott, FRG). Skeletal muscle oxygen tension (tPO_2) was measured in the left sartorius muscle by a microprocessor controlled fast responding ($T_{90} < 500$ msec) polarographic needle probe of 12.5 μ diameter.[14] Every respective measurepoint, 200 single tPO_2 values were determined over a period of five minutes in a conical tissue area of 2–3 cm^3. After insertion in the skeletal muscle in a depth of 20 mm, the probe was driven forward through the tissue in different directions in steps of 0.7 mm each followed by a reverse step of 0.3 mm under control of the microprocessor. This procedure was

repeated every 30 sec following measurements of 20 single tPO_2 values and guaranteed relief of tissue pressure at the tip of the probe and avoided compression of capillaries. Drift corrections in accordance with pre- and post-measurement calibrations were carried out by an integrated calculator. A drift of the probe of more than $0.2\% \cdot min^{-1}$ was not accepted. Reduced susceptibility for humidity of the needle probe due to a permanent connection between the needle probe and the connection plug of the micromanipulator allowed for high reproductability and low drift of measures which has been demonstrated in animal experiments[15] as well as in clinical investigations.[16,17] Each single tPO_2 value was displayed on a monitor. After completion of 200 measurements, the combined frequency distribution of the tPO_2 values was calculated and displayed as histogram by the Sigma-PO_2-Histograph KIMOC 6650 (Eppendorf-Netheler-Hinz, FRG). Pooled histograms were formed for eight animals of each group at the respective measurepoint and consisted of 1,600 single tPO_2-measurements.

Haematocrit values (Hct) were determined five minutes after centrifugation of arterial blood (Haemofuge A, Heraeus Sepatech, FRG). Free haemoglobin in plasma (Hbf) was measured using EDTA (ethylene diamine tetra-acetoacid)-stabilized aterial blood after five minutes centrifugation (5000 g, Biofuge 17 RS, Heraeus Sepatech, FRG). Total haemoglobin (Hbtot) in heparinized arterial blood and Hbf were measured using a six wave length oximeter (OSM 3, Radiometer, Danmark). An oxygen-specific fuel cell (Lex-O_2-Con, Lexington Instruments, Mass.) was used for measurement of arterial (Ca-O_2) and mixed-venous oxygen content (Cv-O_2). Arterial and mixed-venous lactate were measured photometrically after dilution using a specific test kit (Lactat, Boehringer, FRG). Following variables were calculated: cardiac index (CI), systemic vascular resistance (SVR), pulmonary vascular resistance (PVR), oxygen delivery (DO_2), oxygen consumption (VO_2), oxygen extraction ratio (ER-O_2) = VO_2/DO_2.

Protocol

Eight dogs which were randomly allocated to Group 1 underwent plebotomy under sedation for autologous blood donation three weeks before the experiment. Fifteen $ml \cdot kg^{-1}$ blood were withdrawn using 63 mL of CPD solution (26.3 $g \cdot L^{-1}$ sodium citrate, 3.3 $g \cdot L^{-1}$ citrate, 2.5 $g \cdot L^{-1}$ sodium hydrogen phosphate, 25.5 $g \cdot L^{-1}$ dextrose, pH = 5.65) as anticoagulant and substituted by an equal volume of Ringer's solution. The blood was immediatedly separated into plasma and red cell concentrate (RBC) by centrifugation (4000 g, 15 min). The RBCs were stored at +4°C in 200 ml PAGGS-mannitol additive solution (16.1 $mmol \cdot L^{-1}$ phosphate, 0.194 $g \cdot L^{-1}$ adenine, 0.408 $g \cdot L^{-1}$ guanine, 9.4 $g \cdot L^{-1}$ glucose, 96.2 $mmol \cdot L^{-1}$ sodium, 10.0 $g \cdot L^{-1}$ mannitol, pH = 6.0). Prior to transfusion, about 2/3 of the PAGGS-mannitol were removed by discarding the supernatant after centrifugation. On the day of the experiment, all animals received a PCWP controlled isovolaemic haemodilution to haematocrit values of 20%, 15% and 10%. Blood exchange was performed using 6% hetastarch 200,000/0.5 (Hemohes, B. Braun FRG). This entailed progressive removal of blood with infusion of hetastarch in volumes sufficient to monitor PCWP in a range of 8 ± 2 mm Hg. In eight dogs of Group 2, the blood volume removed by isovolaemic haemodilution was stored in CPD bags at room temperature. When a haematocrit of 10% was reached, stepwise transfusion was started in all groups to achieve haemoglobin target values of +1 $g \cdot dl^{-1}$, +2 $g \cdot dl^{-1}$ and +3 $g \cdot dl^{-1}$ compared with the respective haemoglobin value at haematocrit 10%. Animals of Group 1 received their own stored red cells. In Group 2, the dogs received the freshly donated blood. In Group 3, animals received ultrapurified, polymerized bovine haemoglobin (HBOC, Biopure, Boston, Mass.) with a haemoglobin concentration of 13 ± 1 $g \cdot dl^{-1}$ (methaemoglobin and oxyhaemoglobin ≤10%) and an oncotic pressure of 17 mmHg. The HBOC was prepared from bovine red cells by lysis, filtration, chromatography and polymerization with glutaraldehyde ($65{,}000 < MW < 500{,}000$). The sterile pyrogen free solution contains <0.5 $EU \cdot ml^{-1}$ endotoxin and <3 nM phospholipid and physiological concentrations of electrolytes.

All variables were recorded after an equilibration period of 20 min. The time between the respective measurement-points was 60 min. At the end of the study the animals were euthanized by intravenous injection of potassium chloride 7.45%.

Statistics

Data are reported as mean values ± SD. Skeletal muscle tPO_2-values are plotted as 10th-, 50th- and 90th-percentiles. The tPO_2-values were tested using the Mann Whitney U-test. Differences within groups of other variables were tested by one-way ANOVA and post-hoc comparison by Student's t test. Differences between groups were tested by two-way ANOVA and post-hoc comparison with Bonferoni's correction for alpha. All differences were considered significant at $P < 0.05$.

Results

Blood gases

In all groups, temperature and arterial blood gases did not change over time. During haemodilution pH, stan-

TABLE I Haemodynamic variables during IHD to haematocrit (Hct) target levels of 20%, 15%, 10% and stepwise transfusion of 1 g·dl^{-1} haemoglobin using stored red blood cells (Group 1), fresh donated blood (Group 2) or HBOC (Group 3)

	HR (*beats·min^{-1}*)	*MAP* (*mmHg*)	*CVP* (*mmHg*)	*PAP* (*mmHg*)	*PVR* (*dyne·s·cm^{-5}*)	*SVR* (*dyne·s·cm^{-5}*)	*CO* (*L·min^{-1}*)	*a Lactate* (*mg·dl^{-1}*)
Group 1								
Baseline	72 ± 13	113 ± 17	2 ± 3	14 ± 3	133 ± 82	2471 ± 426	3.6 ± 0.3	7.8 ± 4.1
Hct 20%	91 ± 27	103 ± 17	2 ± 3	12 ± 3	54 ± 16*	1436 ± 431*	5.9 ± 1.4*	6.1 ± 2.5
Hct 15%	103 ± 21*	98 ± 20	3 ± 2	14 ± 3	63 ± 27*	1119 ± 271*	6.9 ± 0.9*	6.2 ± 2.1
Hct 10%	140 ± 36*	92 ± 20*	3 ± 2	16 ± 4	68 ± 33	766 ± 237*	9.7 ± 1.9*	6.9 ± 3.7
+1 g Hb	127 ± 34*	100 ± 17	3 ± 3	14 ± 4	75 ± 46	1106 ± 389*	7.5 ± 1.6*	6.1 ± 1.2
+2 g Hb	115 ± 27*	100 ± 18	2 ± 2	15 ± 4	111 ± 67	1337 ± 486*	6.3 ± 1.6*	7.4 ± 1.5
+3 g Hb	108 ± 27*	102 ± 19	3 ± 1	15 ± 5	106 ± 68	1354 ± 322*	6.0 ± 2.1*	7.6 ± 1.6
Group 2								
Baseline	85 ± 15	114 ± 26	2 ± 3	12 ± 3	117 ± 42	2203 ± 572	4.2 ± 1.1	8.4 ± 3.9
Hct 20%	113 ± 44	101 ± 21	3 ± 2	12 ± 4	75 ± 21*	1272 ± 458*	7.5 ± 4.6	6.2 ± 4.0
Hct 15%	137 ± 50*	100 ± 26	6 ± 3*†	16 ± 6	82 ± 36	980 ± 387*	9.5 ± 6.4	5.5 ± 2.7
Hct 10%	148 ± 40*	99 ± 25	6 ± 4*	15 ± 6	75 ± 27*	874 ± 339*	10.1 ± 6.2*	5.5 ± 1.7
+1 g Hb	140 ± 38*	95 ± 22	3 ± 3	14 ± 3	83 ± 46	955 ± 356*	9.2 ± 6.2	6.0 ± 2.1
+2 g Hb	137 ± 40*	96 ± 27	3 ± 2	14 ± 2	95 ± 52	1047 ± 392*	8.9 ± 5.3*	5.9 ± 2.3
+3 g Hb	121 ± 28*	107 ± 22	4 ± 2	16 ± 2*	115 ± 48	1156 ± 348*	8.0 ± 4.0	7.4 ± 3.0
Group 3								
Baseline	83 ± 14	122 ± 27	1 ± 2	12 ± 3	112 ± 27	3044 ± 1092	3.6 ± 0.9	9.5 ± 2.2
Hct 20%	94 ± 17	102 ± 16	3 ± 3*	12 ± 3	66 ± 29*	1352 ± 315*	6.6 ± 1.5*	8.5 ± 2.3
Hct 15%	110 ± 20*	102 ± 18	4 ± 2*	14 ± 3	72 ± 32*	1240 ± 607*	7.4 ± 1.6*	8.1 ± 2.9
Hct 10%	124 ± 36*	100 ± 16	3 ± 2*	13 ± 3	52 ± 27*	817 ± 270*	9.9 ± 2.0*	7.7 ± 1.9
+1 g Hb	109 ± 34	107 ± 19	3 ± 3*	15 ± 4	95 ± 29	1297 ± 211*‡	7.2 ± 1.7*	8.1 ± 2.5
+2 g Hb	107 ± 27*	106 ± 20	2 ± 3	14 ± 4	94 ± 48	1326 ± 343*	6.6 ± 1.5*	8.4 ± 2.3
+3 g Hb	108 ± 22*	109 ± 15	2 ± 1	12 ± 3‡	97 ± 59	1492 ± 580*	5.9 ± 1.6*	9.1 ± 5.1

HR: heart rate; MAP: mean arterial pressure; CVP: central venous pressure; PAP: mean pulmonary artery pressure; PVR: pulmonary vascular resistance; SVR: systemic vascular resistance; CO: cardiac output; a Lactate: arterial lactate concentration. Values are expressed as mean ± SD. *$P < 0.05$ compared with baseline; †$P < 0.05$ compared with Group 1; ‡$P < 0.05$ compared with Group 2.

dard bicarbonate (SB) and base excess (BE) decreased when compared with baseline ($P < 0.05$). The SB and BE continued to remain at lower values than baseline during transfusion in all groups ($P < 0.05$). However, this effect was more pronounced in Groups 1 (SB: 15.1 ± 2.3; BE: −11.5 ± 3.2) and 2 (SB: 16.1 ± 1.5; BE: −10.3 ± 2.0) than in Group 3 (SB: 17.2 ± 0.8; BE: −8.7 ± 0.9, $P < 0.05$).

Haemodynamics

Hemodynamic varibles are given in Table I. The HR and CO increased during haemodilution and remained at a higher level than baseline during transfusion in all groups ($P < 0.05$). The PCWP as a parameter for isovolaemic conditions did not change over time in all groups. The SVR was continuously decreased during haemodilution ($P < 0.05$) and increased during transfusion but remained below baseline ($P < 0.05$).

Oxygen transport

The mean haemoglobin concentration of banked packed RBCs was 29.1 ± 1.2 g·dl^{-1}, of freshly donated blood 9.0 ± 1.8 g·dl^{-1} and of HBOC 13.2 ± 0.5 g·dl^{-1} ($P < 0.01$ Group 1 vs 2, 3; $P < 0.01$ group 2 vs 3). The HBOC solution had a higher pH (7.5 ± 0, $P < 0.05$) than RBC units (pH = 6.3 ± 0.1) and fresh blood (pH = 6.8 ± 0.1).

The Hct and Hbtot values decreased and increased in parallel to haemodilution and transfusion in Groups 1 and 2 (Table II). In contrast, Hct remained unchanged at 10% during increasing Hbf values of 0.6 ± 0.5 g·dl^{-1}, 1.5 ± 0.5 g·dl^{-1} and 2.7 ± 1.2 g·dl^{-1} ($P < 0.01$ vs baseline). The HBOC showed a contribution to arterial oxygen content of 17% after the first augmentation of Hbtot, 23% after the second and 42% after the final augmentation. The calculated oxygen transport capacity (Huefner index) was 1.37 g·dl^{-1} for canine erythrocytes and 1.16 g·dl^{-1} for HBOC. Arterial and mixed- venous oxygen content as well as DO_2 decreased during haemodilution ($P < 0.05$) and slightly increased during transfusion in all groups, while VO_2 only increased in Group 3 after final HBOC-transfusion ($P < 0.05$). The ERO_2 increased during haemodilution in all groups ($P < 0.05$). In contrast to Groups 1 and 2, ERO_2 remained elevated during transfusion of HBOC in Group 3 ($P <$

TABLE II Oxygen transport during IHD to haematocrit (Hct) target levels of 20%, 15%, 10% and stepwise transfusion of 1 $g \cdot dl^{-1}$ haemoglobin using stored red blood cells (Group 1), freshly donated blood (Group 2) or HBOC (Group 3)

	DO_2 ($ml \cdot min^{-1}$)	VO_2 ($ml \cdot min^{-1}$)	ERO_2 (%)	Hct (Vol%)	Hbtot ($g \cdot dl^{-1}$)	C_a-O_2 (Vol%)	C_v-O_2 (Vol%)	v Lactate ($mg \cdot dl^{-1}$)
Group 1								
Baseline	679 ± 162	159 ± 34	23.5 ± 4	44 ± 5	14.1 ± 1.5	18.7 ± 2.0	14.4 ± 1.0	7.2 ± 2.9
Hct 20%	453 ± 72*	156 ± 35	35.5 ± 9*	19 ± 1*	5.8 ± 0.6*‡	7.8 ± 0.7*‡	5.0 ± 0.7*	5.9 ± 2.3
Hct 15%	405 ± 95*	175 ± 48	43.7 ± 10*	15 ± 1*	4.3 ± 0.5*	5.9 ± 0.6*	3.4 ± 0.9*	6.3 ± 1.8
Hct 10%	424 ± 70*	208 ± 89	48.6 ± 16*	10 ± 1*	3.0 ± 0.5*	4.5 ± 1.1*	2.2 ± 0.7*	5.8 ± 1.2
+1 g Hb	420 ± 104*	181 ± 56	44.7 ± 14*	13 ± 1*	4.1 ± 0.6*	5.6 ± 0.7*	3.1 ± 0.9*	6.4 ± 1.3
+2 g Hb	461 ± 142*	182 ± 58	41.7 ± 14*	17 ± 2*	5.4 ± 0.7*	7.3 ± 0.9*	4.3 ± 1.0*	7.3 ± 1.1
+3 g Hb	548 ± 252	189 ± 87	37.1 ± 13*	21 ± 3*	6.7 ± 1.0*	9.0 ± 1.3*	5.6 ± 1.4*	7.4 ± 1.8
Group 2								
Baseline	776 ± 198	173 ± 41	22.5 ± 3	44 ± 6	14.5 ± 2.1	19.7 ± 2.9	15.4 ± 1.6	8.2 ± 4.8
Hct 20%	555 ± 182	198 ± 37	37.2 ± 7*	20 ± 1*	6.7 ± 0.6*	9.1 ± 0.8*	5.9 ± 1.7*	6.2 ± 3.9
Hct 15%	476 ± 310	232 ± 82	56.0 ± 20*	13 ± 2*	4.0 ± 0.8*	5.8 ± 1.2*	2.7 ± 1.3*	5.7 ± 3.0
Hct 10%	421 ± 195*	199 ± 63	50.7 ± 7*	10 ± 1*	3.3 ± 0.3*	4.9 ± 0.6*	2.5 ± 0.4*	5.6 ± 2.3
+1 g Hb	459 ± 250*	216 ± 75	45.7 ± 20*	14 ± 2*	4.3 ± 0.6*	6.1 ± 1.3*	3.2 ± 1.0*	5.8 ± 4.2
+2 g Hb	529 ± 257	172 ± 50	35.4 ± 10*	17 ± 2*	5.2 ± 0.7*	7.1 ± 0.9*	4.6 ± 0.7*	5.5 ± 2.2
+3 g Hb	552 ± 180*	171 ± 45	31.6 ± 5*	20 ± 3*	6.2 ± 0.7*	8.3 ± 0.9*	5.7 ± 0.9*	6.6 ± 2.8
Group 3								
Baseline	748 ± 162	165 ± 40	22.3 ± 4	48 ± 5	15.8 ± 1.6	20.8 ± 2.4	16.2 ± 2.5	9.5 ± 3.3
Hct 20%	567 ± 161*	191 ± 46	34.4 ± 6*	19 ± 1*	6.0 ± 0.7*	8.5 ± 1.0*	5.6 ± 0.9*	7.6 ± 2.0
Hct 15%	429 ± 95*	166 ± 35	39.1 ± 3*	14 ± 2*	4.1 ± 0.6*	5.9 ± 1.1*	3.6 ± 1.1*	7.2 ± 1.3
Hct 10%	418 ± 117*	215 ± 60	52.1 ± 10*	10 ± 1*	3.0 ± 0.6*	4.2 ± 0.8*	2.0 ± 0.6*	6.6 ± 1.6
+1 g Hb	372 ± 158*	204 ± 93	56.5 ± 14*	10 ± 2*†‡	3.7 ± 0.9*	5.2 ± 1.5*	2.3 ± 1.1*	8.0 ± 1.9
+2 g Hb	377 ± 107*	203 ± 65	53.5 ± 7*†‡	10 ± 2*†‡	4.7 ± 0.9*	5.8 ± 1.4*†‡	2.7 ± 0.8*†‡	7.9 ± 1.6‡
+3 g Hb	401 ± 101*	235 ± 53*	59.4 ± 8*†‡	10 ± 3*†‡	5.6 ± 1.2*	6.9 ± 1.3*†‡	2.8 ± 1.2*†‡	7.5 ± 1.7

DO_2: oxygen delivery; VO_2: oxygen consumption; ERO_2: oxygen extraction ratio; Hct: arterial haematocrit; Hb tot: total haemoglobin concentration; Ca-O_2: arterial oxygen content; Cv-O_2: venous oxygen content; v Lactate: mixed-venous lactate concentration. Values are expressed as mean ± SD.

*$P < 0.05$ compared with baseline; †$P < 0.05$ compared with Group 1; ‡$P < 0.05$ compared with Group 2.

0.05) with lower arterial and venous oxygen contents than in Groups 1 and 2 ($P < 0.05$). Arterial and mixed-venous lactate concentrations did not change in all groups.

Muscular tissue oxygenation

All pooled tPO_2 histograms, consisting of 1,600 single measurements, show a shift to the left because of continuously decreasing tPO_2 values during severe acute isovolaemic anaemia (Figure 1). In all groups, the mean tPO_2 decreased at Hct 10% when compared with baseline ($P < 0.01$). Transfusion provided a shift of the pooled histograms to the right in all groups, but mean tPO_2 values were higher in Group 3 than in Groups 1 and 2 ($P < 0.01$). There was a more pronounced shift of the histogram to the right in Group 3 than in other groups. In contrast to Groups 1 and 2, no tPO_2 value <7.5 mmHg was seen in Group 3 during transfusion of HBOC. The percentage increases of mean tPO_2 during transfusion were higher in Group 3 than in Groups 1 and 2 when compared with baseline ($P < 0.01$, Figure 2). In Group 3, the baseline tPO_2 was restored by a haemoglobin elevation of 0.7 $g \cdot dl^{-1}$, while in Groups 1 and 2 a haemoglobin rise of 2.7 $g \cdot dl^{-1}$ and 2.1 $g \cdot dl^{-1}$ was required ($P < 0.01$). Figure 3 shows the course of the 10th percentile with higher tPO_2 values in Group 3 than in Groups 1 and 2 during transfusion ($P < 0.01$). The highest elevation of the 10th percentile was seen after the first gram augmentation by HBOC in Group 3.

Discussion

The present dog model mimics a clinical situation of severe blood loss volumetrically managed by hetastarch and consecutive resucitation from haemorrhage using RBCs or bovine stroma free haemoglobin. The present data show that smaller doses of HBOC resulted in higher tissue oxygen tensions after severe isovolaemic haemodilution than are observed with transfusing autologous stored red cells. The lower arterial oxygen content and oxygen transport capacity of HBOC compared with groups receiving RBC transfusion can be explained by a different oxygen saturation curve of HBOC which

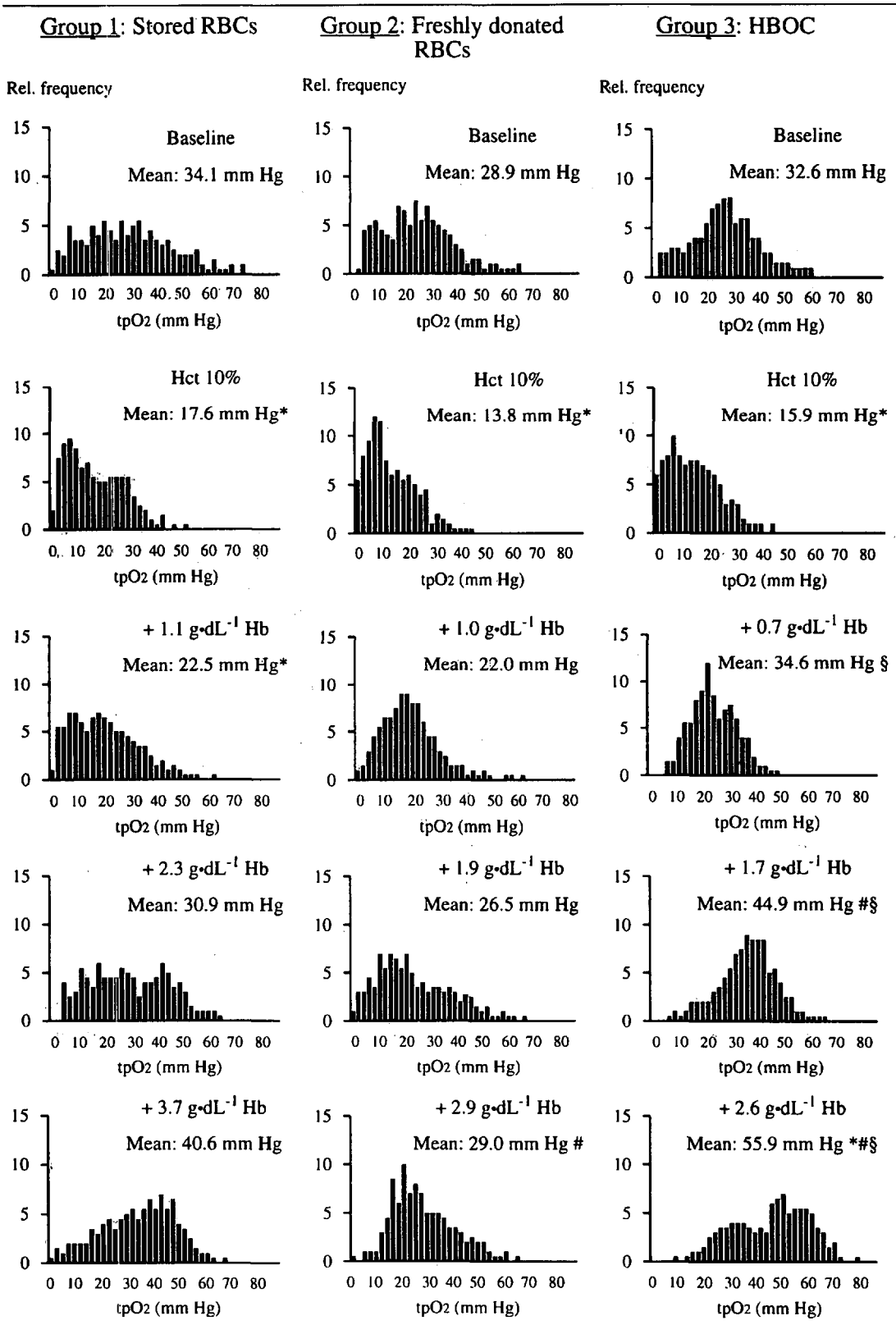

FIGURE 1 Pooled histograms and mean tpO_2 values during isovolaemic haemodilution to haematocrit 10% and stepwise transfusion of stored red cells (Group 1), freshly donated blood (Group 2) and HBOC (Group 3). $*P < 0.05$ compared with baseline; $\#P < 0.05$ compared with Group 1; $\S P < 0.05$ compared with Group 2.

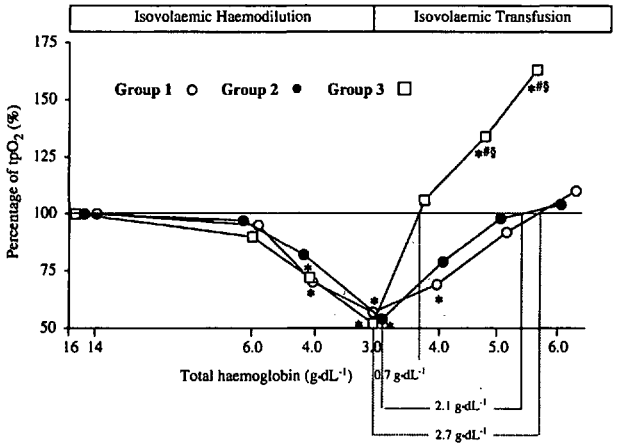

FIGURE 2 Percentage changes of mean tpO_2 values in comparison with baseline during isovolaemic haemodilution to haematocrit target levels of 20%, 15% and 10% and stepwise transfusion of stored red cells (Group 1), freshly donated blood (Group 2) and HBOC (Group 3). Pointed lines give haemoglobin values at which the respective tpO_2 value reaches baseline. $*P < 0.05$ compared with baseline; $\#P < 0.05$ compared with Group 1; $\S P < 0.05$ compared with Group 2.

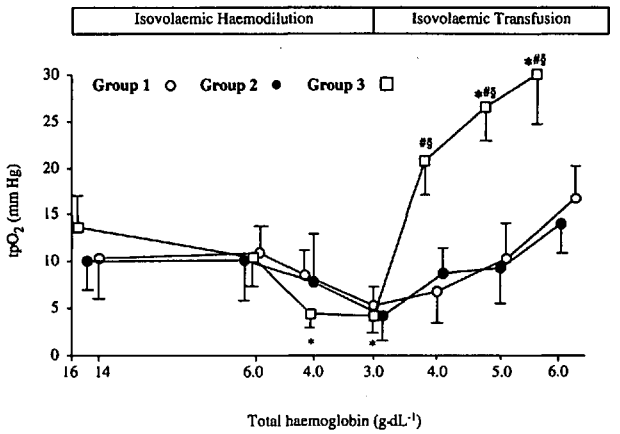

FIGURE 3 Values of 10th percentile during isovolaemic haemodilution to haematocrit target levels of 20%, 15% and 10% and stepwise transfusion of stored red cells (Group 1), freshly donated blood (Group 2) and HBOC (Group 3). Haemoglobin augmentation of 0.7 $g \cdot dl^{-1}$ creates a tpO_2 elevation from 4.2 to 21.6 mmHg in Group 3. Values are expressed as mean ± SEM. $*P < 0.05$ compared with baseline; $\#P < 0.05$ compared with Group 1; $\S P < 0.05$ compared with Group 2.

shows only 85% saturation at a PO_2 of 100 mmHg. Despite this lower oxygen transport capacity, the tPO_2 was higher in HBOC treated animals. In addition, the oxygen extraction ratio and the final oxygen consumption were higher in animals with HBOC transfusion.

This suggests a lower oxygen affinity of bovine haemoglobin than with autologous RBCs. The HBOC has a higher P_{50} of 34 mmHg when compared with the physiological P_{50} of canine haemoglobin of 30 mmHg. The more complete off-loading of oxygen to the tissues may overcompensate the lower oxygen transport capacity of HBOC in comparison to circulating viable canine red cells and provide faster restoration of baseline tPO_2. In addition, physiological concentrations of plasma chloride may enhance the oxygen off-loading from bovine haemoglobin because its oxygen affinity is regulated by chloride ion concentrations rather than by 2,3-DPG concentrations.[18] In contrast, depending on the time of storage, RBCs suffer from 2,3-DPG depletion thus increasing their oxygen affinity.[19] Studies have shown that human RBC loose about 50% of their 2,3-DPG concentrations within seven days of storage at 4°C.[20] In addition, the 24 hr survival rate of human RBCs after transfusion was only 70% following storage of 35 days in CPDA–1 and 78% after 49 days using PAGGS-sorbitol.[21] According to these findings, stored RBCs show a reduced number of vital erythrocytes which have a higher oxygen affinity because of a shift to the left of the oxygen dissociation curve.[22] When extrapolating the changes of the tPO_2 values created by an increase of 1 $g \cdot dl^{-1}$ of total haemoglobin, our data suggest that three-week old stored RBCs have the lowest off loading capacity within the first hour after transfusion in comparison with fresh blood and especially with HBOC. Storage of freshly donated blood at room temperature does not influence concentrations of 2,3-DPG up to seven hours after donation.[23] The lower pH of the transfusion solution in Group 1 may have additionally shifted the oxygen binding curve to the left thus increasing the oxygen affinity of stored RBC haemoglobin. However, when the second elevation of the haemoglobin concentration was reached two hours after the start of RBC transfusion, there was no difference in tPO_2 elevation between Groups 1 and 2. This may be consistent with an intravascular rejuvenation process of RBC after transfusion providing restoration of 2,3-DPG.[22,24] Normalization of the oxygen affinity of stored RBCs can be expected within hours after transfusion.[25] Following the last transfusion, an augmentation of total hemoglobin of 3.7 $g \cdot dl^{-1}$ in Group 1 and only of 2.9 $g \cdot dl^{-1}$ in Group 2 was seen which resulted in higher absolute tPO_2 values in Group 1. However, there was no difference in tissue oxygenation between Groups 1 and 2 if tPO_2 values were related to the respective augmentation of total haemoglobin.

Increases in tPO_2 remained at a higher level in HBOC treated animals compared to other groups with a maximal rise after the first $g \cdot dl^{-1}$ augmentation of haemoglobin. This suggests that the first augmentation is the most important in improving tissue oxygenation in areas of

low oxygen tension. Because the 10th percentile is the respective tPO_2 value at which 10% of all measured tPO_2 values are below this mark, it represents the lower oxygenated and potentially hypoxic tissue areas. An increase of the 10th percentile, as seen during transfusion of HBOC, represents a shift of the lower tPO_2 values to the right to higher values because HBOC may reach muscular tissue areas where RBC flow is not possible. Data from Federspiel[26] suggest that free haemoglobin 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.

This plasmatic oxygen pathway may also overcome potential vasoconstrictive effects which have been demonstrated to be associated with the use of haemoglobin solutions.[27–29] The intensity of vasoconstriction seems to be dependent on the specific features of each haemoglobin formulation and may be related to the degree of purification and tetrameric stabilization.[27,28] In this regard, there were no increases in systemic or pulmonary vascular resistance above baseline in this experiment. All three groups responded to haemodilution and haemoglobin transfusion in a similar manner. The higher oxygen extraction ratio in HBOC treated animals than in the RBC transfused animals was not associated with lower cardiac output thus contradicting potential cardiodepressive side effects of HBOC. This is consistent with data of a canine coronary model.[30]

There is some evidence that animals treated with HBOC had a better rheology because of a reduced blood viscosity in comparison to animals treated with RBCs. Especially in stored RBC units, erythrocytes suffer from a drop in ATP concentrations to 15% of baseline values after a three weeks storage.[31] This ATP depletion creates decreased RBC viality and decreases in deformability of erythrocytes.[32] Although cryopreservation offers an alternative to preserve RBC with high concentrations of ATP and 2,3-DPG even after long-term storage, the clinical application remains still limited because of a more time consuming procedure in comparison to routinely stored RBC units.[33] Rheological effects of HBOC transfusion cannot be excluded in this experiment, because the blood viscosity has not been determined. However, experiments with isovolaemic haemodilution using non-oxygen carrying colloids failed to demonstrate similar increases in tPO_2 as seen after HBOC application at lower haematocrit values.[11,34]

The more complete oxygen release of HBOC may be an advantage in acute tissue hypoxia caused not only by anaemia but also by other low-flow conditions such as arterial stenosis and myocardial or cerebral infarction.[35] There is some evidence that oxygen in the plasma phase may improve tissue oxygenation in cases with reduced haemodynamic conditions or impaired red cell flow, e.g., sickle cell disease.[36]

The experimental finding that HBOC is more potent in tissue oxygenation at small doses than red cells is consistent with a study in human volunteers who received HBOC or autologous RBCs after haemodilution.[37] The volunteers were able to exercise with the same capacity whether they had received RBCs or one third of the amount of haemoglobin in the form of HBOC. Although in human trials side effects related to transient elevations in systemic blood pressure, gastrointestinal disturbances and some enzyme elevations have been reported, none of these effects appear to be major obstacles for clinical use of HBOC.[36–39]

In conclusion our data show, that, in dogs, HBOC provides higher muscular tissue oxygen tensions than stored or freshly donated RBCs after profound haemodilution. The higher oxygen extraction ratio from HBOC enables a relative tissue oxygenation potential that is three to four-fold higher than that of stored autologous red cells.

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