Mild Hypothermia Has Minimal Effects on the Tolerance to Severe Progressive Normovolemic Anemia in Swine

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Background: The benefits of hypothermia during acute severe anemia are not entirely settled. The authors hypothesized that cooling would improve tolerance to anemia.

Methods: Eight normothermic ($38.0 \pm 0.5°C$) and eight hypothermic ($32.0 \pm 0.5°C$) pigs anesthetized with midazolam–fentanyl–vecuronium–isoflurane (0.5% inspired concentration) were subjected to stepwise normovolemic hemodilution (hematocrit, 15%, 10%, 7%, 5%, 3%). Critical hemoglobin concentration (Hgb_{CRIT}) and critical oxygen delivery (DO_{2CRIT}), *i.e.*, the hemoglobin concentration (Hgb) and oxygen delivery (DO_2) at which oxygen consumption (VO_2, independently measured by indirect calorimetry) was no longer sustained, and Hgb at the moment of death, defined prospectively as the point when VO_2 decreased below 40 ml/min, were used to assess the tolerance of the two groups to progressive isovolemic anemia.

Results: At hematocrits of 15% and 10% (Hgb, 47 and 31 g/l), VO_2 was maintained in both groups by an increase ($P < 0.001$) in cardiac output (CO) and extraction ratio (ER; $P < 0.001$) with unchanged mean arterial lactate concentration (L_{art}). At hematocrit of 7% (Hgb, 22 g/l), all normothermic but no hypothermic animals had DO_2-dependent VO_2. No normothermic and three hypothermic animals survived to 5% hematocrit (Hgb, 15 g/l), and none survived to 3%. Hgb_{CRIT} was 23 ± 2 g/l and 19 ± 6 g/l (mean $\pm$ SD) in normothermic and hypothermic animals, respectively ($P = 0.053$). Hgb at death was 19 ± 3 g/l *versus* 14 ± 4 g/l ($P = 0.015$), and DO_{2CRIT} was 8.7 ± 1.7 *versus* 4.6 ± 0.8 ml $\cdot$ kg^{-1} $\cdot$ min^{-1} ($P < 0.001$).

Conclusion: During progressive normovolemic hemodilution in pigs, hypothermia did not significantly change Hgb_{CRIT}, but it decreased the Hgb at death, *i.e.*, short-term survival was prolonged.

ACUTE limitations in the availability of blood products, awareness of potential risks associated with allogeneic transfusion, and religious beliefs have warranted the search for methods that increase tolerance to acute anemia. Induced hypothermia could be such a method because it may improve the balance between oxygen supply and demand when oxygen delivery is acutely restricted. It is a common procedure during cardiopulmonary bypass and has been shown to be beneficial in humans with traumatic brain injury,[1] adult respiratory distress syndrome,[2] and refractory cardiac failure.[3] As to the case of extreme normovolemic anemia, there are several case reports on hypothermia in humans,[4–7] but it is not known whether the reduced oxygen demand will counterbalance the cardiovascular depression induced by hypothermia[8] or compensate for the decreased oxygen availability caused by changes in the hemoglobin affinity for oxygen. Talwar *et al.*[9] have even suggested that cooling might be harmful during acute anemia.

The primary aim of this study was to determine whether mild hypothermia without mechanical or pharmacologic support of the circulation would affect the tolerance to progressive acute normovolemic anemia. We hypothesized that cooling would decrease Hgb at death and Hgb_{CRIT}. A secondary aim was to compare systemic and myocardial hemodynamics and oxygen extraction during anemia in normothermic and cooled animals.

Materials and Methods

The local Ethical Committee on Animal Research approved the study. Sixteen Swedish Landrace pigs of both sexes (weight, 24.6–32.4 kg) were fasted overnight with free access to water. Thirty minutes before starting the preparation, the animals were premedicated intramuscularly with 25 mg midazolam and 0.5 mg atropine. A peripheral catheter was inserted in an auricular vein, and anesthesia was induced with 2–10 mg/kg of intravenous ketamine chloride. The animal was weighed and placed supine on the operating table. A modified CM5 electrocardiogram lead was used to monitor the heart rate and rhythm. Intravenous cloxacillin, 15 mg/kg, was given before a cuffed endotracheal tube was inserted through a tracheotomy performed with local anesthesia with lidocaine, 2%. Mechanical ventilation (tidal volume, 25 ml/kg; respiratory rate, 10 breaths/min) provided by a constant volume ventilator (Servo 900 B; Siemens-Elema, Solna, Sweden) was started. Loading doses of 20 μg/kg fentanyl, 0.3 mg/kg midazolam, and 0.3 mg/kg vecuronium were given and followed by continuous infusions of 20 μg $\cdot$ kg^{-1} $\cdot$ h^{-1} fentanyl, 0.3 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} midazolam, and 0.5 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} vecuronium. Isoflurane at an inspired concentration of 0.5% as measured by a calibrated gas monitor (Servo 910; Siemens-Elema, Solna, Sweden) was added to the air–oxygen mixture (inspiratory fraction of $O_2 = 0.5$, as measured by a calibrated

1189

oximeter). The muscle relaxation was deemed necessary to avoid shivering during hypothermia. Minute ventilation was adjusted to obtain an arterial carbon dioxide tension (Pa_{CO_2}) of 34–38 mmHg as measured at 37°C, *i.e.*, Pa_{CO_2} was regulated according to the α-stat strategy.[10] An end-expiratory pressure of 5 cm water was applied. VO_2 was measured by indirect calorimetry with a Deltatrac metabolic computer (Datex Engström, Helsinki, Finland) after a warm-up period of 30 min and following calibration procedures as recommended by the manufacturer. The whole system was carefully checked for gas leaks. At each stage, the mean of 10 consecutive VO_2 values recorded at 1-min intervals, starting immediately after a prescribed period of stabilization (see Experimental Sequence section), was taken to represent VO_2. Ringer's glucose solution (Na^+, 73.5 mmol; K^+, 2 mmol; Ca^{+2}, 1.15 mmol; Cl^-, 77.8 mmol; glucose 25 g/l; Baxter Medical AB, Kista, Sweden) was given at a rate of $5 \; ml \cdot kg^{-1} \cdot h^{-1}$ during the whole procedure. All animals received 5,000 IE intravenous heparin before insertion of the intravascular monitoring catheters. These were inserted through peripheral incisions after local infiltration with lidocaine, 2%. One catheter was thread into the aorta through the left carotid artery for measuring mean arterial pressure (MAP) and withdrawing blood. An 8-French introducer was placed into the right atrium by direct puncture of the cranial caval vein. A thermodilution catheter (Optometrix; Abbott Laboratories, North Chicago, IL) was inserted through the introducer and advanced with fluoroscopy to the right pulmonary artery for measuring mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), sampling mixed venous blood, temperature registration, and CO measurements. Central venous pressure (CVP) was monitored at the side port of the introducer. A 5-French coronary sinus thermistor catheter (Webster Laboratories, Baldwin Park, CA) was inserted through the right external jugular vein into the coronary sinus, and its tip was advanced 3 cm beyond the confluence with the azygous vein into the great cardiac vein (GCV) for measuring flow (F_{GCV}) and sampling blood. An oxygen saturation of about 25% in the blood sampled from the catheter and the injection of radiopaque contrast medium during fluoroscopy confirmed correct positioning and adequate mixing. The urinary bladder was catheterized through a cystotomy for monitoring urine output.

Hemodynamic Measurements

Intravascular catheters filled with normal saline solution were connected to disposable electronic pressure transducers (Codan Triplus, Stockholm, Sweden) and balanced to atmospheric pressure with the zero reference placed at the mid-thoracic level. All pressures were continuously displayed on monitors and simultaneously printed out on a thermo-array recorder (model WS-682G; Nihon Kohden, Tokyo, Japan). CO was determined by thermodilution

technique (Abbott Laboratories, North Chicago, IL) from the mean value of triplicate injections of 10 ml normal saline solution at room temperature during end-expiration. The values were accepted if within ± 10% of each other. Vascular resistance indices and stroke work indices (left ventricular, right ventricular) were calculated from standard formulae: systemic vascular resistance index = $[MAP - CVP] \cdot CO^{-1} \cdot weight^{-1}$; pulmonary vascular resistance index = $[MPAP - PCWP] \cdot CO^{-1} \cdot weight^{-1}$; left ventricular stroke work index = $SV \cdot [MAP - PCWP] \cdot weight^{-1}$; right ventricular stroke work index = $SV \cdot [MPAP - CVP] \cdot weight^{-1}$.

Coronary perfusion pressure was calculated as the difference between diastolic arterial pressure and PCWP. The rate–pressure product was calculated as the product of heart rate and systolic arterial pressure.

Determination of Oxygen Supply and Uptake

Blood gas tensions, pH, base excess (ABE), bicarbonate concentration, and electrolytes (Na^+, K^+, and Ca^{2+}) were measured by an ABL 505 auto analyzer (Radiometer Medical A/S, Copenhagen, Denmark). Hgb and oxygen saturation in the samples (Sx_{O_2}, "x" = "a," arterial; "v̄," mixed venous or "GCV," great cardiac vein) were determined by an OSM 3 hemoximeter (Radiometer Medical A/S, Copenhagen, Denmark) using internal correction for swine hemoglobin absorption characteristics. P_{50}, *i.e.*, the oxygen tension at half oxygen saturation of Hgb, was also computed by integrating data from the hemoximeter with the ABL 505. The value obtained assuming standard conditions in respect of pH, Pco_2, and fetal and methemoglobin concentration at 37°C is designated as P_{50} (st). The oxygen tension at half oxygen saturation of hemoglobin at the *actual* condition of the mixed venous blood, at the prevailing body temperature, P_{50} (act), is also reported. It was obtained with the help of a software package, Radiometer ABL Calculated Parameters version 1, build 6 (Radiometer Medical A/S, Copenhagen, Denmark). Blood gas tensions, pH, ABE, and Sx_{O_2} are stated as obtained at 37°C by the ABL 505/OSM3 machines, except for oxygen partial pressure in mixed venous blood ($P\bar{v}_{O_2}$) and in GCV blood ($P_{GCV}O_2$), which are reported at 37°C and at the animals' actual temperature. ABE values were corrected for species using the acid–base curve and alignment nomogram for swine blood developed by Weiskopf *et al.*[11] The ABL 505 also reported the hematocrit numerically derived from Hgb. During the pilot studies, we checked these hematocrit values against those obtained with a microcentrifuge. Further, by step diluting an amount of blood with known Hgb and hematocrit with the solution used as blood substitute (see Experimental Sequence section, first paragraph), we could confirm the accuracy of this equipment at very low Hgb and hematocrit values, and stated values are those calculated by the ABL 505/OSM3. Oxygen content (Cx_{O_2}), DO_2, and ER were calculated from standard formulae:

$CxO_2 = Hgb \cdot 1.34 \cdot SxO_2 + 0.03 \cdot PxO_2$; $DO_2 = CO \cdot Cao_2$; $ER = (Cao_2 - C\bar{v}O_2) \cdot Cao_2^{-1}$. F_{GCV}, *i.e.*, flow from the bulk of the left ventricle, was measured by continuous retrograde thermodilution as described by Ganz *et al.*[12] This technique yields reliable results, provided that the flow rate of the indicator is sufficiently high, adequate mixing takes place, and the catheter is not dislodged between measurements. We used a constant infusion pump (Harvard Apparatus, Holliston, MA) delivering 30 ml/min of isotonic saline solution at the room temperature for approximately 20 s. The catheter was secured in place by skin sutures, and its position was rechecked by fluoroscopy before each measurement. The calculation of flow was performed as previously described.[12] Left ventricular oxygen delivery (DO_{2LV}), consumption (VO_{2LV}), and extraction ratio (ER_{LV}) were calculated as follows: $DO_{2LV} = F_{GCV} \cdot Cao_2$; $VO_{2LV} = F_{GCV} \cdot (Cao_2 - C_{GCV}O_2)$; $ER_{LV} = (Cao_2 - C_{GCV}O_2) \cdot Cao_2^{-1}$. Arterial and GCV blood samples for lactate measurements (L_{art} and L_{GCV}, respectively) were simultaneously withdrawn and immediately centrifuged for determination of plasma lactate by a spectrophotometric method (EktaChem 700 XR-C; Eastman Kodak, Rochester, NY). The manufacturer has stated that the coefficient of variation for within-lab precision for this method is between 0.9% and 2.1%. Left ventricular arteriovenous lactate difference (mM) was calculated as $L_{art} - L_{GCV}$, and left ventricular lactate flux ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) was calculated as $F_{GCV} \cdot (L_{art} - L_{GCV})$.

Experimental Sequence

At the end of the surgical preparation, which lasted 60–90 min, the animal was randomly assigned to one of two groups: one normothermic (pulmonary artery blood temperature, $38.5 \pm 0.5°C$) and one hypothermic (pulmonary artery blood temperature, $32.0 \pm 0.5°C$). One hour was allowed to elapse before baseline measurements (stage 1) were taken. Thereafter, in the hypothermic group, central temperature was gradually decreased to $32.0 \pm 0.5°C$ by surface cooling with ice packs and fans. Just before the target temperature ($32°C$), surface cooling was interrupted (ice packs removed and fans turned off), and 15 min was allowed for stabilization, after which measurements were taken (stage 2). In both groups, it was possible to maintain stable body temperature throughout the experiment just by covering the animal with a reflecting blanket and by prewarming substitution solutions. In the normothermic group, measurements were taken at time intervals matched with those of the preceding hypothermic animal.

Dextran–hapten, 0.3 ml/kg, was given intravenously to prevent potential anaphylactic and anaphylactoid reactions to dextran during hemodilution. Blood was then withdrawn at a rate of 50 ml/min from the left carotid artery and simultaneously replaced with equal amounts of a prewarmed (38 or 32°C) solution of 3% Ringer's dextran 60 solution (Braun Medical, Bromma, Sweden) until a hematocrit of approximately 15% was reached. The solution consisted of 30 g dextran 60, 130 mmol Na^+, 4 mmol K^+, 2 mmol Ca^{2+}, 1 mmol Mg^{2+}, approximately 30 mmol Ac^-, and 110 mmol Cl^- per 1,000 ml. Blood gases and hematocrit determinations were repeated after every 500 ml of volume exchange. Thirty minutes was allowed for stabilization, and new measurements were taken (stage 3).

The exchange of blood for fluid was continued *ad mortem*, and measurements were taken at hematocrit 15%, 10%, 7%, and 5% after 30 min of stabilization at each successive level. Some pigs died before reaching a circulatory steady state at 7%, and only a few survived to 5% (see Results section and top of tables 1–3). At hematocrit 3%, those remaining failed to reach steady state. Hgb was measured at the moment of death, defined as the time when VO_2, as measured by the metabolic computer, decreased below 40 ml/min. The limit was determined from pilot studies, which showed that a decrease in VO_2 to this low level always occurred abruptly and signaled simultaneous cardiovascular collapse as judged by the arterial pressure curve and the electrocardiogram. Total cessation of oxygen uptake occurred soon after.

Statistical Analysis

Values are reported as mean $\pm$ SD. Statistical analysis was performed with SigmaStat 2.0 (Jandel Scientific Software, Erkrath, Germany). The absence of differences between groups at baseline (stage 1) was verified with the two-sided Student t test for unpaired data, and the effect of inducing hypothermia (stage 2 *vs.* stage 1) was analyzed with the two-sided t test for paired data. P value less than 0.05 was reported as significant. In case of skewed distribution of data, the corresponding nonparametric test was used. Data describing the effect of progressive hemodilution (stages 2-5) were analyzed with two-way repeated measures analysis of variance (ANOVA) for one factor. Stage 6 (Hgb 15 g/l) was left out because only three animals (all hypothermic) survived that long. When appropriate, *post hoc* analysis with the Student–Newman–Keuls test was used to identify which stages were different from each other.

The data in the tables include only animals surviving through the stabilization period (30 min) at the hematocrit in question. Thus, data obtained from dying animals were excluded from the statistical analysis, with two exceptions: the calculation of critical values, and Hgb at death.

The DO_{2CRIT} was calculated for each animal by determining the intersection of two best-fit regression lines using a least sum of squares technique.[13] DO_2 was used as the independent variable, and VO_2 was used as the

Table 1. Systemic and Pulmonary Hemodynamics

	Stages									
	1 Baseline	2 32°C		3 15% Hct		4 10% Hct		5 7% Hct		6 5% Hct
Animals alive (C, H)	(8, 8)	(8, 8)		(8, 8)		(8, 8)		(6, 8)		(0, 3)
Hemoglobin (g/l)										
C	110 ± 10	110 ± 11		47 ± 2		32 ± 1		22 ± 1		
H	115 ± 14	112 ± 10		47 ± 1		31 ± 1		22 ± 1		15 ± 3
Hematocrit (%)										
C	34.3 ± 3.0	34.0 ± 3.2		14.9 ± 0.6		10.5 ± 0.2		7.4 ± 0.2		
H	35.4 ± 4.2	34.6 ± 3.1		15.0 ± 0.3		10.3 ± 0.3		7.4 ± 0.5		5.3 ± 0.8
Temperature (°C)										
C	38.5 ± 0.5	38.6 ± 0.5		38.5 ± 0.4		38.5 ± 0.4		38.3 ± 0.5		
H	38.3 ± 0.4	32.0 ± 0.1		32.0 ± 0.1		32.2 ± 0.1		32.1 ± 0.1		32.0 ± 0.1
CI (mL · kg^{-1} · min^{-1})										
C	98 ± 19	101 ± 20		148 ± 28	b	174 ± 23	bc	186 ± 27	bcD	
H	85 ± 15	56 ± 16	a†	92 ± 13	b†	103 ± 19	bC†	120 ± 12	bcD†	124 ± 48
HR (beats/min)										
C	106 ± 16	121 ± 14	a	141 ± 24	B	166 ± 25	bC	185 ± 26	bc	
H	91 ± 13	60 ± 11	a†	88 ± 27	b†	109 ± 34	bC†	113 ± 25	bc†	118 ± 11
SVI (ml/kg)										
C	0.9 ± 0.1	0.8 ± 0.1		1.1 ± 0.1	b	1.1 ± 0.1	b	1.1 ± 0.1	b	
H	1.0 ± 0.1	0.9 ± 0.1		1.1 ± 0.1		1.0 ± 0.1		1.1 ± 0.1		1.0 ± 0.2
MAP (mm Hg)										
C	110 ± 14	89 ± 14	A	73 ± 12	b	64 ± 4	bC	51 ± 11	bcD	
H	106 ± 24	102 ± 20		86 ± 14	B	69 ± 9	bC	54 ± 19	bc	41 ± 14
SAP (mm Hg)										
C	126 ± 14	109 ± 15	A	93 ± 13	b	87 ± 7	b	76 ± 15	bc	
H	125 ± 28	117 ± 20		104 ± 17		88 ± 10	bC	81 ± 13	bc	80 ± 16
DAP (mm Hg)										
C	99 ± 14	79 ± 12	A	58 ± 11	b	46 ± 4	bC	31 ± 6	bc	
H	93 ± 23	89 ± 20		71 ± 14	B *	55 ± 8	bC	44 ± 10	bc	42 ± 20
CVP (mm Hg)										
C	6 ± 2	6 ± 2		6 ± 2		6 ± 2		6 ± 2		
H	6 ± 1	5 ± 1		5 ± 2		5 ± 2		5 ± 2		5 ± 3
PCWP (mm Hg)										
C	7.6 ± 2.3	6.6 ± 2.3	A	7.2 ± 2.2		6.9 ± 2.0		7.2 ± 2.2	B	
H	8.2 ± 1.2	6.6 ± 1.5	A	7.0 ± 2.0		6.9 ± 2.0		7.2 ± 2.4		7.0 ± 3.0
MPAP (mm Hg)										
C	22 ± 5	20 ± 4		17 ± 3	b	17 ± 3	B	16 ± 2	B	
H	23 ± 9	19 ± 5		17 ± 3		17 ± 5		17 ± 5		19 ± 8
SVRI (dynes · sec · cm^{-5} · kg^{-1})										
C	110 ± 32	86 ± 26	A	46 ± 8	b	33 ± 5	bC	24 ± 7	bc	
H	120 ± 48	184 ± 75	A†	87 ± 20	b *	63 ± 13	b	45 ± 16	bC	43 ± 20
PVRI (dynes · sec · cm^{-5} · kg^{-1})										
C	15 ± 8	14 ± 6		7 ± 2	B	6 ± 1	B	5 ± 2	B	
H	20 ± 20	25 ± 17		11 ± 6	b	10 ± 6	b	9 ± 5	b	10 ± 6

Hct = hematocrit; CI = cardiac index; HR = heart rate; SVI = stroke volume index; MAP = mean arterial pressure; SAP = systolic arterial pressure; DAP = diastolic arterial pressure; CVP = central venous pressure; PCWP = pulmonary capillary wedge pressure; MPAP = mean pulmonary artery pressure; SVRI = systemic vascular resistance index; PVRI = pulmonary vascular resistance index; C = normothermia; H = hypothermia.

Significance of differences between groups is indicated as $*P < 0.05$ and $†P < 0.01$, respectively. Significance of differences within groups, after correction for multiple comparisons, is indicated by letter symbols where capital letters represent $P < 0.05$ and lower-case letters represent $P < 0.01$ as follows: A, a for stage 1 vs. 2; B, b for stage 2 vs. 3, 2 vs. 4 and 2 vs. 5; C, c for stage 3 vs. 4 and 3 vs. 5; and D, d for stage 4 vs. 5. Temperature and hemoglobin were 'target' parameters; therefore significance of difference is not shown.

dependent variable. Hgb$_{CRIT}$ and critical ER were obtained in a like fashion. For the analysis of Hgb$_{CRIT}$, a larger data set could be used than for DO$_{2CRIT}$ or critical ER because Hgb was measured repeatedly during the transition from one stage to the next to monitor the hemodilution procedure. Survival analysis with Cox F test was performed to assess whether the groups were significantly different in respect of Hgb at death. All other between-group differences were analyzed with the two-sided t test for unpaired data.

Results

There were no significant differences between groups during stage 1. Cooling resulted in the expected hemodynamic and metabolic changes: decreased VO$_2$, DO$_2$, HR,

Table 2. Systemic and Pulmonary Oxygenation

						Stages				
	1 Baseline	2 32°C		3 15% Hct		4 10% Hct		5 7% Hct		6 5% Hct
Animals alive (C, H)	(8, 8)	(8, 8)		(8, 8)		(8, 8)		(6, 8)		(0, 3)
Pa_{O_2} (mm Hg)										
C	262 ± 15	248 ± 15		257 ± 15		258 ± 11		266 ± 17	B	
H	242 ± 26	304 ± 18	a†	300 ± 10	†	300 ± 12	†	293 ± 35	†	305 ± 11
Ca_{O_2} (ml/l)										
C	157 ± 13	155 ± 14		70 ± 3	B	50 ± 1	BC	38 ± 0	BCD	
H	161 ± 18	159 ± 14		72 ± 1	B	51 ± 1	BC	38 ± 2	BCD	30 ± 4
$P\bar{v}_{O_2}$ (mm Hg)										
C	40 ± 5	40 ± 3		30 ± 2	b	26 ± 2	bc	26 ± 2	bc	
H	39 ± 3	41 ± 7		35 ± 3	b†	30 ± 3	bc	28 ± 3	bc	29 ± 1
$P\bar{v}_{O_2}$ (t) (mm Hg)										
C	44 ± 5	45 ± 4		33 ± 3	b	30 ± 2	bc	27 ± 3	bc	
H	43 ± 3	29 ± 4	a†	26 ± 3	b†	22 ± 2	bc†	20 ± 2	bc†	19 ± 2
$S\bar{v}_{O_2}$										
C	0.63 ± 0.07	0.62 ± 0.05		0.44 ± 0.08	b	0.31 ± 0.04	bc	0.24 ± 0.03	bc	
H	0.60 ± 0.04	0.66 ± 0.08	a	0.55 ± 0.07	b†	0.40 ± 0.07	bc†	0.33 ± 0.08	bcD*	0.38 ± 0.06
V_{O_2} (ml · kg^{-1} · min^{-1})										
C	7.2 ± 1.2	7.0 ± 1.2		7.2 ± 1.3		7.1 ± 1.0		5.7 ± 1.4	bcd	
H	5.9 ± 1.5	3.1 ± 1.0	a†	3.2 ± 0.9	†	3.1 ± 0.7	†	3.0 ± 0.5	†	2.5 ± 1.0
D_{O_2} (ml · kg^{-1} · min^{-1})										
C	15.3 ± 2.9	15.5 ± 3.1		10.3 ± 1.9	b	8.8 ± 1.1	bC	6.9 ± 1.0	bcD	
H	13.5 ± 1.1	8.8 ± 2.0	a†	6.5 ± 0.9	b†	5.3 ± 1.0	bC†	4.5 ± 0.4	bc *	3.7 ± 1.7
ER										
C	0.40 ± 0.07	0.40 ± 0.05		0.60 ± 0.07	b	0.72 ± 0.03	bc	0.79 ± 0.03	bc	
H	0.41 ± 0.04	0.37 ± 0.08		0.51 ± 0.06	b *	0.66 ± 0.06	bc *	0.72 ± 0.06	bcd	0.72 ± 0.05
L_{art} (mM)										
C	1.39 ± 0.34	1.11 ± 0.18	A	1.25 ± 0.25		1.66 ± 0.46		4.85 ± 1.86	bcd	
H	1.39 ± 0.31	1.13 ± 0.15	A	1.44 ± 0.26		1.65 ± 0.34		2.30 ± 0.51	bcd†	3.31 ± 1.86
pH										
C	7.49 ± 0.05	7.48 ± 0.04		7.48 ± 0.04		7.48 ± 0.03		7.48 ± 0.08	BCD	
H	7.49 ± 0.04	7.50 ± 0.03		7.51 ± 0.04		7.49 ± 0.03		7.46 ± 0.05	C	7.53 ± 0.01
ABE (mM)										
C	4.6 ± 3.3	4.2 ± 2.5		4.3 ± 2.1		4.2 ± 1.7		−0.4 ± 4.0	bcd	
H	4.2 ± 2.0	3.6 ± 1.6		5.0 ± 2.1		4.2 ± 1.6		2.3 ± 2.3	C	1.0 ± 3.0
P_{50} (act) (mm Hg)										
C	36 ± 2	38 ± 2		37 ± 4		40 ± 2		45 ± 5	bcD	
H	37 ± 2	22 ± 2	a†	23 ± 1	†	25 ± 1	BC†	25 ± 1	bc†	25 ± 3
P_{50} (st) (mm Hg)										
C	35 ± 2	34 ± 1		33 ± 4		35 ± 2		35 ± 2		
H	34 ± 1	33 ± 3		34 ± 1		35 ± 3		35 ± 2		32 ± 1

Hct = hematocrit; Pa_{O_2} = arterial partial pressure of oxygen; C = normothermia; H = hypothermia; Ca_{O_2} = arterial oxygen content; $P\bar{v}_{O_2}$ = mixed venous partial pressure of oxygen; $P\bar{v}_{O_2}$ (t) = mixed venous partial pressure of oxygen at actual temperature; $S\bar{v}_{O_2}$ = mixed venous oxygen saturation; V_{O_2} = systemic oxygen consumption; D_{O_2} = systemic oxygen delivery; ER = systemic extraction ratio; L_{art} = arterial lactate concentration; ABE = arterial base excess; P_{50} (act) = oxygen tension at half oxygen saturation of hemoglobin at the actual condition of the blood; P_{50} (st) = oxygen tension at half oxygen saturation of hemoglobin at standard conditions for pH, Pco_2, fetal and methemoglobin concentrations, at 37°C. Significance of differences between groups are indicated as *$P < 0.05$ and †$P < 0.01$. Significance of differences within groups, after correction for multiple comparisons, is indicated by letter symbols where capital letters represent $P < 0.05$ and lower-case $P < 0.01$ as follows: A, a for stage 1 *vs.* 2; B, b for stage 2 *vs.* 3, 2 *vs.* 4 and 2 *vs.* 5; C, c for stage 3 *vs.* 4 and 3 *vs.* 5; and D, d for stage 4 *vs.* 5.

and rate–pressure product and increased systemic vascular resistance index (tables 1–3).

Hgb at Death and Critical Values

The Hgb at death was 19 ± 3 g/l in the normothermia group and 14 ± 4 g/l in the hypothermia group ($P = 0.015$). Hgb_{CRIT} was 23 ± 2 g/l and 19 ± 6 g/l, respectively ($P = 0.053$). Critical ER was the same in both groups: 0.73 ± 0.03 and 0.73 ± 0.05. In one animal of each group, there were too few data points for the analysis of DO_{2CRIT}, so it was only calculated in 7 + 7

animals and was 8.7 ± 1.7 ml · kg^{-1} · min^{-1} and 4.6 ± 0.8 ml · kg^{-1} · min^{-1} for the normothermia and hypothermia groups, respectively ($P < 0.001$).

Acute Normovolemic Hemodilution to 10% Hematocrit

Systemic and Pulmonary Hemodynamics and Oxygenation. Hemodilution to 15% and then to 10% hematocrit (Hgb, 47 and 31 g/l) was tolerated in both groups. Cardiac index increased but did not fully compensate for the decreased Ca_{O_2} (fig. 1). V_{O_2} was thus

Table 3. Left Ventricular Myocardial Blood Flow and Oxygenation

	Stages					
	1 Baseline	2 32°C	3 15% Hct	4 10% Hct	5 7% Hct	6 5% Hct
Animals alive (C, H)	(8, 8)	(8, 8)	(8, 8)	(8, 8)	(6, 8)	(0, 3)
VO_{2LV} (ml · kg^{-1} · min^{-1})						
C	0.18 ± 0.08	0.14 ± 0.05	0.14 ± 0.05	0.14 ± 0.05	0.12 ± 0.08	
H	0.13 ± 0.04	0.10 ± 0.05	0.08 ± 0.02	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.02
DO_{2LV} (ml · kg^{-1} · min^{-1})						
C	0.23 ± 0.09	0.17 ± 0.06	0.18 ± 0.06	0.18 ± 0.07	0.14 ± 0.09	
H	0.17 ± 0.06	0.12 ± 0.06 a	0.11 ± 0.04	0.18 ± 0.21	0.12 ± 0.07	0.10 ± 0.03
ER_{LV}						
C	0.80 ± 0.07	0.78 ± 0.09	0.79 ± 0.06	0.80 ± 0.04	0.88 ± 0.03	
H	0.79 ± 0.08	0.81 ± 0.05	0.78 ± 0.09	0.78 ± 0.03	0.82 ± 0.06	0.79 ± 0.05
F_{GCV} (ml · kg^{-1} · min^{-1})						
C	1.4 ± 0.6	1.1 ± 0.3	2.6 ± 0.9 b	3.6 ± 1.4 bC	3.8 ± 2.0 bc	
H	1.1 ± 0.3	0.8 ± 0.3	1.4 ± 0.5	2.0 ± 0.7 b*	2.7 ± 1.0 bc	3.8 ± 0.7
LVSWI (g · m^{-1} · kg^{-1})						
C	1.28 ± 0.18	0.96 ± 0.28 a	0.93 ± 0.22	0.84 ± 0.15	0.6 ± 0.25	
H	1.28 ± 0.41	1.19 ± 0.25	1.21 ± 0.43	0.86 ± 0.26 bC	0.74 ± 0.42 BC	0.5 ± 0.27
RVSWI (g · m^{-1} · kg^{-1})						
C	0.20 ± 0.05	0.19 ± 0.06	0.16 ± 0.05	0.15 ± 0.05	0.15 ± 0.05	
H	0.21 ± 0.10	0.19 ± 0.08	0.18 ± 0.07	0.18 ± 0.07	0.20 ± 0.08	0.20 ± 0.10
L_{art}–L_{GCV} (mM)						
C	0.49 ± 0.38	0.33 ± 0.22	0.24 ± 0.15	0.34 ± 0.11	−0.28 ± 0.22 bcd	
H	0.61 ± 0.22	0.33 ± 0.16 a	0.30 ± 0.19	0.25 ± 0.07	−0.02 ± 0.38 bCD	−0.07 ± 0.28
LV lactate flux (μM · kg^{-1} · min^{-1})						
C	0.74 ± 0.5	0.40 ± 0.34	0.60 ± 0.23	1.13 ± 0.25 BC	−0.72 ± 0.23 bcd	
H	0.66 ± 0.33	0.26 ± 0.16 a	0.39 ± 0.33	0.68 ± 0.57	0.21 ± 0.78	0.21 ± 0.20
$S_{GCV}O_2$						
C	0.19 ± 0.06	0.23 ± 0.10	0.23 ± 0.06	0.22 ± 0.05	0.15 ± 0.04	
H	0.22 ± 0.08	0.19 ± 0.05	0.25 ± 0.10	0.26 ± 0.04	0.22 ± 0.08	0.27 ± 0.08
$P_{GCV}O_2$ (mmHg)						
C	20 ± 4	23 ± 5	22 ± 3	23 ± 2	21 ± 3	
H	22 ± 4	21 ± 3	23 ± 5	23 ± 5	22 ± 2	26 ± 2
$P_{GCV}O_2$ (t) (mm Hg)						
C	23 ± 4	25 ± 5	24 ± 3	25 ± 2	23 ± 3	
H	24 ± 5	15 ± 2 a†	17 ± 3 †	17 ± 2 †	16 ± 2 †	19 ± 2
RPP (beats · min · mmHg^{-1} · 10^{-3})						
C	13.5 ± 2.7	13.0 ± 1.4	13.2 ± 3.5	14.4 ± 2.3	14.2 ± 3.8	
H	11.3 ± 2.1	6.9 ± 1.7 a†	8.9 ± 2.2 †	9.6 ± 3.0 †	8.9 ± 1.8 †	9.5 ± 2.2
CPP (mm Hg)						
C	91 ± 14	72 ± 12 A	51 ± 11 b	39 ± 3 bc	24 ± 6 bcD	
H	85 ± 23	82 ± 20	64 ± 15 B*	48 ± 9 bc	37 ± 9 bc	35 ± 18

Hct = hematocrit; VO_{2LV} = left ventricular oxygen consumption; C = normothermia; H = hypothermia; DO_{2LV} = left ventricular oxygen delivery; ER_{LV} = left ventricular extraction ratio; F_{GCV} = great coronary vein flow/kg; LVSWI = left ventricular stroke work index; RVSWI = right ventricular stroke work index; L_{art} - L_{GCV} = left ventricular arterial-venous lactate difference; LV = left ventricular; $S_{GCV}O_2$ = great coronary vein oxygen saturation; $P_{GCV}O_2$ = great coronary vein partial pressure of oxygen; RPP = rate-pressure product; CPP = coronary perfusion pressure. Significance of differences between groups indicated by *$P < 0.05$ and †$P < 0.01$. Significance of differences within groups, after correction for multiple comparisons, is indicated by letter symbols where capital letters represent $P < 0.05$ and lower-case $P < 0.01$ as follows: A, a for stage 1 *vs.* 2; B, b for stage 2 *vs.* 3, 2 *vs.* 4 and 2 *vs.* 5; C, c for stage 3 *vs.* 4 and 3 *vs.* 5; and D, d for stage 4 *vs.* 5.

sustained by increasing ER. Heart rate was significantly less ($P < 0.001$) in the hypothermic group. MAP and systemic vascular resistance index decreased in both groups. $S\bar{v}O_2$ was less in the normothermic group than in the hypothermic group (tables 1 and 2). At 15% hematocrit (Hgb, 47 g/l), the DO_2:VO_2 ratio was 1.46 ± 0.3 and 2.16 ± 0.5, respectively ($P = 0.006$); at 10% hematocrit (Hgb, 31 g/l), it was 1.26 ± 0.2 and 1.79 ± 0.5 ($P = 0.009$).

Myocardial Hemodynamics and Oxygenation

The F_{GCV} increased during hemodilution in both groups. Rate–pressure product was lower in the hypo-

thermic animals throughout the experiment, whereas coronary perfusion pressure was at least as high or higher (table 3).

Progressive Hemodilution below 10% Hematocrit

During the attempt to further lower the hematocrit to 7% (Hgb, 22 g/l), two of the normothermic animals died of myocardial ischemia (increased left ventricular lactate production, ST segment elevation, bradycardia, and finally ventricular fibrillation). One died at an Hgb of 24 g/l, and the other died at an Hgb of 23 g/l before reaching steady state (fig. 2). In the remaining six animals, the compensatory mechanisms became insuffi-
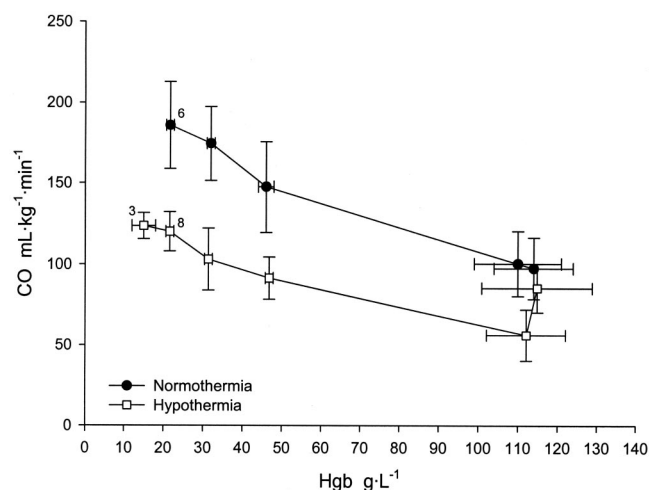
Fig. 1. Hemoglobin concentration (Hgb) *versus* cardiac output (CO). The symbols are mean ± SD. The digit beside the symbol indicates the number of surviving animals at that stage. The first depicted point (baseline) for the hypothermic animals was measured before inducing hypothermia.
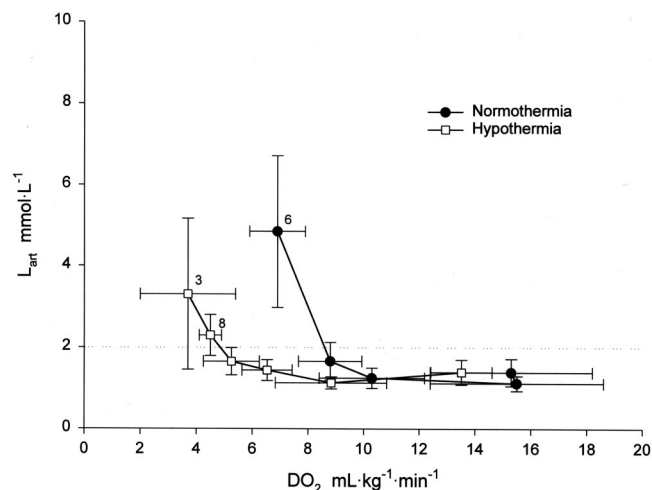

Fig. 3. Oxygen delivery (DO$_2$) *versus* L$_{art}$ (arterial lactate concentration). The symbols are mean ± SD. The digit beside the symbol indicates the number of surviving animals at that stage. The first depicted point (baseline) for the hypothermic animals was measured before inducing hypothermia.

cient to maintain aerobic metabolism as shown by an increase in L$_{art}$ ($P < 0.001$; fig. 3), a decrease in ABE ($P < 0.001$), and a decrease in VO$_2$ ($P < 0.01$; figs. 4 and 5). Thus, VO$_2$ became DO$_2$-dependent in all normothermic animals (figs. 4 and 5). Further, all normothermic animals showed myocardial lactate production (fig. 1). No normothermic animal survived to stage 6 (hematocrit, 5%; Hgb, 16 g/l).

In the hypothermic group, all animals survived hemodilution to 7% hematocrit (Hgb, 22 g/l). The increase in CI and ER was enough to maintain systemic VO$_2$ in all, and although L$_{art}$ increased ($P < 0.001$), it was only about half that in the control group ($P < 0.01$). Three animals developed myocardial lactate production (fig.

2). When hematocrit was reduced to 5% (15 g/l), five of eight hypothermic animals died in heart failure caused by myocardial ischemia (fig. 2), as evidenced by ST segment changes in the electrocardiogram. Two of the three surviving animals showed signs of inadequate DO$_2$ with increased L$_{art}$ and decreased ABE, and one of those had a decreased VO$_2$ (figs. 3–5). In two survivors, VO$_{2LV}$ became supply dependent as indicated by myocardial lactate production. None survived to stage 7 (hematocrit 3%).

The VO$_2$:DO$_2$ ratio at 7% hematocrit (Hgb, 22 g/l) was 1.26 ± 0.3 and 1.54 ± 0.3 for normothermic and hypothermic animals, respectively ($P < 0.09$).
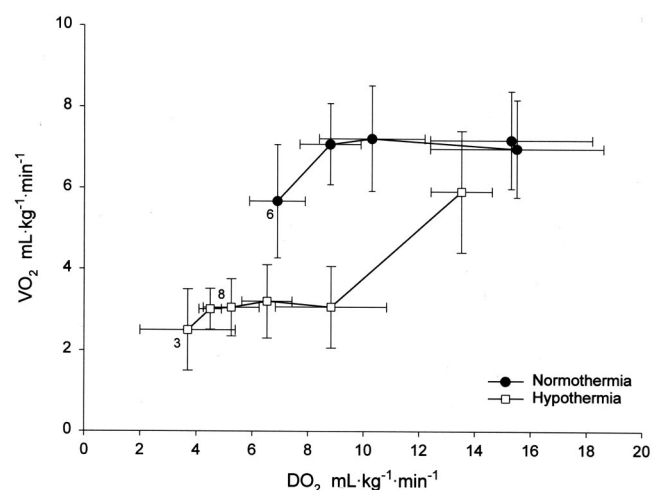

Fig. 2. Hemoglobin concentration (Hgb) *versus* L$_{art}$ − L$_{GCV}$ (left ventricular arterial–venous lactate difference). The symbols are mean ± SD. The digit beside the symbol indicates the number of surviving animals at that stage. The first depicted point (baseline) for the hypothermic animals was measured before inducing hypothermia.
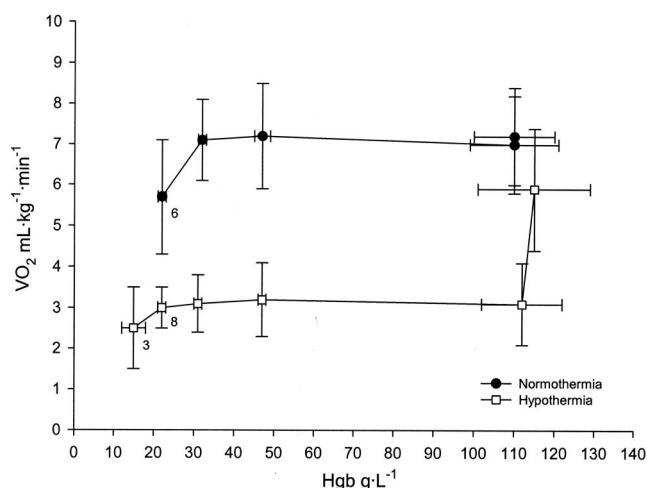

Fig. 4. Oxygen delivery (DO$_2$) *versus* oxygen consumption (VO$_2$). The symbols are mean ± SD. The digit beside the symbol indicates the number of surviving animals at that stage. The first depicted point (baseline) for the hypothermic animals was measured before inducing hypothermia.

Fig. 5. Hemoglobin concentration (Hgb) *versus* oxygen consumption (VO_2). The symbols are mean ± SD. The digit beside the symbol indicates the number of surviving animals at that stage. The first depicted point (baseline) for the hypothermic animals was measured before inducing hypothermia.

Discussion

The main findings in this animal study were that mild hypothermia, induced before progressive acute normovolemic anemia, (1) reduced Hgb at death and thus prolonged short-term survival, but (2) did not significantly decrease Hgb_{CRIT}. A secondary finding was that (3) hypothermia decreased the DO_{2CRIT} and (4) had no overt detrimental effects on oxygen extraction.

We chose a porcine model because of the similarities between swine and man regarding the distribution of the coronary circulation, heart-to-body weight ratios,[14] maximum VO_2, CI, and regional distribution of blood flow.[15] However, there are at least two significant differences between the species that are relevant to this study. One is the lower affinity of swine hemoglobin for oxygen[16] (the P_{50} of porcine hemoglobin is about 8 mmHg greater than in humans) and the smaller leftward shift of the oxygen dissociation curve in response to decreases in temperature.[17] This will increase peripheral oxygen availability during hypothermia in relation to humans. Another difference is that the hemiazygous vein empties directly into the coronary sinus in swine. Therefore, to obtain reliable measurements[18] of cardiac venous flow, the thermodilution coronary catheter was placed far into the coronary sinus, so only blood draining the left ventricular myocardium was measured.

A closed-chest model was used to minimize surgical trauma. In the hypothermic group, the body temperature was reduced to 32°C because this level has been used clinically and is easily achieved.[19] Blood was substituted with Ringer's dextran 60 solution, and PCWP remained stable, suggesting that normovolemia was maintained. An unchanged Hgb (when comparing that taken directly after the completion of each hemodilution step with that obtained some 30 min later, before the next

step) and a constant CVP through all hemodilution stages (table 1) also indicated adequate volume replacement.

The anesthetic method was selected to mimic techniques commonly used in humans while trying to avoid drugs and dosages that could jeopardize or limit the compensatory responses elicited by acute anemia. Fentanyl and midazolam are devoid of direct effects on coronary vascular resistance[20] and allow stable hemodynamics to be maintained. Because the study by Moon *et al.*[21] suggested that fentanyl dosage protocols used by most researchers were inadequate to provide anesthesia in swine and considering that drug concentration may momentarily vary during blood substitution, we decided to complement the anesthetic with isoflurane at an inspired concentration of 0.5%. Muscle relaxation was unavoidable because of the need to inhibit shivering during hypothermia. Further, involuntary muscle activity could dislodge the monitoring catheters or induce arrhythmia. We have used the same anesthetic technique without muscle relaxation in other experimental settings and during our pilot studies in normothermic animals without observing any voluntary movement. There is to date no generally accepted method for measuring anesthetic depth in experimental animals, and because of the complexity of possible interactions, we decided not to make any dose modifications after inducing hypothermia or hemodilution. It is probable that the hypothermic animals were deeper anesthetized than the normothermic ones, but, if so, we cannot tell whether this was detrimental or beneficial. On the one hand, deeper anesthesia could depress the circulation; on the other hand, it may reduce oxygen demand. In this connection, it may be noted that the observed decrease in DO_2 at the start of hypothermia matched the reduction in VO_2 (see stages 1 and 2; table 2), suggesting that the cardiovascular system was not unduly depressed by anesthesia. It may also be noted that the correlation between VO_2 and anesthetic depth is rather weak.[22]

In accordance with the α-stat theory of Reeves,[10] we chose to maintain a constant $Paco_2$ as reported by the blood gas machine at 37°C. However, there is a potential theoretical disadvantage of the α-stat strategy compared with the "pH-stat" strategy, which keeps the pH at the actual temperature of the blood constant during cooling; with the former, the blood will be more alkalotic during hypothermia, resulting in an accentuation of the leftward shift of the oxyhemoglobin dissociation curve.[23] In agreement with our choice of pH management, it was logical to report gas tensions as measured at 37°C. However, there are also good arguments for correcting to actual body temperature, and we, therefore, report corrected and uncorrected values for $P\bar{v}O_2$ and $P_{GCV}O_2$, which are thought to reflect the driving force for oxygen transfer into the tissues. Temperature correction will not affect blood oxygen content, which will be the same

irrespective of the temperature at which it is calculated. The same applies to ER and largely to $S\bar{x}O_2$. $P\bar{v}O_2$ at body temperature was less in the hypothermic animals, but this does not necessarily imply that the ability of the tissues to extract oxygen from the blood had been compromised. If so, ER would not have increased as it did. This is in accordance with Gutierrez *et al.*,[24] who studied hypothermic dogs subjected to progressive hypoxic hypoxia and concluded that hypothermia did not impair oxygen transport to the tissues. P_{50} (st) was unchanged in both groups, suggesting that a compensatory increase in 2,3-diphosphoglycerate did not take place.

Indirect calorimetry was used to measure VO_2, whereas DO_2 was obtained from CO, measured by thermodilution, and CaO_2 and $C\bar{v}O_2$. Thus, we could estimate DO_{2CRIT} without the theoretical drawbacks of mathematical coupling of data.[25] The use of thermodilution for determining CO during hypothermia has been previously validated.[24,26] The higher DO_2:VO_2 ratio in the hypothermic group at 15% and 10% hematocrit is in line with the finding that death was somewhat delayed in the hypothermic animals. However, it is notable that Hgb_{CRIT} was not significantly affected by hypothermia ($P = 0.053$). A clear-cut effect should have been disclosed even with the limited number of subjects we used in each group. Taking into consideration the dissimilarities between the hemoglobin characteristics of pigs in relation to humans, an even more modest protective effect of hypothermia could be expected in humans.

It is obviously not possible to directly apply our findings to the clinical setting. Our results only to a limited extent support those earlier case reports[4-7] that suggest that hypothermia is of value during extreme acute normovolemic anemia. Because hypothermia can be hazardous,[27,28] not the least regarding coagulation,[27] and the gains in terms of prolonged survival were so modest in the present study, it seems unwise to decrease body temperature during uncontrolled diffuse bleeding. There could be some theoretical advantage of hypothermia in respect to increased protection of the central nervous system during periods of decreased DO_2,[29] but this aspect was not covered by our experiment.

In conclusion, apart from the finding that short-term survival was prolonged, we could not demonstrate a clear-cut protective effect of mild hypothermia during progressive acute normovolemic anemia.

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