Assessing anemia and hypovolemia related altered oxygen balance

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Full paper publications related to the thesis

I. **Kocsi S,** Demeter G, Fogas J, Erces D, Kaszaki J, Molnár Z. Central venous oxygen saturation is a good indicator of altered oxygen balance in isovolemic anemia. *Acta Anaesthesiol Scand* 2012; 56(3):291-297.

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II. **Kocsi S**, Demeter G, Erces D, Nagy E, Kaszaki J, Molnar Z. Central venous-to-arterial CO_2 gap is a useful parameter in monitoring hypovolemia-caused altered oxygen balance: animal study. *Crit Care Res Pract* 2013; 2013: 583598.

IF: 2013 still computing

III. **Kocsi S**, Demeter G, Erces D, Kaszaki J, Molnar Z. Central venous-to-arterial CO_2-gap may increase in severe isovolemic anemia. *PLoS One* 2014; 9(8): e105148.

IF: 3.534

IV. **Kocsi Sz**, Molnár Zs. Sebészeti betegek perioperatív folyadékterápiája és hemodinamikai monitorozása. *Aneszteziológia és Intenzív Terápia* 2010; 40.

Presentations related to the thesis

V. **Kocsi S**, Demeter G, Fogas J, Erces D, Kaszaki J, Molnar Z. The diagnostic role of central venous oxygen saturation ($ScvO_2$) and central venous-to-arterial carbon dioxide defference (dCO_2) in hypovolemia *Intensive Care Med* 2011; 37(S1): S1 .

IF: 5.168

VI. **Kocsi** S, Demeter G, Erces D, Kaszaki J, Molnar Z. Central venous to arterial carbon dioxide gap ($dcvCO_2$) as an indicator of oxygen debt in isovolemic anemia *Crit Care* 2011; A564.

IF: 4.93

VII. **Kocsi S**, Demeter G, Erces D, Soos K, Nagy E, Kaszaki J, Molnar Z. The relationship between central venous oxygen stauration ($ScvO_2$) and oxygen debt in normovolaemic anaemia *Intensive Care Med* 2010; 36(S2): S238.

IF: 5.168

VIII. **Kocsi S**, Koczka I, Soos K, Frei N, Molnar Z. Central venous oxygen saturation ($ScvO_2$): the physiologic transfusion trigger? *Intensive Care Med* 2010; 36(S2): S351.

IF:5.168

IX. **Kocsi Sz**, Demeter G, Nagy E, Érces D, Kaszaki J, Molnár Zs. A centrális vénás oxigén szaturáció ($ScvO_2$) és a centrális vénás-artériás vér CO_2 különbségének (dCO_2) diagnosztikai jelentősége hipovolémiában. *Aneszteziológia és Intenzív Terápia* 2011; 41 (S1): 1. (Boros Mihály Ösztöndíj első díját nyerte)

X. **Kocsi Sz.** Ezt kutattuk 2010-ben. Szegedi Intenzíves Találkozó – Szezonzáró Szegeden 2010.

XI. **Kocsi** Sz, Demeter G, Nagy E, Érces D, Kaszaki J, Molnár Zs. A centrális vénás oxigén szaturáció ($ScvO_2$) és az oxigénadósság kapcsolata normovolémiás anémiában. *Aneszteziológia és Intenzív Terápia* 2010; 40 (Suppl 1): 1. (Boros Mihály Ösztöndíj első díját nyerte)

XII. **Kocsi Sz**, Hannauer P, Molnar Zs. Magas centrális vénás-artériás CO_2-rés (dCO_2) kinetikájának értelmezése kritikus állapotú betegekben. *Aneszteziológia és Intenzív Terápia* 2010; 40(S1):EA28.

XIII. **Kocsi Sz**, Koczka I, Molnar Zs. Centrális vénás oxigén szaturáció ($ScvO_2$) – az élettani transzfúziós küszöb? *Aneszteziológia és Intenzív Terápia* 2010; 40(S1):EA29.

XIV. **Kocsi Sz**, Brzózka V, Molnar Zs. Megbízhatóak-e a vérgázgéppel meghatározott hemoglobin (Hb) és hematokrit (Htk) értékek? *Aneszteziológia és Intenzív Terápia* 2010; 40(S1):P46.

XV. Demeter G, **Kocsi** Sz, Soós K, Érces D, Molnar Zs, Kaszaki J. Hydroxyaethyl keményítő (HES) és gelatin (GEL) oldattal végzett reszuszcitáció hemodinamikai hatásai normovolémiás anaemiában. *Aneszteziológia és Intenzív Terápia* 2010; 40(S1):EA27.

XVI. **Kocsi Sz**, Demeter G, Érces D, Kaszaki J, Molnár Zs. A centrális vénás-artériás CO_2 különbség (dCO_2) és az oxigénadósság kapcsolata normovolémiás anémiában. Magyar Sebészeti Társaság Kísérletes Sebészeti Szekció 2011. évi XXIII. Kísérletes Sebész Találkozó, Budapest.

XVII. **Kocsi Sz**, Demeter G, Érces D, Kaszaki J, Molnár Zs. A centrális vénás oxigén szaturáció ($ScvO_2$) és a centrális vénás-artériás vér CO_2 különbségének (dCO_2) diagnosztikai jelentősége hipovolémiában. Magyar Sebészeti Társaság Kísérletes Sebészeti Szekció 2011. évi XXIII. Kísérletes Sebész Találkozó, Budapest.

XVIII. **Kocsi Sz**, Demeter G, Soós K, Nagy E, Érces D, Kaszaki J, Molnár Zs. A centrális vénás oxigén szaturáció ($ScvO_2$) és az oxigénadósság kapcsolata normovolémiás anémiában. A Magyar Élettani Társaság (MÉT) LXXIV. Vándorgyűlése és a Magyar Kísérletes és Klinikai Farmakológiai Társaság (MFT) második közös tudományos konferenciája Szeged, 2010.

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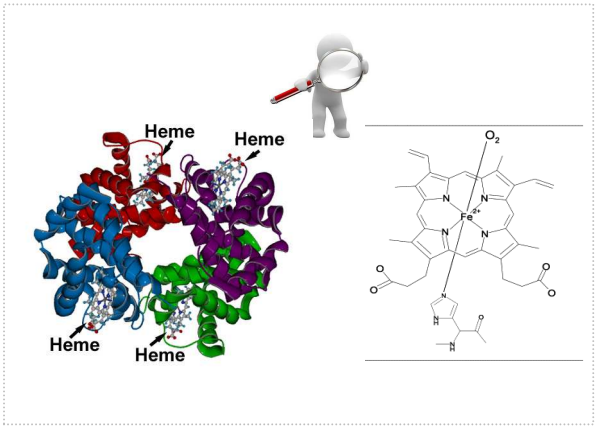
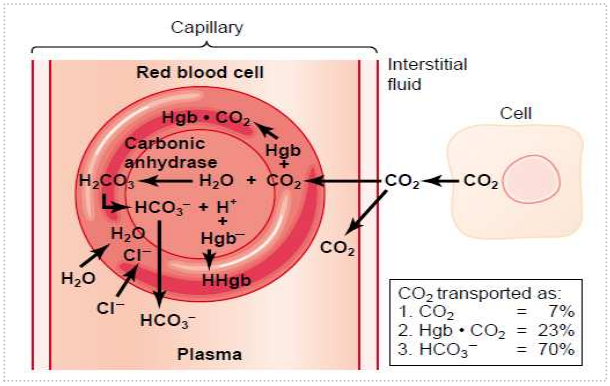
Introduction

Physiological background

Oxygen is one of the major essential components required for sustaining life. Under physiologic conditions pulmonary ventilation provides oxygen for the body through the respiratory tree to the alveoli. During alveolar ventilation oxygen diffuses through the respiratory membrane from the alveoli to the pulmonary blood. Oxygen is transported to the tissues in chemical combination with hemoglobin in the red blood cells and in the dissolved state. Were it not for the hemoglobin molecule only a small fraction of oxygen could be transported.



Hemoglobin consists of four chains and each has an atom of iron with which one molecule of oxygen loosely and reversibly binds. In 100 millilitres of blood there is 15 grams of hemoglobin. Each gram of hemoglobin may bind with a maximum of 1.34 (1.39 when chemically pure) millilitres of oxygen, thus in 100 millilitres of blood there is ~ 20 millilitres of oxygen bound when the hemoglobin saturation is 100 per cent. Under normal circumstances ~ 4 ml/kg/min of oxygen is consumed by the tissues to ensure aerobic energy release in the cells.



As oxygen is used up by the cells, carbon dioxide is produced. Transport of carbon dioxide takes place in exactly the opposite direction as that of oxygen. In each 100 millilitres of blood ~ 4 ml of carbon dioxide is transported from the cells towards the pulmonary capillaries in three forms; in the dissolved state, as bicarbonate ion and bound to hemoglobin. Carbon dioxide is transported from the cells to the alveoli where the excess amount of it is exhaled.[1]

Critically Ill

Tissue oxygenation is determined by the balance between the rate of oxygen transport to the tissues (oxygen delivery) and the rate at which the oxygen is used by the tissues (oxygen consumption). Standard formulae to determine oxygen delivery and oxygen consumption are the following:

$$DO_2 = SV * HR * \left(Hb * 1.34 * SaO_2 + \left(0.003 * PaO_2\right)\right) = CO * \left(CaO_2\right)$$
$$VO_2 = CO * \left(CaO_2 - \left(Hb * 1.34 * SvO_2 + \left(0.003 * PvO_2\right)\right)\right) = CO * \left(CaO_2 - CvO_2\right)$$

DO_2 - oxygen delivery, SV – stroke volume, HR – heart rate, Hb – hemoglobin, SaO_2 – arterial hemoglobin oxygen saturation, PaO_2 – arterial oxygen partial pressure, CO – cardiac output, CaO_2 – arterial oxygen content, VO_2 – oxygen consumption, SvO_2 – mixed venous hemoglobin oxygen saturation, PvO_2 – venous oxygen partial pressure, CvO_2 – venous oxygen content

From the above formulae one can calculate the oxygen delivery at rest:

$$CO = 70 \text{ mL} * 72/\text{min} = 5 \text{ L/min}$$
$$CaO_2 = \left(150 \text{ g/L} * 1.34 \text{ mL} * 1\right) + \left(0.003 * 100 \text{ mm Hg}\right) = 201.30 \text{ mL/L}$$
$$DO_2 \sim 1000 \text{ mL/min}$$

Oxygen consumption at rest:

$$CvO_2 = \left(150 \text{ g/L} * 1.34 \text{ mL} * 0.75\right) + \left(0.003 * 40 \text{ mm Hg}\right) = 150.87 \text{ mL/L}$$
$$VO_2 = 5 \text{ L/min} * \left(201.30 \text{ mL/L} - 150.87 \text{ mL/L}\right) \sim 250 \text{ mL/min}$$

$$\text{Oxygen extraction}\left(VO_2/DO_2 * 100\right) = 250 \text{ mL/min} / 1000 \text{ mL/min} * 100 = 25\%$$

In the critically ill there is often an imbalance between oxygen delivery and consumption.[2,3,4] Oxygen delivery may be inadequate based on two grounds; **arterial oxygen content** and/or **cardiac output** may be reduced.

One of the most common causes of decreased **arterial oxygen content** in the critically ill is anemia.[5] The prevalence of anemia among critically ill patients could be as high as 95 % by day three. A number of guidelines are of help in transfusion practice, however the criteria for the optimal management of anemia are not clearly defined. [6,7] In most guidelines the transfusion trigger, i.e. the indication and timing of blood transfusion, is a certain level of hemoglobin, usually 70-100 g/L.[8,9,10] It was recently suggested that hemoglobin level should not be the only factor on which the indication of blood transfusion is based.[8,9] There is increasing evidence that transfusion is a double-edged sword: untreated anemia can be associated with a worse outcome and increased mortality, while transfusion may cause various infectious and non-infectious adverse effects.[11-13] There is a clear need for additional quantitative parameters that would give information on anemia related altered oxygen extraction and hence the need for blood administration.[9,14]

A common cause for decreased **cardiac output** in the intensive care unit is hypovolemia. Diagnosing hypovolemia is an everyday challenge in critical care. Clinicians utilize a large array of tools from simple clinical signs to invasive hemodynamic measurements, but a universally accepted gold standard parameter remains elusive.[15] Although, diagnosis may prove difficult, early recognition of hypovolemia is of utmost importance. By the time macro-hemodynamic changes manifest, the microcirculation may already be damaged.[16-18] Furthermore, fluid therapy is ambiguous, on the one hand fluid resuscitation can save lives, but on the other hand a cumulative positive fluid balance is an independent risk factor for mortality.[19,20] Deciding on the level of monitoring (non-invasive, ‘less’ invasive, invasive), and which parameter to monitor in order to keep the critically ill patient normovolemic remains uncertain.

Central venous oxygen saturation ($ScvO_2$), the hemoglobin oxygen saturation measured in a central vein, is a potentially useful physiological parameter. It is easily obtained as a blood gas sample from the vena cava superior via the central venous catheter already *in situ* in most critically ill patients. The main factors influencing $ScvO_2$ are hemoglobin, arterial oxygen saturation of hemoglobin, cardiac output and oxygen consumption. Its normal value varies between 73-82 %. Although, the value reflects the oxygen extraction of the brain and the upper extremities, it is considered a reasonable surrogate marker of mixed-venous oxygen saturation (SvO_2) – the parameter representing the whole body oxygen extraction - in the clinical setting. Thence, it is often used as a marker of the balance between oxygen delivery and consumption. [14,21-24]

Changes in $ScvO_2$ reflect systemic oxygen metabolism, but may fail to detect regional hypoxia and may also be false-negative when >70%. Under these conditions the central venous-to-arterial carbon dioxide difference (CO_2-gap) has been proposed as an alternative. It is as easily obtained as $ScvO_2$, only a simultaneous arterial and central venous blood gas sampling is needed. The physiological threshold of CO_2-gap is <5 mmHg, but this may be higher in low flow states. A concept based on the CO_2 stagnation phenomenon considers the cause of an increased CO_2-gap to be low blood flow, and an inverse correlation was found between CO_2-gap and CO in non-septic and septic circulatory failures. Moreover, literature findings indicate that the amount of CO_2 produced is negligible when anaerobic respiration is present and therefore CO_2-gap cannot serve as a marker of tissue hypoxia.[25-28]

Our aims were the following:

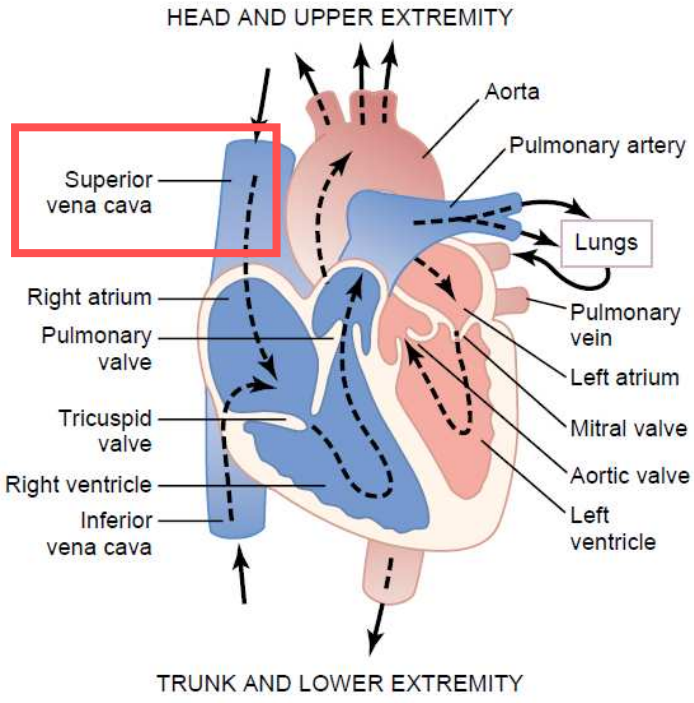
1. To evaluate the change in $ScvO_2$ before and after transfusion in a retrospective study and to test whether the combination of Hb and $ScvO_2$ may reflect anemia caused altered oxygen balance better than Hb alone.

2. To monitor the changes of $ScvO_2$ in isovolemic anemia using a large animal model and test whether an altered VO_2/DO_2 balance, which is due solely to a decreased Hb level, can be detected by $ScvO_2$.

3. To investigate how CO_2-gap changes during experimental isovolemic anemia and how it contributes to $ScvO_2$.

4. To determine the effect of hypovolemia on $ScvO_2$ and CO_2-gap and the association between $ScvO_2$, CO_2-gap and indicators of microcirculatory blood flow.

$ScvO_2$


HEAD AND UPPER EXTREMITY
Aorta
Pulmonary artery
Superior vena cava
Lungs
Right atrium
Pulmonary valve
Pulmonary vein
Left atrium
Tricuspid valve
Mitral valve
Aortic valve
Right ventricle
Left ventricle
Inferior vena cava
TRUNK AND LOWER EXTREMITY

1. Changes in $ScvO_2$ before and after transfusion - a retrospective study

1.1. Materials and Methods

This retrospective study was conducted in four intensive care units of the University of Szeged over a six month period.

All the patients who received blood transfusions were searched via the medical network system. Only those patients were included in the study who had arterial and central venous blood gas analysis before and after transfusion. The parameters of interest were: hemoglobin, $ScvO_2$, arterial oxygen saturation of haemoglobin (SaO_2), mean arterial pressure (MAP), heart rate (HR), central venous pressure (CVP), lactate and the simplified oxygen extraction ratio (ERO_2).

The standard formula for ERO_2 is: $(SaO_2 - ScvO_2)/ SaO_2 \times 100$.

Data are reported as medians (interquartile ranges), unless indicated otherwise. To test for normal distribution the Kolmogorov-Smirnov test was used. For between-groups analysis the Mann-Whitney test and for before-after comparison the Wilcoxon test were used. For statistical analysis SPSS version 18.0 for Windows (SPSS, Chicago, IL) was used and $p<.05$ was considered statistically significant.

1.2. Results

Over the study period 128 transfusion events were recorded of which $ScvO_2$ was measured in 50 events in 41 patients. The data of these 41 patients were included in further analysis. Demographics are summarised in Table 1.

Table 1 Demographic data	
Patient number	41
Age (ys)	63(49-73)
Gender (m/f)	27/14
Survival (y/n)	26/15
ICU stay (days)	7(3-13)
Major diagnosis:	
postoperative	22
sepsis	7
observation	6
pneumonia	6

After transfusion Hb levels increased significantly, which was accompanied by significant changes in all the other parameters but SaO_2 (Table 2).

Table 2 Before and after transfusion		
	Before	After
Hb (g/L)	7.7(7.1-8.2)	9.1(7.9-9.7)*
$ScvO_2$ (%)	67(60-76)	75(69-80)*
SaO_2 (%)	99(95-99)	99(98-100)
MAP (mmHg)	72(65-80)	80(71-90)*
HR (beat/min)	100(90-	90(80-109)*
CVP mmHg)	8(4-10)	9(7-11)*
Lactate (mmol/L)	1.1(0.9-2.2)	0.9(0.6-2.1)*
ERO_2 (%)	31(22-39)	22(19-28)*

*p <.05 Wilcoxon test.

The median $ScvO_2$ was 71%, therefore we divided the patients into two groups: low group (LG, $ScvO_2$<70%), n=27; and high group (HG, $ScvO_2$>70%), n=23. Before transfusion ERO_2 and lactate levels were significantly higher in the LG as compared to the HG. After transfusion Hb increased significantly in both groups. There were no significant changes in $ScvO_2$ and ERO_2 in the HG, while on the contrary in the LG $ScvO_2$ and ERO_2 improved significantly. Regarding other cardiorespiratory variables, there were variably significant changes in MAP, HR, CVP, SaO_2, lactate but values remained in the physiologic range in both groups.

Table 3 LG ($ScvO_2$≤70%) vs. HG ($ScvO_2$>70%)

	LG		HG	
	Before	After	Before	After
Hb (g/L)	7.8(7.1-	9.2(8.0-9.9)*	7.7(7.1-8.1)	8.9(7.9-9.6)*
$ScvO_2$ (%)	62(57-64)	70(66-72)*	77(75-80)#	79(76-82)
SaO_2 (%)	99(95-99)	100(99-100)	98(95-99)	98(97-99)
MAP (mmHg)	70(64-88)	80(70-90)	75(67-80)	78(71-91)*
HR (beat/min)	96(90-125)	87(74-113)*	100(88-110)	90(80-102)*
CVP (mmHg)	8(6-10)	10(9-11)	8(4-9)	9(5-12)*
Lactate (mmol/L)	1.7(0.9-	1.2(0.9-3.3)	1.0(0.8-1.2)#	0.7(0.5-1.1)*
ERO_2 (%)	38(35-48)	27(20-29)*	21(16-24)#	20(15-22)

*p <.05 Wilcoxon test; #p <.05 Mann-Whitney test.

1.3. Discussion

The main finding of this retrospective study is that patients with low Hb but normal $ScvO_2$ levels may have received unnecessary blood transfusion, as transfusion was not accompanied by any significant changes in $ScvO_2$ and ERO_2.

The value of $ScvO_2$ depends on hemoglobin, arterial oxygen saturation of hemoglobin, cardiac output and oxygen consumption as previously described. We found that arterial oxygen saturation of hemoglobin remained in the normal range. As far as the change of macro-hemodynamic parameters (mean arterial pressure, heart rate) indicate the change of cardiac output, it did not clinically significantly change either.

As lactate levels differed significantly between the two groups before transfusion, oxygen consumption may have been increased only in LG and not in HG. Moreover, for transfusion $ScvO_2$ increased significantly only in LG, not in HG. Thus, blood transfusions in HG may be arguable.

For the retrospective study design and the limitation of parameters measured, we decided to perform an animal experiment in order to test the current findings under controlled circumstances.

2. Changes of $ScvO_2$ in isovolemic anemia – an animal experiment

2.1. Materials and Methods

The study protocol was approved by the local ethics committee at the University of Szeged, and the study was carried out in the research laboratory of the Institute of Surgical Research.

Animals and Instrumentation.

Vietnamese mini-pigs (n=13) weighing 24±3 kg underwent a 24-hr fast preoperatively but with water *ad libitum*. Anesthesia was induced with an intramuscular injection of a mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) and maintained with a continuous infusion of propofol (6 mg/kg/hr iv.). A tracheal tube was inserted and the animals' lungs were ventilated mechanically. The tidal volume was set at 13±2 mL/kg, and the respiratory rate was adjusted to maintain the end-tidal carbon dioxide and the partial pressure of arterial carbon dioxide in the range of 35-45 mm Hg and the arterial pH between 7.35 and 7.45. The adequacy of the depth of anesthesia was assessed by monitoring the jaw tone. After the initiation of anesthesia, the right carotid artery and jugular vein and the right femoral artery and vein were dissected and catheterized. The animals underwent suprapubic urinary catheter placement and laparotomy for splenectomy. Splenectomy in swine haemorrhage models are performed because of the distensibility of the spleen and the resultant variation in the amounts of sequestered blood.[29] The core temperature was maintained at 37±1 ^{o}C through use of an external warming device.

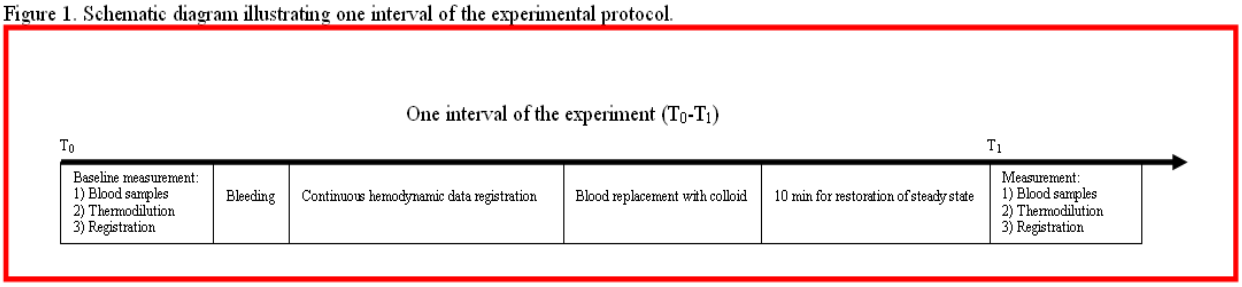
For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter (PiCCO, PULSION Medical Systems AG, Munich, Germany) was placed in the femoral artery and a pulmonary artery catheter (PV2057 VoLEF Catheter, PULSION Medical Systems AG, Munich, Germany) by pressure tracings via the femoral vein. The latter was also used to draw mixed venous blood gas samples. The femoral artery served as the site of arterial blood gas samples and the central venous line was used for central venous blood gas sampling

and for the injection of cold saline boluses for thermodilution measurements. During the experiment blood was drained from the catheter in the right carotid artery, which was also used to replace the blood loss with the same amount of colloid, in order to avoid a sudden increase in right ventricular preload.

Experimental Protocol.

The most important steps in the experiment are outlined in Figure 1. At baseline (T_0) hemodynamic and blood gas parameters were recorded and heparin sulphate (200 IU/kg) was administered through the central venous line. Isovolemic anemia was achieved in five intervals (T_1-T_5). During each interval 10 % of the estimated total blood volume was withdrawn over a 5-10-minutes period. Hemodynamic parameters were recorded and the amount of blood drained off was immediately replaced by an equal volume of colloid (hydroxyethyl starch 130 kDa/0.4, 6%, Voluven, Fresenius, Germany). To achieve a steady state, the animals were allowed to rest for 10 minutes between intervals. At the end of each cycle, hemodynamic and blood gas parameters were measured. At the end of the experiment the animals were humanely euthanized.

Figure 1. Schematic diagram illustrating one interval of the experimental protocol.



Hemodynamic Measurements.

Cardiac output, global end-diastolic volume, extravascular lung water, stroke volume, stroke volume variation, index of left ventricular contractility, heart rate, and mean arterial pressure were measured by transpulmonary thermodilution and pulse contour analysis at

baseline and at the end of each interval. Detailed description of transpulmonary thermodilution and pulse contour analysis is provided elsewhere.[29,30] All hemodynamic parameters were indexed for body surface area. The averages of three random measurements following 10 mL bolus injections of ice-cold 0.9 % saline were recorded. Continuous variables of invasive blood pressure measurements and pulse contour analysis, such as cardiac output, mean arterial pressure, heart rate, stroke volume, stroke volume variation and index of left ventricular contractility were measured and recorded at the end of each bleeding episode and at the same times as the other hemodynamic variables. The central venous pressure was monitored continuously and registered with a computerized data-acquisition system (SPELL Haemosys; Experimetria Ltd., Budapest, Hungary).

Arterial, central venous and mixed venous blood gas samples (Cobas b 221, Roche Ltd, Basel, Switzerland) were drawn and analysed by cooximetry simultaneously at baseline and at the end of each cycle (Figure 1).

From these parameters oxygen delivery, oxygen consumption, oxygen extraction and the simplified oxygen extraction were calculated according to previously described standard formulae.

Data Analysis and Statistics.

Data are reported as median (interquartile ranges), unless indicated otherwise. To test for normal distribution, the Kolmogorov-Smirnov test was used. The changes in all parameters throughout the experiment were tested by repeated measures analysis of variance (ANOVA); and the number of degrees of freedom was adjusted to Greenhouse-Geisser epsilon when needed. For pairwise comparisons, Pearson's correlation was used. To evaluate the performance of $ScvO_2$ in the detection of altered oxygen extraction with a threshold of 30% of VO_2/DO_2, receiver operating characteristics (ROC) curve analysis was performed, and sensitivity, specificity, and positive predictive (PPV) and negative predictive values (NPV) were also determined (Appendix B.). To model the linear relationship between VO_2/DO_2 and the possible indicator of altered oxygen extraction, linear regression model was used. Post-hoc calculation showed a power of 86% with an effect of 25% increase in VO_2/DO_2, for a sample size of 13 and α=0.05. For statistical analysis SPSS version 18.0 for Windows (SPSS, Chicago, IL) was used and $p < .05$ was considered statistically significant.

2.2. Results

Hemodynamic Effects of Isovolemic Anemia.

All 13 animals survived the study. The blood loss was on average 150 ± 33 mL in each phase. Hemodynamic data are presented in Table 4.

The bleeding caused a gradual decrease in hemoglobin level after each phase and by the end of the experiment it had fallen by 61% of the baseline value. The preload as indicated by global end-diastolic volume and central venous pressure values did not change significantly. Heart rate, index of left ventricular contractility and cardiac index were increased significantly after the first bleeding and remained so for the rest of the experiment. Mean arterial pressure decreased significantly from T_2, but the median remained >70 mmHg throughout. Other macro-hemodynamic variables did not change significantly during the experiment.

Table 4 Hemodynamic Effects of Isovolemic Anemia

	T_0	T_1	T_2	T_3	T_4	T_5
Hb (g/L)	125(113-134)	102(90-109)*#	79(73-93)*#	68(60-76)*#	59(53-67)*#	49(43-55)*#
HR (beats/min)	125(91-135)	119(100-138)*	123(102-146)*	129(110-159) *	139(118-179) *	147(131-177)*
MAP (mm Hg)	91(79-105)	89(79-101)	83(75-98)*	82(68-90)*	72(59-85)*	72(63-86)*
CVP (mm Hg)	6(5-8)	8(5-9)	7(4-9)	7(5-9)	7(5-9)	7(3-10)
CI (L/min/m^2)	2.6(2.3-2.8)	3.3(2.7-3.6)*#	3.6(2.9-3.8)*#	3.6(3.3-4.1)*	3.5(3.2-4.0)*	3.9(3.6-4.1)*
GEDI (mL/m^2)	270(243-284)	271(245-320)	276(248-298)	274(236-305)	268(227-302)	261(232-298)
ITBI (mL/m^2)	335(307-352)	335(305-400)	343(303-373)	342(295-383)	334(282-375)	333(285-375)
ELWI (mL/kg)	9(9-10)	10(10-10)	9(9-10)	10(9-10)	10(9-10)	10(9-11)
SVI (mL/m^2)	21(18-29)	26(23-31)	27(24-31)	28(25-31)	25(21-33)	28(22-31)
SVV (%)	17(14-21)	15(12-21)	19(9-21)	15(11-20)	19(11-25)	14(11-27)
dPmx (mm Hg/s)	540(485-790)	700(540-985)*	800(570-1075)*	810(540-1480)*	880(560-1360)*	975(562-1275)*

Hb- Hemoglobin, HR- Heart rate, MAP- Mean arterial pressure, CVP- Central venous pressure, CI- Cardiac index, GEDI- Global end-diastolic volume index, ITBI- Intrathoracic blood volume index, ELWI- Extravascular lung water index, SVI- Stroke volume index, SVV- Stroke volume variation, dPmx- Index of left ventricular contractility. T_0- Baseline measurement, T_1-T_5- Five intervals of bleeding.
*p <.05 compared with T_0; #p <.05 compared with previous; GLM repeated measures ANOVA

Effects on Oxygen Balance.

Variables related to oxygen balance are listed in Table 5.

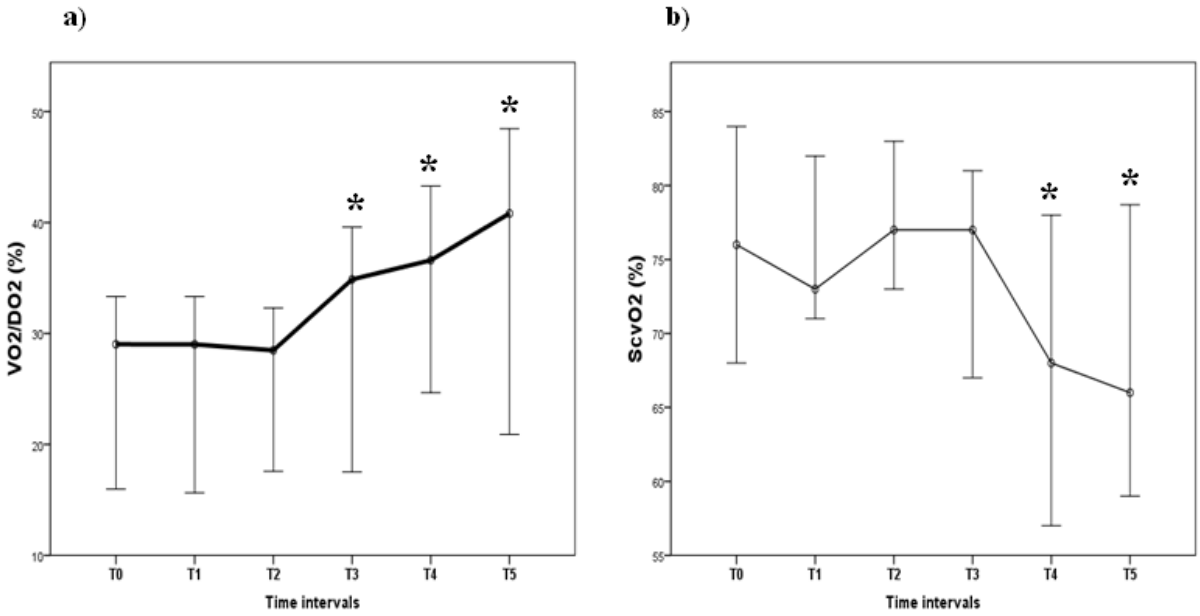
Table 5 Effects of Isovolemic Anemia on Oxygen Balance

	T_0	T_1	T_2	T_3	T_4	T_5
SaO_2 (%)	95(92-97)	96(94-97)	96(95-97)	96(95-97)	97(97-97)	97(97-97)
DO_2 (mL/min/m^2)	431(362-474)	438(323-524)	378(302-412)*#	344(252-376)*	284(236-333)*	247(216-292)*#
VO_2 (mL/min/m^2)	119(82-139)	130(77-151)	93(66-136)	113(67-141)	98(72-120)	105(70-120)
VO_2/DO_2 (%)	29(18-33)	29(17-33)	29(18-32)	35(21-40)*	37(26-43)*	41(27-47)*
ERO_2 (%)	19(13-26)	19(14-24)	20(14-22)	21(16-28)	30(22-37)*	32(21-39)*
SvO_2 (%)	68(64-77)	67(64-77)	68(63-79)	64(58-76)	62(55-72)*	58(52-72)*
$ScvO_2$ (%)	76(69-83)	73(72(82)	77(75-83)	77(68-81)	68(61-76)*	66(60-76)*
Lactate (mmol/L)	4.5(3.2-5.3)	4.2(3.0-5.1)	5.0(3.2-6.0)	4.1(2.9-6.0)	4.2(2.9-6.5)	4.0(3.0-6.4)
pH	7.44(7.40-7.50)	7.43(7.40-7.50)	7.43(7.41-7.50)	7.43(7.39-7.49)	7.44(7.42-7.49)	7.44(7.40-7.47)
PaO_2 (mm Hg)	76(66-80)	75(72-80)	76(73-80)	77(72-82)	79(75-85) *	81(77-90) *
$PaCO_2$ (mm Hg)	39(35-44)	38(35-43)	37(34-45)	39(34-46)	37(34-42)	38(35-41)
$aHCO_3$ (mmol/L)	25(24-27)	24(24-26)	25(23-27)	25(23-27)	25(22-27)	25(21-25)
aBE (mmol/L)	0.90(-0.05-2.50)	0.40(-0.85-2.25)	0.60(-0.9-2.45)	0.80(-0.45-3.15)	0.90(-1.45-2.35)	0.70(0.43-1.08)

SaO_2- Arterial oxygen saturation, DO_2- Oxygen delivery, VO_2- Oxygen consumption, VO_2/DO_2- Oxygen extraction ratio, ERO_2- Simplified oxygen extraction ratio, SvO_2- Mixed venous oxygen saturation, $ScvO_2$- Central venous oxygen saturation, PaO_2 – Arterial oxygen partial pressure, $PaCO_2$ – Arterial carbon dioxide partial pressure, $aHCO_3$ – Arterial bicarbonate, aBE – Arterial base excess
T_0- Baseline measurement, T_1-T_5- Five intervals of bleeding.
*p <.05 compared with T_0; #p <.05 compared with previous; GLM repeated measures ANOVA

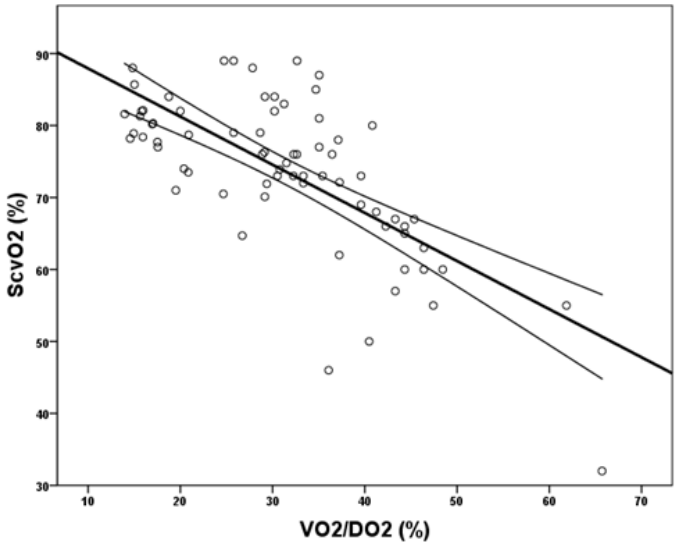
Arterial oxygen saturation of hemoglobin remained in the normal range throughout the experiment. DO_2 fell significantly from T_2, VO_2 at T_4, VO_2/DO_2 increased significantly from T_3, and exceeded the physiologic threshold of 30%. The change in $ScvO_2$ displayed a similar pattern as VO_2/DO_2 and changed significantly and also fell below 70% only at T_4. The other parameters did not change significantly. The pattern of changes of VO_2/DO_2 and $ScvO_2$ over time is demonstrated in Figure 2.

Figure 2. Change of VO_2/DO_2 and $ScvO_2$ over time.



We determined the association between VO_2/DO_2 and $ScvO_2$, and found a strong, negative correlation ($r=-.71$, $p<.001$) (Figure 3).

Figure 3. Correlation between VO_2/DO_2 and $ScvO_2$.



ROC analysis revealed the same tendency as the correlation. With 30% taken as the physiologic threshold for VO_2/DO_2, the area under the curve (AUC), its standard error and that of the 95% confidence interval were >0.5 for $ScvO_2$ (AUC=0.768 ± 0.056 [0.657-0.878] p<.001). It was also important to determine the best cut-off for $ScvO_2$ to detect the significant increase in VO_2/DO_2, therefore, sensitivity, specificity, PPV and NPV for $ScvO_2$ levels of 70% and 75% were calculated. A $ScvO_2$ level of 70% had better specificity and PPV, while a $ScvO_2$ level of 75% had better sensitivity and NPV (Table 6). Furthermore, linear regression revealed a significant relationship between $ScvO_2$ (r=-.71, r^2=.50, p<.001) and VO_2/DO_2.

Table 6 Performance of $ScvO_2$ in Detecting VO_2/DO_2>30%

	$ScvO_2$ < 70%	$ScvO_2$ < 75%
Sensitivity (%)	45	68
Specificity (%)	97	77
PPV (%)	95	79
NPV (%)	58	65

$ScvO_2$- Central venous oxygen saturation, VO_2/DO_2- Oxygen extraction ratio,
PPV – positive predictive value, NPV – negatíve predictive value

2.3. Discussion

Maintaining adequate tissue oxygenation by improving oxygen delivery is the rational for blood transfusion. Despite this fact, treatment of anemia with blood transfusion in the absence of acute bleeding is recommended at certain levels of hemoglobin, regardless of actual oxygen delivery and need, thus resulting in possible excess blood administration.[8,9] In our study in isovolaemic anemia, we found that $ScvO_2$ is a sensitive indicator of oxygen balance and may thus serve as a rational guide to therapy in this all too common problem.

Oxygen balance and $ScvO_2$.

In certain clinical conditions, an $ScvO_2$ value of ~70% has been used as a goal to therapeutic intervention in attempts at improving oxygen delivery.[19,23,31,32] In one study in septic patients goal directed therapy according to $ScvO_2$ values, saw an absolute 16% reduction in in-hospital mortality as compared to conventional therapy.[19] Two recent studies have also demonstrated that a low $ScvO_2$ predicts peri- and postoperative morbidity and complications in high-risk surgery.[23,31] It may also serve as a useful tool to assist the weaning process in mechanically ventilated patients.[32]

In our experiment, despite a continuous and significant drop in hemoglobin levels, the value of VO_2/DO_2 increased significantly only from T_3, and exceeded the physiologic threshold of 30%. The change in $ScvO_2$ displayed a similar pattern as VO_2/DO_2 and fell below 70% only at T_4. If we translate that into clinical practice, the reduced hemoglobin concentrations could have indicated blood transfusion from T_2, however, we found no evidence of impaired VO_2/DO_2 until the hemoglobin was well below the current recommended transfusion threshold.

About two decades ago it was found during haemorrhage in animal and human experimental models that $ScvO_2$ may be useful for the identification of patients with occult or ongoing clinically significant blood loss.[33,34] In a prospective human interventional study it was found that in acute isovolaemic anemia of hemoglobin 50 g/L in conscious healthy resting humans did not produce evidence of inadequate systemic DO_2 and oxygen imbalance

was accompanied by a significant drop in mixed venous saturation.[35] These results were reinforced by a retrospective analysis of a prospective observational study in which $ScvO_2$ was found to be a good indicator of transfusion.[36] Our results give further evidence that anemia-induced change in oxygen balance can be monitored by $ScvO_2$.

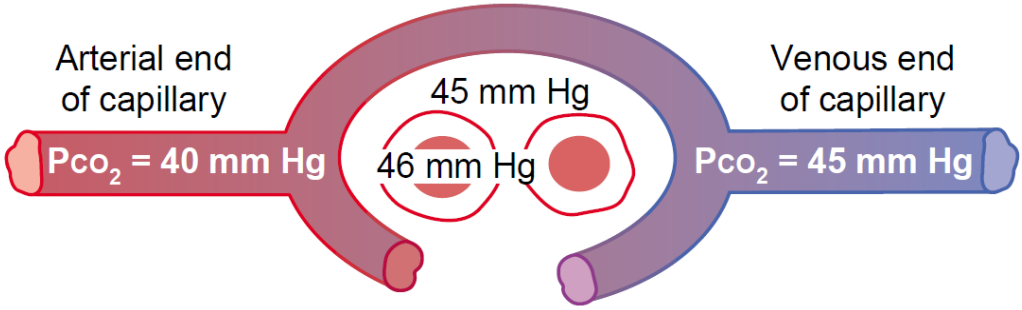
Hemodynamic effects of Isovolaemic Anemia.

Our goal of maintaining isovolemia was achieved as parameters of preload and central blood volume, such as global end-diastolic volume and central venous pressure did not change throughout the experiment. A significant increase in cardiac index was found, due to increased heart rate, as stroke volume did not change significantly over time. This increase was in agreement with the hemodynamic changes of a recent study by Krantz et al. with a similar experimental setting, but with a different hypothesis.[37] Myocardial contractility as indicated by the index of left ventricular contractility value also increased significantly, most likely in response to isovolemic anemia. Although we did not measure catecholamine levels, a theoretical explanation could be that the bleeding caused an enhanced stress response resulting in the observed positive inotropic effect.[38,39]

Limitations of the study.

One of the possible limitations of our study was that the length of the recovery time after splenectomy may not have been satisfactory, and therefore the levels of lactate were above the normal range, although they did not change significantly and the relevance of this is questionable. The steady-state periods may also have been relatively short, although, the same time intervals have been used previously.[40] Further, the type of fluid replacement may be important, and we cannot exclude the possibility that the use of different types of colloid solutions would affect the results. Finally, these data were obtained in anesthetized animals, and may not be applicable to conscious animals.

CO_2-gap


Arterial end
of capillary
Pco2 = 40 mm Hg
45 mm Hg
46 mm Hg
Venous end
of capillary
Pco2 = 45 mm Hg

3. Changes of CO_2-gap in isovolemic anemia – an animal experiment

3.1. Materials and Methods

The study protocol was approved by the local ethics committee at the University of Szeged and the study was carried out in the research laboratory of the Institute of Surgical Research.

This experiment complements the previously described data on the relationship of $ScvO_2$ and isovolemic anemia. The animals and instrumentation, the experimental protocol (Figure 1) and the hemodynamic measurements are detailed above.

Arterial, central venous and mixed venous blood gas samples (Cobas b 221, Roche Ltd., Basel, Switzerland) were drawn and analyzed by cooximetry simultaneously at baseline and at the end of each cycle. From these parameters the oxygen delivery (DO_2), oxygen consumption (VO_2), oxygen extraction ratio (VO_2/DO_2) and the simplified oxygen extraction ratio (ERO_2) were calculated according to standard formulae:

$$DO_2 = SV * HR * \left[Hb * 1.34 * SaO_2 + \left(0.003 * PaO_2 \right) \right]$$

$$VO_2 = CO * \left[CaO_2 - \left(Hb * 1.34 * SvO_2 + \left(0.003 * PvO_2 \right) \right) \right]$$

$$ERO_2 = \left(SaO_2 - ScvO_2 \right) / SaO_2$$

Central venous-to-arterial CO_2-gap ($_{cv}CO_2$-gap), mixed venous-to-arterial CO_2-gap ($_{v}CO_2$-gap) were also calculated from the arterial, central venous and mixed venous blood gas samples.

These were calculated according to standard formulae:

$$_{cv}CO_2\text{-gap} = P_{cv}CO_2 - P_aCO_2$$

$$_{v}CO_2\text{-gap} = P_vCO_2 - P_aCO_2$$

Data Analysis and Statistics.

Data are reported as median (interquartile ranges), unless indicated otherwise. For testing normal distribution the Kolmogorov-Smirnov test was used. Changes in all parameters throughout the experiment were tested by Friedman test and repeated measures analysis of variance (RM ANOVA), and the number of degrees of freedom was adjusted to Greenhouse-Geisser epsilon when needed. For pairwise comparisons Pearson's correlation was used. To evaluate the performance in detecting altered oxygen extraction of >30% (considered as the "physiological threshold"), receiver operating characteristics (ROC) curve analysis was performed. Post-hoc calculation showed a power of 86% with an effect of 25% increase in VO_2/DO_2, for a sample size of 13 and α=0.05. For statistical analysis SPSS version 20.0 for Windows (SPSS, Chicago, IL, USA) was used and $p < .05$ was considered statistically significant.

3.2. Results

All 13 animals survived the study. The bleeding caused a gradual decrease in hemoglobin level after each phase and by the end of the experiment it had fallen by 61% of the baseline value. The hemodynamic parameters are summarized in Table 4. The SaO_2 remained in the normal range throughout the experiment. DO_2 fell significantly from T_2, VO_2 at T_4, VO_2/DO_2 increased significantly from T_3, and exceeded the physiologic threshold of 30% (Table 5). The change in $ScvO_2$ displayed a similar pattern as VO_2/DO_2 and changed significantly and also fell below 70% only at T_4. There was strong negative correlation between VO_2/DO_2 and $ScvO_2$ (Figure 3).

The CO_2-gap was calculated for both, central venous ($_{cv}CO_2$-gap) and mixed venous blood ($_{v}CO_2$-gap). By T_4 $_{cv}CO_2$-gap increased significantly, however $_{v}CO_2$-gap did not change (Table 7).

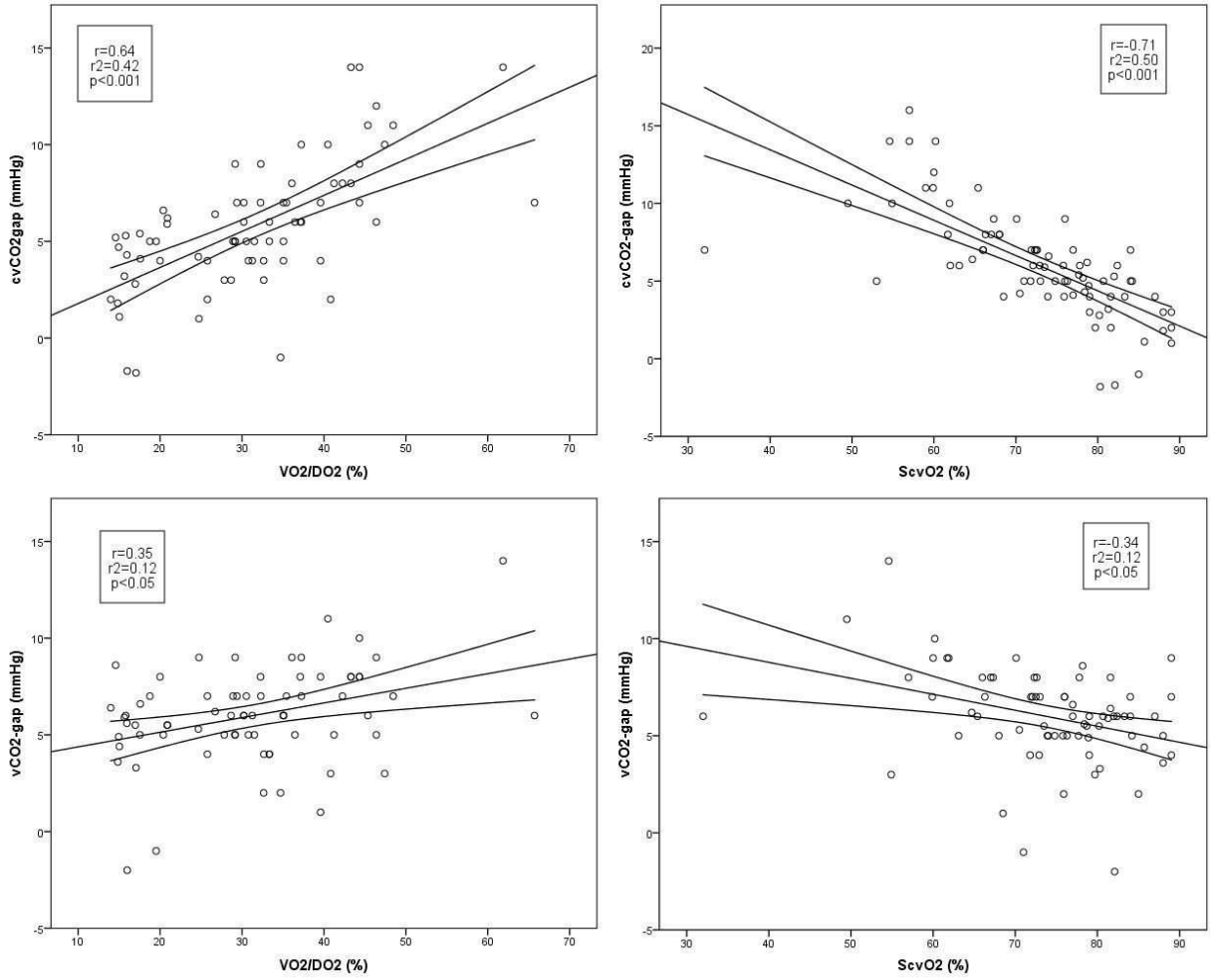
Table 7 Effects of Isovolaemic Anemia on Oxygen Balance

	T_0	T_1	T_2	T_3	T_4	T_5
$_{cv}CO_2$-gap (mmHg)	5.0(2.6-8.5)	6.0(3.1-7.0)	5.0(3.5-5.5)	5.4(4.4-7.0)	8.0(4.3-8.5)*	6.3(5.9-11.0)*
$_{v}CO_2$-gap (mmHg)	5.5(4.0-9.0)	6.5(4.5-7.8)	6.5(5.1-7.0)	5.5(3.7-6.0)	5.4(5.0-8.0)	6.2(5.5-8.0)
Lactate (mmol/L)	4.5 (3.2–5.3)	4.2 (3.0–5.1)	5.0 (3.2–6.0)	4.1 (2.9–6.0)	4.2 (2.9–6.5)	4.0 (3.0–6.4)
vLactate (mmol/L)	4.6(3.7-5.3)	4.3(3.3-5.3)	4.4(3.1-5.4)	4.4(2.8-5.2)	4.4(3.0-5.2)	4.1(3.0-6.4)
cvLactate (mmol/L)	4.5(3.5-5.5)§	3.9(3.4-5.4)§	4.2(3.3-6.3)§	4.1(3.1-5.6)§	3.9(2.9-5.7)§	3.9(3.0-6.4)§

$_{cv}CO_2$-gap: central venous-to-arterial carbon dioxide difference; $_{v}CO_2$-gap: mixed venous-to-arterial carbon dioxide difference;
* p< .05 as compared to baseline, § p< .05 significant difference between mixed venous and central venous blood with Friedman and Wilcoxon tests,

The correlations of VO_2/DO_2 and $ScvO_2$ were significant with $_{cv}CO_2$-gap, while there were only weak correlations with $_{v}CO_2$-gap (Figure 4).

Figure 4. Correlation between $_{cv}CO_2$-gap and VO_2/DO_2 and $ScvO_2$; $_{v}CO_2$-gap and VO_2/DO_2 and $ScvO_2$.



ROC analysis revealed the same tendency as the correlation. With 30% taken as the physiologic threshold for VO_2/DO_2, the area under the curve (AUC), its standard error and that of the 95% confidence interval were >0.5 only for $_{cv}CO_2$-gap and $ScvO_2$ (Table 8).

Table 8 ROC analysis for determining VO_2/DO_2>30%

Test Result Variable(s)	Area	Std. Error	Sig.	95% CI	
$_{cv}CO_2$-gap	,769	,078	,007	,617	,921
$_{v}CO_2$-gap	,553	,097	,598	,363	,742
$ScvO_2$	,768	,056	,000	,657	,879
SvO_2	,986	,010	,000	,967	1,000
Lactate	,517	,078	,867	,363	,670

$_{cv}CO_2$-gap: central venous-to-arterial carbon dioxide difference;
$_{v}CO_2$-gap: mixed venous-to-arterial carbon dioxide difference;
$ScvO_2$: central venous oxygen saturation;
SvO_2: mixed venous oxygen saturation;

Linear regression revealed a significant relationship between $ScvO_2$ ($r=0.71$, $r^2=0.50$, $p<.001$) and VO_2/DO_2. This relationship became significantly stronger when $_{cv}CO_2$-gap was added to $ScvO_2$ ($r=0.74$, $r^2=0.54$, $p<.015$). According to the Pratt's importance coefficient, $ScvO_2$ was responsible for this increase in 63% and $_{cv}CO_2$-gap in 37%.

3.3. Discussion

Our results in this isovolemic anemia animal model show that besides $ScvO_2$, only central venous-to-arterial CO_2-gap correlated well with changes in anemia caused increase in VO_2/DO_2. Furthermore, mixed venous blood driven indices, such as $_vCO_2$-gap failed to indicate changes in oxygen extraction. When oxygen extraction ratio started to increase (from T_3) it was followed by a decrease of $ScvO_2$ and an increase of $_{cv}CO_2$-gap, and both performed well in the ROC analysis, with the $_{cv}CO_2$-gap's AUC being marginally better. In addition, in our experiment neither $_vCO_2$-gap nor lactate could detect the increase in VO_2/DO_2>30% as revealed by ROC analysis.

An interesting finding of our experiment is that although isovolemia was maintained as indicated by the stable global end diastolic volume index values and in fact, cardiac output and stroke volume both increased, we observed a rise in $_{cv}CO_2$-gap. This observation seemingly contradicts previously published results to some extent. The occurrence of increased CO_2-gap has fundamentally been explained by the CO_2 stagnation phenomenon [25]. This was based on the finding that there was inverse correlation between CO_2-gap and cardiac index during non-septic and septic low flow states [25,26,41]. Moreover, it was also found that the amount of CO_2 produced is negligible when anaerobic respiration is present and CO_2-gap therefore cannot serve as a marker of tissue hypoxia [41]. The paramount study on this theory by Vallet et al. used an isolated hind limb model and reached hypoxia either by decreasing flow or decreasing arterial oxygen content [41]. They found that occurrence of an increased CO_2-gap during ischemia was related to decreased blood flow and impaired carbon dioxide washout; moreover, dysoxia *per se* was not sufficient to increase CO_2-gap. However, the latter could also be due to Haldane's effect. As the carbon dioxide dissociation curve is influenced by the saturation of hemoglobin with oxygen, the lower the saturation of hemoglobin with oxygen, the higher the saturation of hemoglobin with carbon dioxide for a given carbon dioxide partial pressure [42]. In our experiment arterial oxygen saturation and PaO_2 remained in the normal range and did not change over time, hence the CO_2 dissociation curve was not influenced by low saturation of hemoglobin with oxygen.

Nevertheless, anemia resulted in increased VO_2/DO_2 above the baseline and also above the physiological 30% after the 3rd bleeding event, which was followed by the

significant decrease of SvO_2 and $ScvO_2$. (It is important to note that there is mathematical coupling between VO_2 and SvO_2, this is not the case with $ScvO_2$). The most interesting finding of the current study is the increase of $_{cv}CO_2$-gap during the last two stages of the experiment, without any change in the $_{v}CO_2$-gap. One of the possible reasons for this difference is that due to isovolemia cardiac output was maintained to avoid low flow in the systemic circulation, which is also reinforced by the unchanged lactate levels. Therefore when CO_2 was measured in the mixed venous blood it was unchanged and within the normal range almost throughout. As central venous blood driven variables mostly reflect blood flow and metabolism of the brain [14], our hypothesis is that anemia reached such a degree by T_4 that it caused tissue hypoxia and consecutive anaerobic respiration with CO_2 production. However, due to the low hemoglobin levels the Haldane effect could not take effect, hence there was a significant increase in central venous pCO_2. But these changes in the brain did not have significant effects on the systemic level, to be picked up in mixed venous blood. As anemia has greater influence on arterial oxygenation than hypoxemia [43], this might explain the observed increase in $_{cv}CO_2$-gap. We also measured mixed venous and central venous lactate levels and found that central venous lactate was significantly lower than in the mixed venous blood, which might give further proof to this hypothesis [44,45]. In a previous animal experiment by Hare *et al*, it was found that hemodilutional isovolemic anemia led to cerebral hypoxia, and they also reported a gradual increase in the jugular venous pCO_2 with a CO_2-gap of 2.9 to 7.8 mmHg (mean) 60 minutes after hemodilution in the traumatic brain injured animals [46]. This finding was not discussed in the article, as the authors mainly focused on oxygenation, nevertheless this is in accord with our results and gives some support to our hypothesis.

There is increasing evidence that untreated anemia can be associated with a worse outcome and increased mortality, while transfusion may cause various infectious and non-infectious adverse effects [11,13]. $_{cv}CO_2$-gap may be an additional quantitative parameter, beyond Hb and $ScvO_2$, that would give information on anemia related altered oxygen extraction and hence the need for blood administration. $_{cv}CO_2$-gap is a choice of plausible alternatives as it can be easily obtained via the central venous and arterial catheters already *in situ* in most critically ill patients and no additional invasive device is needed; moreover we found that mixed venous blood driven indices failed to indicate changes in oxygen extraction.

There are several limitations of our study. As the experiment was not designed to measure the effects of isovolemic anemia specifically on the brain, our hypothesis cannot be

supported by specific measurements, such as regional cerebral blood flow, cerebral tissue oxygen and carbon dioxide tension. Furthermore, splenectomy and the length of the preparation of the animals may have been too long, which resulted in increased levels of lactate from baseline to the end of the experiment. The steady-state periods may also have been relatively short, although, the same time intervals have been used previously [40]. Another concern might be the type of fluid replacement, as one cannot exclude the possibility that the use of different types of colloid or crystalloid solutions would affect these results.

4. $ScvO_2$ and CO_2-gap in moderate hypovolemia – animal experiment

4.1. Materials and Methods

The study protocol was approved by the local Ethics Committee and the Institutional Animal Care and Use Committee at the University of Szeged and the study was conducted in the research laboratory of the Institute of Surgical Research in a manner that does not inflict unnecessary pain or discomfort upon the animal.

Animals and Instrumentation.

Vietnamese mini-pigs (n=15) weighing 28±4 kg underwent a 24-hr fast preoperatively but with free access to water. Anesthesia was induced by intramuscular injection of a mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) and maintained with a continuous infusion of propofol (6 mg/kg/hr iv.), while analgesia was maintained with nalbuphine (0.1 mg/kg). A tracheal tube was inserted and the animals' lungs were ventilated mechanically. The tidal volume was set at 10 mL/kg, and the respiratory rate was adjusted to maintain the end-tidal carbon dioxide and partial pressure of arterial carbon dioxide in the range of 35-45 mmHg and the arterial pH between 7.35 and 7.45. The adequacy of the depth of anesthesia was assessed by monitoring the jaw tone. After induction of anesthesia, the right jugular vein and the right femoral artery and vein were dissected and catheterized. Tonometric probes and catheters were placed simultaneously into the stomach and the small bowel. A suprapubic urinary catheter was also inserted to monitor urine output. Animals were kept warm (35±1^{o}C) by an external warming device.

For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter (PiCCO, PULSION Medical Systems SE, Munich, Germany) was placed in the femoral artery and a pulmonary artery catheter (PV2057 VoLEF Catheter, PULSION Medical Systems SE, Munich, Germany) was placed in the femoral vein. The latter was also used to draw mixed venous blood gas samples with which the oxygen consumption (VO_2) was calculated. The femoral artery served as the site for arterial blood gas sampling and the central venous line

was used for taking central venous blood gas samples and for the injection of cold saline boluses for the thermodilution measurements.

For continuous noninvasive visualization of the microcirculation in the sublingual region an intravital orthogonal polarization spectral (OPS) imaging technique (Cytoscan A/R, Cytometrics, Philadelphia, PA, USA) was used.[16,47] A 10x objective was introduced onto the sublingual serosa, and microscopic images were recorded with an S-VHS video recorder (Panasonic AG-TL 700, Osaka, Japan).

For the tonometry special probes (Tonosoft Medical –Technical and R&G Ltd.) were used and monitoring was performed with a Sidestream Microcap Handheld Capnograph (Oridion Medical Ltd, Jerusalem, Israel) instrument.[48]

To assess further biochemical changes in the microcirculation, plasma big-endothelin-1 (BigET) levels were determined. BigET is a 38 amino acid containing protein, the precursor of endothelin-1, which becomes elevated in tissue hypoxia.[49, 50]

Hemodynamic Measurements.

Cardiac output (CO), global end-diastolic volume index (GEDI), stroke volume (SV), heart rate (HR), and mean arterial pressure (MAP) were measured by transpulmonary thermodilution and pulse contour analysis at baseline and at the end of each interval. Detailed description of transpulmonary thermodilution and pulse contour analysis is provided elsewhere.[29,30] All hemodynamic parameters were indexed for body surface area or bodyweight. The average of three measurements following 10 mL bolus injections of ice-cold 0.9% saline was recorded. Central venous pressure (CVP) was monitored continuously and registered with a computerized data-acquisition system (SPELL Haemosys; Experimetria Ltd., Budapest, Hungary).

Arterial, central venous and mixed venous blood gas samples (Cobas b 221, Roche Ltd, Basel, Switzerland) were drawn and analyzed by cooximetry simultaneously at baseline and at the end of each cycle.

Monitoring the Microcirculation.

Microcirculatory evaluation of the sublingual region was performed off-line by frame-to-frame analysis of the videotaped images. Capillary red blood cell velocity (RBCV) and capillary perfusion rate (CPR) were determined in three separate fields using a computer-assisted image analysis system (IVM Pictron, Budapest, Hungary). All OPS-measurements were performed by one investigator.

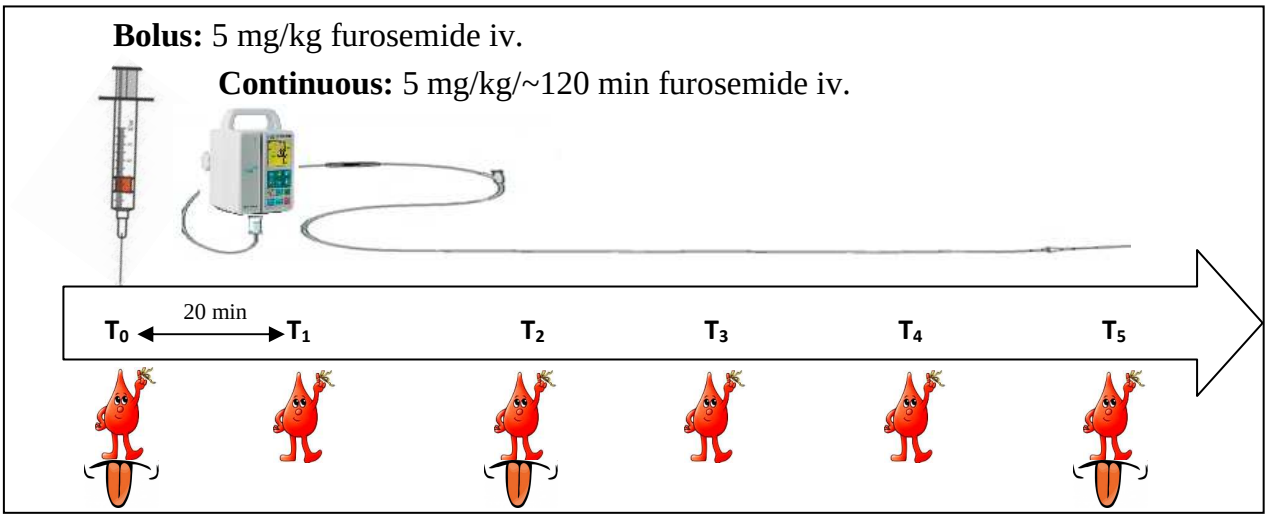
Gastric and small bowel changes in partial pressure of carbon dioxide (ΔPCO_2) were calculated by subtracting tonometric PCO_2 from arterial PCO_2 (gastric-arterial: Ga-PCO_2; bowel-arterial: Ba-PCO_2).[48]

For measurements of BigET, blood samples of 2 ml were drawn from the jugular vein into chilled polypropylene tubes containing EDTA (1 mg/mL). The samples were centrifuged at 1200g for 10 min at 4°C. The plasma samples were then collected and stored at -70°C until assay.

Experimental Protocol.

At baseline (T_0) hemodynamic, blood gas and microcirculatory parameters were recorded (Figure 5). Hypovolemia was induced via a bolus followed by a continuous infusion of furosemide (5 mg/kg and 5 mg/kg/2h, respectively) in a group of 10 animals – hypovolemic group (HG). After the administration of bolus furosemide measurements were recorded in five stages with 20 minutes interval between each measurement (T_1-T_5). When the preload parameter (GEDI) decreased by >20% its baseline value, OPS imaging and BigET sampling were performed, which were repeated only at the end of the experiment (Figure 5).

Figure 5. Schematic diagram illustrating the experimental protocol.



Measurements we performed at baseline (T_0) and then in every 20 minutes (T_{1-5}); symbols indicate hemodynamic/blood gas ( ) and microcirculatory ( ) measurements.

There were 5 anaesthetised, ventilated animals in the sham group (SG), who did not receive any furosemide, but maintenance infusion of lactated Ringer (4mL/kg/h) and hemodynamic, microcirculatory and blood gas parameters were recorded in the same fashion as described previously. At the end of the experiment all animals were humanely euthanized.

Data Analysis and Statistics.

Data are reported as means ± standard deviations unless indicated otherwise. For testing normal distribution the Kolmogorov-Smirnov test was used. Changes in all parameters throughout the experiment were tested by repeated measures analysis of variance (RM ANOVA); and the number of degrees of freedom was adjusted to Greenhouse-Geisser epsilon when needed. Mann-Whitney U-test with Bonferroni correction was used for between-groups analysis. For pairwise comparisons Pearson's correlation was used. To evaluate the performance of $ScvO_2$, CO_2-gap and microcirculatory parameters in detecting altered oxygen extraction with a threshold of 30%, receiver operating characteristics (ROC) curve analysis was performed, and sensitivity, specificity, positive predictive (PPV) and negative predictive values (NPV) were also determined. Post-hoc calculation showed a power of 83% with an effect of 36% decrease in GEDI for a sample size of 10 and α=0.05. For statistical analysis SPSS version 18.0 for Windows (SPSS, Chicago, IL) was used and $p<.05$ was considered statistically significant.

4.2. Results

Hemodynamic Effects of Hypovolemia.

Urine output in the hypovolemic group following the bolus and the onset of infusion was 176±160 mL at T_1, which increased to 647±231 mL at T_5. In contrast, in the sham group, urine output was 74±74 mL at T_1 and had increased to 325±175 mL by T_5. All other hemodynamic data are summarized in Table 9. Preload, as indicated by GEDI, decreased significantly after each phase in the hypovolemic group compared to baseline and dropped by 36% of its baseline value by the end of the experiment. The change of the other macro-hemodynamic variables followed a similar pattern. When comparing the sham versus hypovolemic animals, variables differed between the two groups from T_1, but significant differences over time continued only for GEDI, CVP and SVI. In the sham group there were no significant changes over time throughout the experiment.

Table 9 Hemodynamic Changes

		T_0	T_1	T_2	T_3	T_4	T_5
GEDI mL/m^2)	HG	349±51	293±53*‡#	257±47*‡#	246±53*#	233±42*#	223±31*#
	SG	350±26	387±43	360±22	365±76	356±51	349±46
CVP (mmHg)	HG	7±4	4±3*‡#	4±3*#	4±3*‡#	3±3*#	4±3*#
	SG	8±1	7±1	7±1	7±2	7±1	7±2
MAP (mmHg)	HG	121±15	107±17*‡	94±19*‡	86±15*‡	85±15*	83±17*
	SG	120±13	121±14	117±15	113±16	121±24	111±25
HR (1/beats)	HG	78±14	83±15*‡	93±17*	102±19*	130±28*‡	142±28*‡#
	SG	74±12	75±12	74±9	78±10	83±16	80±9
CI (L/min/m^2)	HG	2.30±0.35	1.78±0.31*‡	1.54±0.32*‡	1.46±0.35*‡	1.52±0.41*	1.58±0.36*
	SG	2.38±0.50	2.65±0.65	2.42±0.48	2.38±0.76	2.32±0.50	2.27±0.27
SVI (mL/m^2)	HG	29±5	19±2*‡#	15±4*#	14±4*#	12±4*#	12±3*#
	SG	33±4	34±6	33±3	32±6	31±8	28±5

GEDI- Global end-diastolic volume index, CVP- Central venous pressure, MAP- Mean arterial pressure, HR- Heart rate, CI- Cardiac index, SVI- Stroke volume index.

T_0- Baseline measurement, T_1-T_5- Five intervals.

*p< .05 as compared to T_1; ‡p< .05 as compared to the previous value; RM ANOVA;

#p< .05 HG vs SG; Mann-Whitney U-test with Bonferroni correction

Effects on Oxygen Balance.

Variables relating to oxygen balance are listed in Table 10. In the hypovolemic group DO_2 fell significantly from T_1 and remained so for the rest of the experiment. The VO_2/DO_2 increased significantly over 30% from T_1, while $ScvO_2$ and CO_2-gap followed this change only after T_2. Lactate changed significantly from T_3. There were no significant changes in the sham group throughout the experiment.

Table 10 Changes of Oxygen Balance

		T_0	T_1	T_2	T_3	T_4	T_5
SaO_2	HG	94±6	95±5	95±2	96±2	94±3	94±2
(%)	SG	95±5	94±8	96±5	94±8	95±5	94±7
Hb	HG	122±9	129±13	132±7*	134±7*‡	137±7*‡	138±8*
(g/L)	SG	119±10	115±9	110±6	108±11	111±9	102±10
DO_2I	HG	361±39	302±52*‡	268±47*‡	258±55*	269±65*	284±55*
($mL/min/m^2$)	SG	365±78	396±88	346±73	337±67	324±55	298±36
VO_2/DO_2	HG	28±5	31±6*‡	34±7*‡	39±9*‡	41±9*	40±10*
(%)	SG	25±4	25±6	26±7	29±5	27±6	30±7
$ScvO_2$	HG	74±10	71±10	67±11*‡	64±14*	59±13*‡	57±14*
(%)	SG	77±8	76±10	76±9	75±11	73±14	73±12
CO_2-gap	HG	4.3±2.3	7.5±3.3	7.1±2.6*	8.3±2.8*	7.3±2.9*	10.1±5.5*#
(mmHg)	SG	4.1±2.4	3.5±1.9	4.5±1.3	3.9±2.9	4.6±1.9	4.4±1.9
Lactate	HG	3.8±1.4	3.9±1.3	4.3±0.9	4.7±0.9*‡	5.1±1.2*‡	5.3±1.5*
(mmol/L)	SG	3.8±0.9	3.9±1.2	4.2±1.8	4.6±2.1	4.7±2.7	4.9±3.2
VO_2I	HG	98±11	93±16	88±9	96±7‡	104±10‡	109±16
($mL/min/m^2$)	SG	88±16	96±6	85±12	96±8	85±12	88±14

SaO_2- Arterial oxygen saturation, Hb- Hemoglobin, DO_2- Oxygen delivery, VO_2/DO_2- Oxygen extraction ratio, $ScvO_2$- Central venous oxygen saturation, CO_2-gap- venous-to-arterial carbon dioxide difference, VO_2- Oxygen consumption.

T_0- Baseline measurement, T_1-T_5- Five intervals.

*p< .05 as compared to T_1; ‡p< .05 as compared to the previous value; RM ANOVA;

#p< .05 HG vs SG; Mann-Whitney U-test with Bonferroni correction

In the hypovolemic group there were significant correlations between VO_2/DO_2 and $ScvO_2$, and CO_2-gap (Figure 6A and 6B, respectively). Lactate also showed a significant, but weak correlation with VO_2/DO_2 ($r = .38$, $r^2 = .14$; $p < .05$).

Figure 6A. Correlation between VO_2/DO_2 and $ScvO_2$.

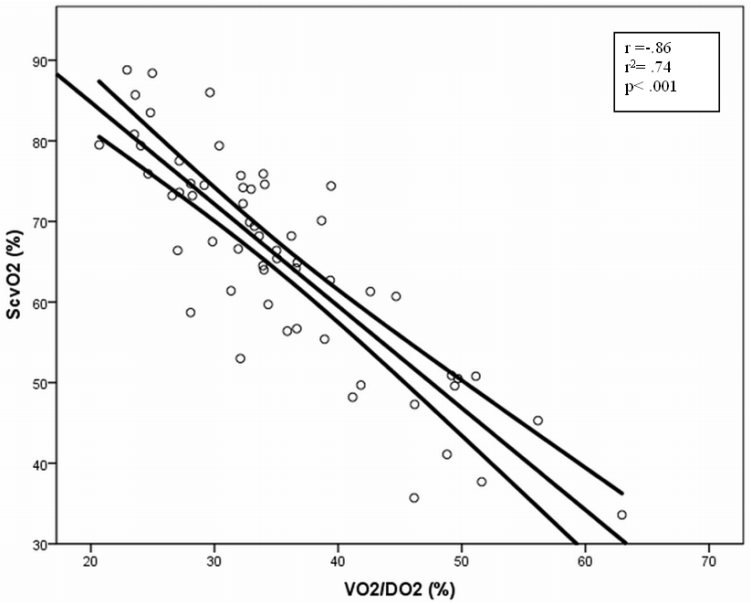
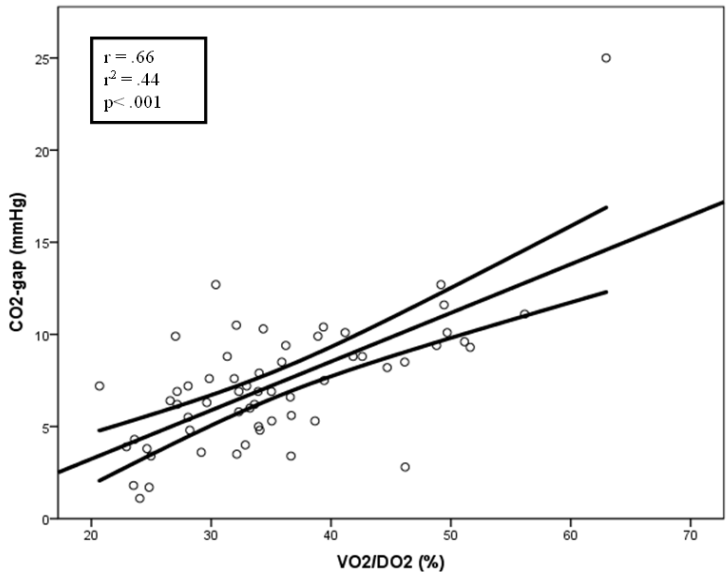


Figure 6B. Correlation between VO_2/DO_2 and CO_2-gap.



With Receiver Operator Characteristic (ROC) curves for $ScvO_2$, CO_2-gap and lactate to detect a VO_2/DO_2>30%, the area under the curves (AUC) was significant for $ScvO_2$, CO_2-gap, (AUC±SE=0.887±0.046; 0.783±0.062; p<.05, respectively), while lactate did not reach statistical significance.

The cut-off values to give the best sensitivity and specificity for $ScvO_2$ and CO_2-gap were: 73% and 6.5 mmHg, respectively. Sensitivity, specificity, positive predictive and negative predictive values for $ScvO_2$ and CO_2-gap are summarized in Table 11. Taking $ScvO_2$ and CO_2-gap values together to predict a VO_2/DO_2>30% the false positive and false negative values were reduced.

Table 11 Complemetation of $ScvO_2$ with CO_2-gap.

	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)
$ScvO_2 \leq 73$ %	78	83	91	63
CO_2-gap > 6 mm Hg	71	72	85	52
$ScvO_2$ + CO_2-gap (≤73%) (>6 mm Hg)	58	100	100	72

$ScvO_2$- Central venous oxygen saturation, CO_2-gap- venous-to-arterial carbon dioxide difference, PPV- positive redictive value, NPV- Negative predictive value

Effects on Microcirculation.

Variables relating to microcirculation are listed in Table 12.

Table 12 Changes in Microcirculation.

		T_0	T_1	T_2	T_3	T_4	T_5
Ba-PCO_2	HG	24±8	35±16*‡	35±17	36±13*	33±13*	37±16*
(mmHg)	SG	19±5	22±9	20±6	22±11	22±10	20±8
Ga-PCO_2	HG	40±12	43±14	42±14	39±14	36±10	37±11
(mmHg)	SG	36±22	37±24	34±21	32±21	34±20	32±18
CPR	HG	82±15	-	53±11*‡#	-	-	45±16*‡#
(%)	SG	91±50		81±80			83±70
RBCV	HG	887±141	-	509±120*‡#	-	-	463±209*#
(μm/s)	SG	1054±141		848±194			963±51
BigET	HG	1.44±0.53	-	1.97±0.84*‡	-	-	2.29±0.89*#
(fmol/mL)	SG	1.36±0.93		1.49±1.27			0.98±0.92*

Ba-pCO_2- Small bowel-to-arterial carbon dioxide difference, Ga-pCO_2- Gastric-to-arterial carbon dioxide difference, CPR – Capillary perfusion rate, RBCV- Red blood cell velocity, BigET- Big-endothelin.

T_0- Baseline measurement, T_1-T_5- Five intervals.

*$p < .05$ as compared to T_1, ‡$p < .05$ as compared to the previous value, GLM repeated measures ANOVA;

#$p < .05$ HG vs SG, Mann-Whitney U-test with Bonferroni correction

In the hypovolemic group tonometry increased significantly only when measured in the intestines. Capillary perfusion rate and red blood cell velocity gradually and significantly decreased over time. This change was accompanied by a significant increase in BigET levels. In contrast, the sham group BigET decreased significantly by T_5, while the other parameters remained unchanged. There was a significant difference in capillary perfusion rate, red blood cell velocity and BigET between the two groups. (Figure 7A and 7B, respectively).

Figure 7A. Significant decrease of capillary perfusion rate.

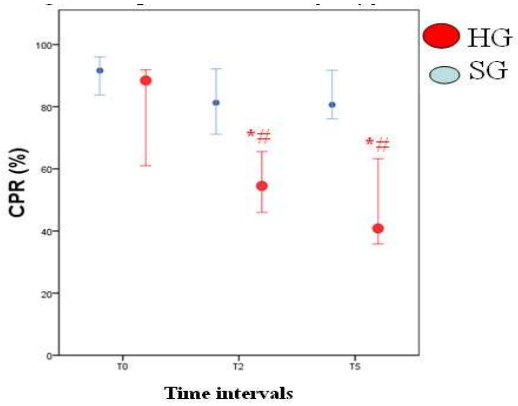
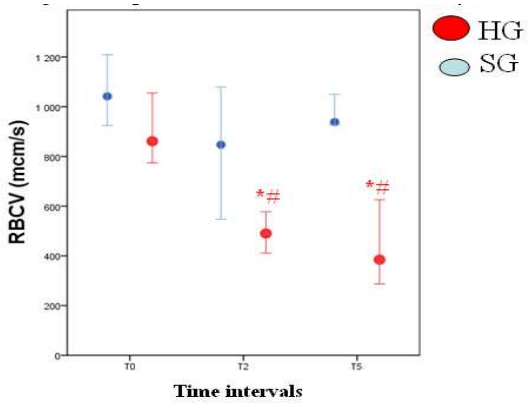


Figure 7B. Significant decrease of red blood cell velocity.



The ROC curves for predicting VO_2/DO_2>30% proved to be significant for capillary perfusion rate and red blood cell velocity in the hypovolemic group. The area under the curve did not differ significantly between these two parameters (AUC±SE=0.848±0.084; 0.848±0.092; p<.05, respectively).

The correlation between $ScvO_2$ and the microcirculatory parameters proved to be significant apart from Ga-PCO_2 ($ScvO_2$ - Ba-PCO_2, - CPR, - RBCV, - BigET: r=-.38, r^2=.15; r=.49, r^2=.24; r=.40, r^2=.16; r=-.47, r^2= .23; p<.05, respectively). CO_2-gap showed significant correlations with Ba-PCO_2 and CPR (r=.48, r^2=.23; r=-.51, r^2=.26; p<.05, respectively).

4.3. Discussion

The main finding of our study is that it provides further evidence that low or decreasing $ScvO_2$, as well as high or increasing CO_2-gap can reflect changes and may be complementary in global oxygen balance and altered microcirculatory blood flow in hypovolemia.

Hemodynamic changes.

Our goal of achieving hypovolemia was reached as global end diastolic index, central venous pressure and stroke volume decreased significantly throughout the experiment, which resulted in a significant drop in cardiac index in the hypovolemic group. This decrease was notable until T_3. Due to this change VO_2/DO_2 increased significantly from T_1.

CO_2-gap and $ScvO_2$ in Hypovolemia.

Up until now, there has been consensus neither on the most accurate hemodynamic marker of hypovolemia, nor on the end-points for optimal fluid therapy.[15,51,52] Many recent studies have suggested that fluid therapy should be based on dynamic (such as: cardiac output, pulse pressure variation, stroke volume variation) rather than static hemodynamic variables (such as: CVP, pulmonary artery occlusion pressure), because they are better predictors of fluid responsiveness in ICU patients. However, pulse pressure variation and stroke volume variation are limited to patients who are fully ventilated and have no arrhythmias.[53,54] Although it is not strictly a hemodynamic variable but in certain clinical conditions an $ScvO_2$ value of ~70% has been used as a therapeutic end-point to improve oxygen delivery.[19,23,31-32] In a recent study it was found that change in $ScvO_2$ is a reliable parameter to define fluid responsiveness at the bedside in critically ill patients.[21] Similar results have been reported in two other studies demonstrating a close relationship between $ScvO_2$ and cardiac index.[22,55] However, one has to bear in mind that fluid resuscitation reduces hemoglobin levels, which may result in no change or decrease in $ScvO_2$ therefore, the above may only hold true for hypovolemic patients with low $ScvO_2$.

In our experiment the change in VO_2/DO_2, which increased significantly from T_1 was accompanied by a fall in $ScvO_2$, which is in accord with previous findings. The change in VO_2/DO_2 was also accompanied by an increase in CO_2-gap from T_2. There is some evidence that CO_2-gap increases in certain low flow states.[25,27] The pathophysiology of increased CO_2-gap may be due to the CO_2 stagnation phenomenon. When cardiac output decreases, blood flow is slow and the washout is impaired, therefore more CO_2 is accumulated in the tissues and as CO_2 diffuses easily and quickly the CO_2-gap increases.[41]

$ScvO_2$ showed very good sensitivity and specificity with a threshold of 73% for determining VO_2/DO_2>30%, which was further improved when CO_2-gap>6.5 mmHg was added, leading to less false negative and false positive results. The $ScvO_2$ and CO_2-gap showed a significant and strong negative correlation. It is also important to note that lactate showed a significant but substantially weaker correlation to VO_2/DO_2 as compared to $ScvO_2$ or CO_2-gap. This highlights the limitation of lactate levels as therapeutic endpoint for resuscitation. This finding is in accordance with previously published data showing the limitation of lactate levels as therapeutic endpoint for resuscitation.[56]

In cases when due to microcirculatory and/or mitochondrial defects oxygen uptake is insufficient, $ScvO_2$ may be elevated (i.e.: false negative). Previous studies have suggested that under such circumstances the increased value of CO_2-gap (>5mmHg), may help the clinician in detecting inadequate DO_2 to tissues.[25,26-27] Our results lend further support to this theory. Furthermore, adding the CO_2-gap to $ScvO_2$ for identifying VO_2/DO_2>30%, there was an improvement in specificity, positive predictive and negative predictive values.

We are not aware of any studies that have tailored hemodynamic support based on or supported by changes in CO_2-gap, therefore its clinical relevance remains unclear. However, our data clearly shows, and to our knowledge this is the first experiment to show that an altered VO_2/DO_2 caused by hypovolemia is reflected by an increase in CO_2-gap. Therefore its value may be an important alarm signal for the clinician and would help decision making at the bedside, especially when considering a fluid challenge or where commencing advanced hemodynamic monitoring are concerned.

Microcirculation.

In any shock like states the microcirculation plays a vital role, as the most devastating effects of oxygen debt occur here in the cells.[16,55] It has been demonstrated that microcirculatory disturbances can occur not only in cases of severe hypovolemic shock, but also in cases of a moderate hypovolemia without severe hypotension in human patients.[57] Recently, Bartels et al. evaluated the alteration of the sublingual microcirculation in response to controlled, central hypovolemia using sidestream dark field imaging in human subjects with intact autoregulation. They confirmed that despite adequate compensation of hypovolemia it can still be associated with decreased microcirculatory response, consequently with decreased oxygen delivery to the tissues.[58]

In a prospective observational study in patients with septic shock it was found that the capillary perfusion rate was different in survivors (in whom it was increased) as compared to non-survivors. Moreover, it was the only factor to differentiate survivors and patients dying of multiple system organ failure after the shock had resolved.[16] In accordance with these results we saw a gradual and significant decrease in both capillary perfusion rate and red blood cell velocity over time. There was also a very good area under curve when defining VO_2/DO_2>30%, and good correlation with $ScvO_2$ and CO_2-gap. All these changes were observed only in the hypovolemic, but not in the sham group.

According to the microcirculatory parameters measured in this experiment, the inflicted hypovolemia resulted in significant changes to the microcirculation. Tonometry showed a significant increase in Ba-PCO_2 due to hypovolemia, indicating decreased blood flow in the intestines. This is in accordance with previously published reports.[59] In contrast, there was no significant change in the Ga-PCO_2. Regarding the importance and the value of gastric mucosal pH is controversial and its routine use has declined in intensive care over the last decades.[60-62] The difference between Ga-PCO_2 and Ba-PCO_2 is an interesting observation and also difficult to explain. However monitoring both has already been suggested, in order to give a small additional value in the diagnosis of possible mismatch in splanchnic perfusion.[63,64] There was also a significant correlation between Ba-PCO_2 both with $ScvO_2$ and CO_2-gap.

Little is known about BigET, but there is some evidence that ET-1 reflects tissue hypoxia.[49,65] However, in contrast to the insignificant change in BigET found in healthy volunteers suffering from acute hypoxia, our results showed a significant difference of BigET levels between the hypovolemic and the sham groups, which indicates that there is an effect of hypovolemia induced tissue hypoxia on BigET levels.[66]

Limitations of the study.

One of the possible limitations of our study is the long preparation period which might have resulted in the slightly elevated lactate levels, although this was observed in both groups. Its clinical impact may be limited as a decreased $ScvO_2$ and elevated CO_2-gap may be influenced by several factors other than hypovolemia, including heart failure, severe sepsis/septic shock, multiple trauma etc., thus these results can only be applied when these conditions are unlikely to be present, for example in postoperative critical care. Furthermore, these data were obtained in anesthetized animals, and may not be the same in conscious human subjects. Finally, as there are no gold standards for hypovolemic animal experiments, one cannot exclude that the choice of furosemide-caused hypovolemia may not be the most appropriate model. The disadvantage of this model is that it does not replicate real life clinical diseases. Traumatic hypovolemia and hypovolemia associated with sepsis are associated with profound microcirculatory changes which become superimposed on the changes following hypovolemia. This is particularly important in patients with sepsis, where $ScvO_2$ is known to be a poor marker of tissue oxygenation. Indeed, in patients with sepsis, a high rather than a low $ScvO_2$ is predictive of mortality.

Conclusions

- In nearly 50% of patients *transfusion* was not followed by an increase in central venous oxygen saturation, which implies *unnecessary* transfusions and highlights the need for additional physiological transfusion triggers.

- Central venous oxygen saturation reflects changes of oxygen extraction in isovolaemic anemia. Furthermore, compared to hemoglobin concentration alone, changes in *central venous oxygen saturation* better *identify* the point when compensatory mechanisms fail and oxygen delivery begins to decline.

- Central venous-to-arterial CO_2 difference may increase due to altered oxygen extraction caused by anemia, and may support the change in central venous oxygen saturation.

- In addition to central venous oxygen saturation, central venous-to-arterial CO_2 difference may also be used as a simple and valuable indicator of hypovolemia.

In diagnosing inadequate oxygen delivery due to anemia related decreased arterial oxygen content or hypovolemia related low cardiac output the values of central venous oxygen saturation and central venous-to-arterial carbon dioxide difference may be favorable.

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References

1. Guyton AC, Hall JE. Textbook of Phisiol. *Elsevier* 2006; Unit VII, *pp*.504-533.
2. Vallet B, Tavernier B, Lund N. Assessment of tissue oxygenation in the critically ill. *Eur J Anaesthesiol* 2000; 17: 221–29.
3. Reinhardt K, Bloos F. The value of venous oximetry. *Curr Opin Crit Care* 2005; 11: 259–63.
4. Shoemaker WC, Appel PL, Kram HB. Role of oxygen debt in the development of organ failure sepsis, and death in high-risk surgical patients. *Chest* 1992; 102:208-215.
5. Gattinoni L, Chiumello D. Anemia in the intensive care unit: how big is the problem? *Transfus Altern Transfus Med* 2002; 4: 118–20.
6. Corwin HL, Parsonnet KC, Gettinger A. RBC transfusion in the ICU. Is there a reason. *Chest* 1995; 108: 767–71.
7. Vincent JL, Baron JF, Reinhart K et al. ABC Investigators. Anemia and blood transfusion in critically ill patients. *JAMA* 2002; 288: 1499–507.
8. Blood Observational Study Investigators of ANZICS-Clinical Trials Group, Westbrook A, Pettilä V, Nichol A et al. Transfusion practice and guidelines in Australian and New Zealand intensive care units. *Intensive Care Med* 2010; 36: 1138–46.
9. Vallet B, Robin E, Lebuffe G. Venous oxygen saturation as a physiologic transfusion trigger. *Crit Care* 2010; 14: 213.
10. Retter A, Wyncoll D, Pearse R et al. British Committee for Standards in Haematology. Guidelines on the management of anemia and red cell transfusion in adult critically ill patients. *Br J Haematol* 2013; 160(4): 445-64.

11. Vincent JL, Piagnerelli M. Transfusion in the intensive care unit. *Crit Care Med* 2006; 34: S96–S101.
12. Hébert PC, Wells G, Blajchman MA et al. A multi-center, randomized, controlled clinical trial of transfusion requirements in critical care. *N Engl J Med* 1999; 340: 409–17.
13. Galvin I, Ferguson ND. Acute lung injury in the ICU: focus on prevention. In: Vincent JL ed. Annual update in intensive care and emergency medicine 2011. Berlin: *Springer Science+Business Media LLC*, 2011: 117–28.
14. Maddirala S, Khan A. Optimizing hemodynamic support in septic shock using central and mixed venous oxygen saturation. *Crit Care Clin* 2010; 26: 323–33.
15. Perner A. Diagnosing Hypovolemia in the critically ill. *Crit Care Med* 2009; 37(9):2674-2675.
16. Sakr Y, Dubois MJ, De Backer D et al. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004; 32(9):1825-1831.
17. Dubin A, Pozo MO, Casabella CA et al. Increasing arterial blood pressure with norepinephrine does not improve microcirculatory blood flow: a prospective study. *Cril Care* 2009; 13(3): R92.
18. Donati A, Domizi R, Damiani E et al. From macrohemodynamic to the microcirculation. *Crit Care Res Pract* 2013; 2013:892710.
19. Rivers E, Nguyen B, Havstad S et al. Early Goal-Directed Therapy Collaborative Group. Early Goal-Directed Therapy in the Treatment of Severe Sepsis and Septic Shock. *N Engl J Med* 2001; 345:1368-1377.

20. Sakr Y, Vincent JL, Reinhart K et al. on behalf of the Sepsis Occurrence in Acutely Ill Patients (SOAP) investigators. High Tidal Volume and Positive Fluid Balance are Associated with Worse Outcome in Acute Lung Injury. *Chest* 2005; 128:3098-3108.
21. Giraud R, Siegenthaler N, Gayet-Ageron A et al. $ScvO_2$ As a Marker to Define Fluid Responsiveness. *J Trauma* 2005; 70(4):802-807.
22. Krantz T, Warberg J, Secher NH. Venous Oxygen Saturation During Normovolaemic Haemodilution in the Pig. *Acta Anesthesiol Scand* 2005; 49:1149-1156.
23. Collaborative Study Group on Perioperative $ScvO_2$ Monitoring. Multicentre Study on Peri- and Postoperative Central Venous Oxygen Saturation in High-Risk Surgical Patients. *Crit Care* 2006; 10:R158.
24. Vallet B, Futier E. Perioperative oxygen therapy and oxygen utilization. *Curr Opin Crit Care* 2010; 16: 359–64
25. Vallée F, Vallet B, Mathe O et al. Central Venous-to-arterial Carbon Dioxide Difference: an Additional Target for Goal-directed Therapy in Septic Shock? *Intensive Care Med* 2008; 34:2218-2225.
26. Lamia B, Monnet X, Teboul JL. Meaning of arterio-venous PCO_2 difference in circulatory shock. *Minerva Anestesiol* 2006; 72:597–604.
27. Futier E, Robin E, Jabaudon M et al. Central Venous O_2 Saturation and Venous-to-arterial CO_2 difference as Complementary Tools for Goal-directed Therapy During High-risk Surgery. *Crit Care* 2010; 14:R193.
28. Vallet B, Lebuffe G. How to Titrate Vasopressors against Fluid Loading in Septic Shock. *Adv Sepsis* 2007; 6:34-40.

29. Phillips CP, Vinecore K, Hagg DS et al. Resuscitation of haemorrhagic shock with normal saline vs. lactated Ringer's: effects on oxygenation, extravascular lung water and haemodynamics. *Crit Care* 2009; 13: R30
30. Saugel B, Umgelter A, Schuster T et al. Transpulmonary thermodilution using femoral indicator injection: a prospective trial in patients with a femoral and a jugular central venous catheter. *Crit Care* 2010; 14: R95
31. Pearse R, Dawson D, Fawcett J et al. Changes in central venous saturation after major surgery, and association with outcome. *Crit Care* 2005; 9:R694-699.
32. Teixeira C, da Silva NB, Savi A et al. Central venous saturation is a predictor of reintubation in difficult-to-wean patients. *Crit Care Med* 2010; 38: 491-496.
33. Scalea TM, Holman M, Fuortes M et al. Central venous – blood oxygen saturation – an early, accurate measurement of volume during hemorrhage. *J Trauma* 1988; 28: 725–32.
34. Scalea TM, Hartnett RW, Duncan AO et al. Central venous oxygen-saturation: a useful clinical tool in trauma patients. *J Trauma* 1990; 30: 1539–43
35. Weiskopf RB, Viele MK, Feiner J et al. Human cardiovascular and metabolic response to acute, severe isovolemic anemia. *JAMA* 1998; 279: 217–21.
36. Adamczyk S, Robin E, Barreau O et al. Contribution of central venous oxygen saturation in postoperative blood transfusion decision. *Ann Fr Anesth Reanim* 2009; 28: 522–30.
37. Krantz T, Warberg J, Secher NH. Venous oxygen saturation during normovolemic haemodilution in the pig. *Acta Anaesthesiol Scand* 2005; 49: 1149–56.
38. Nagy S, Nagy A, Adamicza A et al. Histamine level changes in the plasma and tissues in hem-orrhagic shock. *Circ Shock* 1986; 18: 227–39.

39. Adamicza A, Tarnoky K, Nagy A et al. The effect of anaesthesia on the haemodynamic and sympathoadrenal responses of the dog in experimental haemorrhagic shock. *Acta Physiol Hung* 1985; 65: 239–54.
40. Meletti JFA, Módolo NSP. Hemorrhagic shock hemody-namic and metabolic behavior: experimental study in dogs. *Rev Bras Anestesiol* 2003; 53: 623–32.
41. Vallet B, Teboul JL, Cain S et al. Venoarterial CO_2 Difference During Regional Ischemic or Hypoxic Hypoxia. *J Appl Physiol* 2000; 89:1317-1321.
42. West JB. Gas transport to the periphery. In: West JB. Baltimore MD, editor, Respiratory Physiology: The Essentials (4th ed.), Williams and Wilkins, 1990; pp. 69–85.
43. Marino PL. Systemic Oxygenation. In: Marino PL, editor, The ICU Book. (4th ed.) Wolters Kluwer Health/Lippincot Williams and Wilkins, 2014; pp. 171-192.
44. Jalloh I, Helmy A, Shannon RJ et al. Lactate uptake by the injured human brain: evidence from an arteriovenous gradient and cerebral microdialysis study. *J Neurotrauma* 2013; 30(24): 2031-2037.
45. Gallagher CN, Carpenter KL, Grice P et al. The human brain utilizes lactate via the tricarboxylic acid cycle: a 13C-labelled microdialysis and high-resolution nuclear magnetic resonance study. *Brain* 2009; 132(Pt10): 2839-2849.
46. Hare GM, Mazer CD, Hutchison JS et al. Severe hemodilutional anemia increases cerebral tissue injury following acute neurotrauma. *J Appl Physiol* 2007; 103: 1021-9.
47. Groner W, Winkelman JW, Harris AG et al. Orthogonal Polarization Spectral Imaging: A New Method for Study of the Microcirculation. *Nat Med* 1999; 5(10): 1209-1212.

48. Boda D, Kaszaki J, Tálosi G. A New Simple Tool for Tonometric Determination of the PCO_2 in the Gastrointestinal Tract: *in vitro* and *in vivo* Validation Studies. *Eur J Anaesthesiol* 2006; 23: 680-685.
49. Kourembanas S, Marsden PA, McQuillan LP et al. Hypoxia induces endothelin gene expression and secretion in cultured human endothelium. *J Clin Invest* 1991; 88:1054-1057.
50. Korzonek-Szlacheta I, Gwóźdź B. Effects of endothelin-1 on prevention of microvascular endothelium injuries in hemorrhagic shock in rats. *Pharmacological Reports* 2007; 59(1): 98–106.
51. Barros JM, do Nascimento P Jr, Marinello JL et al. The effects of 6% hydroxyethyl starch-hypertonic salin in resuscitation of dogs with hemorrhagic shock. *Anesth Analg* 2011; 112(2): 395-404.
52. Vallet B. Intravascular volume expansion: which surrogate markers could help the clinician to assess improved tissue perfusion? *Anesth Analg* 2011; 112(2):258-259.
53. Michard F, Teboul JL. Predicting Fluid Responsiveness in ICU Patients: A Critical Analysis of the Evidence. *Chest* 2002; 121:2000-2008.
54. Monnet X, Osman D, Ridel C et al. Predicting Volume Responsiveness by Using the End-expiratory Occlusion in Mechanically Ventilated Intensive Care Unit Patients. *Crit Care Med* 2009; 37(3):951-956.
55. Liakopoulos OJ, HO JK, Yezbik A et al. An Experimental and Clinical Evaluation of a Novel Central Venous Catheter with Integrated Oximetry for Pediatric Patients Undergoing Cardiac Surgery. *Anesth Analg* 2007; 105:1598-1604.
56. Jansen TC, Van Bommel J, Bakker J. Blood lactate monitoring in critically ill patients: a systematic health technology assessment. *Crit Care Med* 2009; 37(10): 2827–2839.

57. Ward KR, Tiba MH, Ryan KL et al. Oxygen Transport Characterisation of a Human Model of Progressive Hemorrhage. *Resuscitation* 2010; 81:987-993.

58. Bartels SA, Bezemer R, Milstein DMJ et al. The Microcirculatory Response to Compensated Hypovolemia in a Lower Body Negative Pressure Model. *Microvasc Res* 2011; 82(3):374-380.

59. Walley KR, Friesen BP, Humer MF et al. Small Bowel Tonometry is More Accurate than Gastric Tonometry in Detecting Gut Ischemia. *J Appl Physiol* 1998; 85:1770-1777.

60. Creteur J, DeBacker D, Vincent JL. Does gastric tonometry monitor splanchnic perfusion? *Crit Care Med* 1999; 27(11): 2480–2484.

61. Palizas F, Dubin A, Regueira T et al. Gastric tonometry versus cardiac index as resuscitation goals in septic shock: a multicenter, randomized, controlled trial. *Crit Care* 2009; 3(2): R44.

62. Steiner LA, Staender S, Sieber CC et al. Effects of simulated hypovolemia on haemodynamics, left ventricular function, mesenteric blood flow and gastric PCO_2. *Acta Anaesthesiol Scand* 2007; 51(2): 143–150.

63. Otte JA, Huisman AB, Geelkerken RH et al. Jejunal tonometry for the diagnosis of gastrointestinal ischemia. Feasibility, normal values and comparison of jejunal with gastric tonometry exercise testing. *Eur J Gastroenterol Hepatol* 2008; 20(1): 62–67

64. Thorén A , Jakob SM, Pradl R et al. Jejunal and gastric mucosal perfusion versus splanchnic blood flow and metabolism: an observational study on postcardiac surgical patients, *Crit Care Med* 2000; 28(11):3649–3654.

65. Lal H, Yu Q, Williams KI, Woodward B. Hypoxia Augments Conversion of Big-endothelin-1 and Endothelin ET_B Receptor-mediated Actions in Rat Lungs. *Eur J Pharmacol* 2000; 402:101-110.

66. Lenz T, Nadansky M, Gossmann J et al. Exhaustive Exercise-induced Tissue Hypoxia Does Not Change Endothelin and Big Endothelin Plasma Levels in Normal Volunteers. *AJH* 1998; 11:1028-1031.

Appendix

I.

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Central venous oxygen saturation is a good indicator of altered oxygen balance in isovolemic anemia

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Background: Red blood cell transfusion is done primarily as a means to improve oxygen delivery (DO_2). Current transfusion guidelines are based solely on hemoglobin levels, regardless of actual DO_2 need. As central venous oxygen saturation ($ScvO_2$) may reflect imbalances in DO_2 and consumption (VO_2) the aim of this study was to investigate the value of $ScvO_2$ as an indicator of oxygen balance in isovolemic anemia.
Methods: After splenectomy, anesthetized Vietnamese mini pigs ($n = 13$, weight range: 18–30 kg) underwent controlled bleeding in five stages (T_0–T_5). During each stage approximately 10% of the estimated starting total blood volume was removed and immediately replaced with an equal volume of colloid. Hemodynamic measurements and blood gas analysis were then performed.
Results: Each stage of bleeding resulted in a significant fall in hemoglobin, T_0: 125 (113–134) to T_5: 49 (43–55) g/l [T_0: 7.7 (6.9–8.2) to T_5: 3.0 (2.6–3.4) mmol/l]. The O_2-extraction (VO_2/DO_2) increased significantly only from T_3: 35 (21–40) %, $P < 0.05$. The change of $ScvO_2$ showed a similar pattern and dropped below the physiological threshold of 70% at T_4: 68 (61–76) %. At this point, hemoglobin was below the recommended transfusion trigger value, 59 (53–67) g/l [3.6 (3.3–4.1) mmol/l]. There was a strong significant association between $ScvO_2$ (< 70%) and VO_2/DO_2 (> 30%): $r = -0.71$, $r^2 = 0.50$, $P < 0.001$.
Conclusion: The results of this study show that $ScvO_2$ reflects changes of VO_2/DO_2 in isovolemic anemia better than Hb alone, therefore it may be used as an additional indicator of blood transfusion in clinical practice.

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The adequacy of tissue oxygenation is determined by the balance between oxygen delivery (DO_2) and consumption (VO_2).[1] In the critically ill there is often an imbalance between the two arms: DO_2 may be too low, frequently to the accompaniment of an increased VO_2. After a critical threshold, severe oxygen debt and shock may occur.[2] One of the most common causes of inadequate DO_2 in the intensive care unit is anemia requiring red blood cell transfusions.[3] Although the prevalence of anemia among critically ill patients could be as high as 95% by day 3, the transfusion trigger, i.e. the indication and timing of the necessity of blood transfusion, is still uncertain, and the detection of altered oxygen extraction caused by anemia is therefore of great clinical importance.[4,5]

A number of guidelines are of help in transfusion practice, but the criteria for the optimal management of anemia are not clearly defined. In most guidelines the transfusion trigger is a certain level of hemoglobin (Hb), usually 70–100 g/l (4.3–6.1 mmol/l).[6,7] It was recently suggested that Hb level should not be the only factor on which the indication of the need for transfusion is based.[6,7] There is increasing evidence that transfusion is a double-edged sword: untreated anemia can be associated with a worse outcome and increased mortality, whereas transfusion may cause various infectious and non-infectious adverse effects.[8–10] As macrohemodynamic changes are not informative enough, there is a clear need for additional quantitative parameters that would give information on anemia-related altered oxygen extraction and hence the need for blood administration.[7,11] One of the potentially useful physiological parameters is the central venous oxygen saturation ($ScvO_2$). The $ScvO_2$ has been found to be slightly higher than the mixed venous oxygen saturation (SvO_2), with normal values 73–82%.[12] Importantly, the $ScvO_2$ closely trends the changes in SvO_2, and is considered a

reasonable surrogate marker of mixed venous saturation in the clinical setting.[12] As such $ScvO_2$, which is easily measured at the bedside, has been found reflect the balance between DO_2 and VO_2 in many clinical settings.[11,12]

DO_2 is dependent on in part of the concentration of Hb. The value of using changes in $ScvO_2$ to detect anemia-related changes in oxygen balance in clinical practice is still unclear. The aim of our study therefore was to monitor the changes of $ScvO_2$ in isovolemic anemia using a large animal model, and test whether an altered VO_2/DO_2 balance, which is due solely to a decreased Hb level, can be detected by $ScvO_2$.

Materials and methods

The study protocol was approved by the local ethics committee at the University of Szeged, and the study was carried out in the research laboratory of the Institute of Surgical Research.

Animals and instrumentation

Vietnamese mini pigs ($n = 13$) weighing 24 ± 3 kg underwent a 24-h fast preoperatively but with water ad libitum. Anesthesia was induced with an intramuscular injection of a mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) and maintained with a continuous infusion of propofol (6 mg/kg/h i.v.). A tracheal tube was inserted and the animals' lungs were ventilated mechanically. The tidal volume was set at 13 ± 2 ml/kg, and the respiratory rate was adjusted to maintain the end-tidal carbon dioxide and the partial pressure of arterial carbon dioxide in the range of 35–45 mmHg (4.6–5.9 kPa) and the arterial pH between 7.35 and 7.45. The adequacy of the depth of anesthesia was assessed by monitoring the jaw tone. After the initiation of anesthesia, the right carotid artery and jugular vein and the right femoral artery and vein were dissected and catheterized. The animals underwent suprapubic urinary catheter placement and laparotomy for splenectomy. Splenectomy in swine hemorrhage models are performed because of the distensibility of the spleen and the resultant variation in the amounts of sequestered blood.[13] The core temperature was maintained at 37 ± 1 °C through use of an external warming device.

For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter (PiCCO, PULSION Medical Systems AG, Munich, Germany) was placed in the femoral artery and a pulmonary artery catheter (PV2057 VoLEF Catheter, PULSION Medical Systems AG) by pressure tracings via the femoral vein. The latter was also used to draw mixed venous blood gas samples. The femoral artery served as the site of arterial blood gas samples and the central venous line was used for central venous blood gas sampling and for the injection of cold saline boluses for thermodilution measurements. Central venous catheter was positioned by using guidewire attached intracavital ECG. During the experiment blood was drained from the catheter in the right carotid artery, which was also used to replace the blood loss with the same amount of colloid, in order to avoid a sudden increase in right ventricular preload.

Experimental protocol

The most important steps in the experiment are outlined in Fig. 1. At baseline (T_0) hemodynamic and blood gas parameters were recorded, and heparin sulfate (200 IU/kg) was administered through the central venous line. Isovolemic anemia was achieved in five intervals (T_1–T_5). During each interval 10% of the estimated total blood volume was withdrawn over a 5- to 10-min period. Hemodynamic parameters were recorded and the amount of blood drained off was immediately replaced by an equal volume of colloid (hydroxyethyl starch 130 kDa/0.4, 6%, Voluven, Fresenius, Germany). To achieve a steady state, the animals were allowed to rest for 10 min between intervals. At the end of each cycle, hemodynamic and blood gas parameters were measured. At the end of the experiment the animals were humanely euthanized.

Hemodynamic measurements

Cardiac output (CO), global end-diastolic volume, intrathoracic blood volume, extravascular lung water, stroke volume (SV), SV variation (SVV), index of left ventricular contractility (dPmx), heart rate (HR), and mean arterial pressure (MAP) were measured by transpulmonary thermodilution and pulse contour analysis at baseline and at the end of each interval. Detailed description of transpulmonary thermodilution and pulse contour analysis are provided elsewhere.[13,14] All hemodynamic parameters were indexed for body surface area. The averages of three random measurements following 10 ml bolus injections of ice-cold 0.9% saline were recorded. Continuous variables of invasive blood pressure measurements and pulse contour analysis, such as CO, MAP, HR, SV, SVV, and dPmx, were measured and recorded at the end of each bleeding episode and at the same times as the other hemodynamic

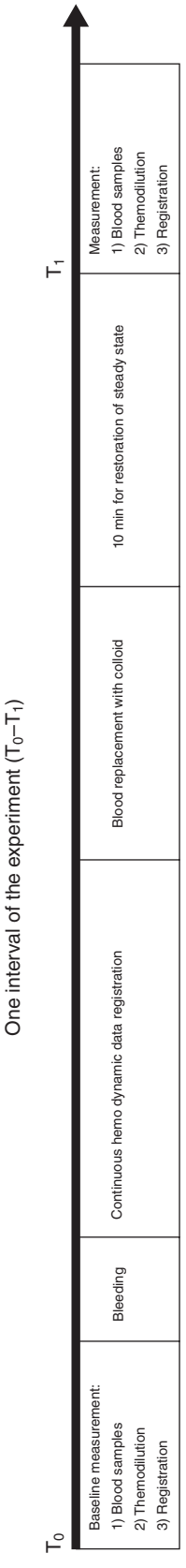

Fig. 1. Schematic diagram illustrating one interval of the experimental protocol. After recording the results of the baseline measurements, 10% of the estimated total blood volume was withdrawn from the animals. Immediately after recording the continuous hemodynamic variables, the withdrawn blood was replaced with the same amount of colloid. After 10 min for restoration of steady state, another set of measurements were recorded.

variables. The central venous pressure (CVP) was determined by the pulmonary artery catheter at the end of each bleeding episode and at the same times as the other hemodynamic variables.

Arterial, central venous, and mixed venous blood gas samples (Cobas b 221, Roche Ltd., Basel, Switzerland) were drawn and analyzed by cooximetry simultaneously at baseline and at the end of each cycle (Fig. 1).

From these parameters the following variables were calculated according to standard formulas:

$$DO_2 = SV * HR * [Hb * 1.34 * SaO_2 + (0.003 * PaO_2)] = CO * [CaO_2]$$

$$VO_2 = CO * [CaO_2 - (Hb * 1.34 * SvO_2 + (0.003 * PvO_2))] = CO * [CaO_2 - CvO_2]$$

$$\text{Oxygen extraction } (VO_2/DO_2) = CO * [CaO_2 - CvO_2] / CO * [CaO_2] * 100$$

$$\text{Simplified oxygen extraction } (O_2ER) = (SaO_2 - ScvO_2)/SaO_2$$

Data analysis and statistics

Data are reported as medians (interquartile ranges) or means ± standard deviations, unless indicated otherwise. To test for normal distribution, the Kolmogorov-Smirnov test was used. The changes in all parameters throughout the experiment were tested by repeated measures analysis of variance (ANOVA); and the number of degrees of freedom was adjusted to Greenhouse–Geisser epsilon when needed. For pairwise comparisons, Pearson's correlation was used. To evaluate the performance of $ScvO_2$ in the detection of altered oxygen extraction with a threshold of 30% of VO_2/DO_2, receiver operating characteristics (ROC) curve analysis was performed, and sensitivity, specificity, and positive predictive (PPV) and negative predictive values (NPV) were also determined. To model the linear relationship between VO_2/DO_2 and the possible indicator of altered oxygen extraction, linear regression model was used. Post hoc calculation showed a power of 86% with an effect of 25% increase in VO_2/DO_2, for a sample size of 13 and $\alpha = 0.05$. For statistical analysis SPSS version 18.0 for Windows (SPSS, Chicago, IL, USA) was used and $P < 0.05$ was considered statistically significant.

Table 1

Hemodynamic effects of isovolemic anemia.

	T_0	T_1	T_2	T_3	T_4	T_5
Hb (g/l)	125 (113–134)	102 (90–109)*†	79 (73–93)*†	68 (60–76)*†	59 (53–67)*†	49 (43–55)*†
(mmol/l)	7.7 (6.9–8.2)	6.3 (5.5–6.7)	4.8 (4.5–5.7)	4.2 (3.7–4.7)	3.6 (3.3–4.1)	3.0 (2.6–3.4)
HR (beats/min)	125 (91–135)	119 (100–138)*	123 (102–146)*	129 (110–159)*	139 (118–179)*	147 (131–177)*
MAP (mm Hg)	91 (79–105)	89 (79–101)	83 (75–98)*	82 (68–90)*	72 (59–85)*	72 (63–86)*
CVP (mm Hg)	6 (5–8)	8 (5–9)	7 (4–9)	7 (5–9)	7 (5–9)	7 (3–10)
CI (L/min/m^2)	2.6 (2.3–2.8)	3.3 (2.7–3.6)*†	3.6 (2.9–3.8)*†	3.6 (3.3–4.1)*	3.5 (3.2–4.0)*	3.9 (3.6–4.1)*
GEDI (ml/m^2)	270 (243–284)	271 (245–320)	276 (248–298)	274 (236–305)	268 (227–302)	261 (232–298)
ITBI (ml/m^2)	335 (307–352)	335 (305–400)	343 (303–373)	342 (295–383)	334 (282–375)	333 (285–375)
ELWI (ml/kg)	9 (9–10)	10 (10–10)	9 (9–10)	10 (9–10)	10 (9–10)	10 (9–11)
SVI (ml/m^2)	21 (18–29)	26 (23–31)	27 (24–31)	28 (25–31)	25 (21–33)	28 (22–31)
SVV (%)	17 (14–21)	15 (12–21)	19 (9–21)	15 (11–20)	19 (11–25)	14 (11–27)
dPmx (mm Hg/s)	540 (485–790)	700 (540–985)*	800 (570–1075)*	810 (540–1480)*	880 (560–1360)*	975 (562–1275)*

GLM repeated measures ANOVA.
*$P < 0.05$ compared with T_0.
†$P < 0.05$ compared with previous.
Hb, hemoglobin; HR, heart rate; MAP, mean arterial pressure; CVP, central venous pressure; CI, cardiac index; GEDI, global end-diastolic volume index; ITBI, intrathoracic blood volume index; ELWI, extravascular lung water index; SVI, stroke volume index; SVV, stroke volume variation; dPmx, index of left ventricular contractility; T_0, baseline measurement; T_1–T_5, five intervals of bleeding.

Results

Hemodynamic effects of isovolemic anemia

All 13 animals survived the study. The blood loss was on average 150 ± 33 ml in each phase. Hemodynamic data are presented in Table 1.

The bleeding caused a gradual decrease in Hb level after each phase and by the end of the experiment it had fallen by 61% of the baseline value. The preload as indicated by global end-diastolic volume index (GEDI), intrathoracic blood volume index (ITBI), and CVP values did not change significantly. HR, dPmx, and CI were increased significantly after the first bleeding and remained so for the rest of the experiment. Only CI changed significantly until T_3 when comparing the values at each interval with the previous one. MAP decreased significantly from T_2, but the median remained > 70 mmHg throughout. Other macrohemodynamic variables did not change significantly during the experiment.

Effects on oxygen balance

Variables relating to the oxygen balance are listed in Table 2.

SaO_2 remained in the normal range throughout the experiment. DO_2 fell significantly from T_2, VO_2 at T_4, VO_2/DO_2 increased significantly from T_3, and exceeded the physiologic threshold of 30%. The change in $ScvO_2$ displayed a similar pattern as VO_2/DO_2 and changed significantly and also fell below 70% only at T_4. The other parameters did not change significantly. The pattern of changes of VO_2/DO_2 and $ScvO_2$ over time is demonstrated in Fig. 2. Only arterial oxygen partial pressure increased significantly by the end of the experiment, other blood gas parameters did not change significantly.

We determined the association between VO_2/DO_2 and $ScvO_2$, and found a strong, negative correlation ($r = -0.71$, $P < 0.001$) (Fig. 3).

ROC analysis revealed the same tendency as the correlation. With 30% taken as the physiologic threshold for VO_2/DO_2, the area under the curve (AUC), its standard error and that of the 95% confidence interval were > 0.5 for $ScvO_2$ [AUC = 0.768 ± 0.056 (0.657–0.878) $P < 0.001$]. It was also important to determine the best cut-off for $ScvO_2$ to detect the significant increase in VO_2/DO_2, therefore, sensitivity, specificity, PPV, and NPV for $ScvO_2$ levels of 70% and 75% were calculated. An $ScvO_2$ level of 70% had better specificity and PPV, whereas a $ScvO_2$ level of 75% had better sensitivity and NPV (sensitivity: 45%, specificity: 97%, PPV: 95, NPV: 58; sensitivity: 68%, specificity: 77%, PPV: 79, NPV: 65, respectively). Furthermore, linear regression revealed a significant relationship between $ScvO_2$ ($r = 0.71$, $r^2 = 0.50$, $P < 0.001$) and VO_2/DO_2.

Discussion

Maintaining adequate tissue oxygenation by improving DO_2 is the rational for blood transfusion. Despite this fact, treatment of anemia with blood transfusion in the absence of acute bleeding is recommended at certain levels of Hb, regardless of actual DO_2 and need, thus resulting in possible

Table 2

Effects of isovolemic anemia on oxygen balance.

	T_0	T_1	T_2	T_3	T_4	T_5
SaO_2 (%)	95 (92–97)	96 (94–97)	96 (95–97)	96 (95–97)	97 (97–97)	97 (97–97)
DO_2 (ml/min/m2)	431 (362–474)	438 (323–524)	378 (302–412)*†	344 (252–376)*	284 (236–333)*	247 (216–292)*†
VO_2 (ml/min/m2)	119 (82–139)	130 (77–151)	93 (66–136)	113 (67–141)	98 (72–120)	105 (70–120)
VO_2/DO_2 (%)	29 (18–33)	29 (17–33)	29 (18–32)	35 (21–40)*	37 (26–43)*	41 (27–47)*
ERO_2 (%)	19 (13–26)	19 (14–24)	20 (14–22)	21 (16–28)	30 (22–37)*	32 (21–39)*
SvO_2 (%)	68 (64–77)	67 (64–77)	68 (63–79)	64 (58–76)	62 (55–72)*	58 (52–72)*
$ScvO_2$ (%)	76 (69–83)	73 (72 (82)	77 (75–83)	77 (68–81)	68 (61–76)*	66 (60–76)*
Lactate (mmol/l)	4.5 (3.2–5.3)	4.2 (3.0–5.1)	5.0 (3.2–6.0)	4.1 (2.9–6.0)	4.2 (2.9–6.5)	4.0 (3.0–6.4)
pH	7.44 (7.40–7.50)	7.43 (7.40–7.50)	7.43 (7.41–7.50)	7.43 (7.39–7.49)	7.44 (7.42–7.49)	7.44 (7.40–7.47)
PaO_2 (mm Hg)	76 (66–80)	75 (72–80)	76 (73–80)	77 (72–82)	79 (75–85)*	81 (77–90)*
(kPa)	10.1 (8.8–10.7)	10.0 (9.6–10.7)	10.1 (9.7–10.7)	10.3 (9.6–10.9)	10.5 (10.0–11.3)	10.8 (10.3–12.0)
$PaCO_2$ (mm Hg)	39 (35–44)	38 (35–43)	37 (34–45)	39 (34–46)	37 (34–42)	38 (35–41)
(kPa)	5.2 (4.7–5.9)	5.1 (4.7–5.7)	4.9 (4.5–6.0)	5.2 (4.5–6.1)	4.9 (4.5–5.6)	5.1 (4.7–5.5)
$aHCO_3$ (mmol/l)	25 (24–27)	24 (24–26)	25 (23–27)	25 (23–27)	25 (22–27)	25 (21–25)
aBE (mmol/l)	0.90 (–0.05–2.50)	0.40 (–0.85–2.25)	0.60 (–0.9–2.45)	0.80 (–0.45–3.15)	0.90 (–1.45–2.35)	0.70 (0.43–1.08)

GLM repeated measures ANOVA.
*$P < 0.05$ compared with T_0.
†$P < 0.05$ compared with previous.
SaO_2, arterial oxygen saturation; DO_2, oxygen delivery; VO_2, oxygen consumption; VO_2/DO_2, oxygen extraction ratio; ERO_2, simplified oxygen extraction ratio; SvO_2, mixed venous oxygen saturation; $ScvO_2$, central venous oxygen saturation; PaO_2, arterial oxygen partial pressure; $PaCO_2$, arterial carbon dioxide partial pressure; $aHCO_3$, arterial bicarbonate; aBE, arterial base excess; T_0, baseline measurement; T_1–T_5, five intervals of bleeding.

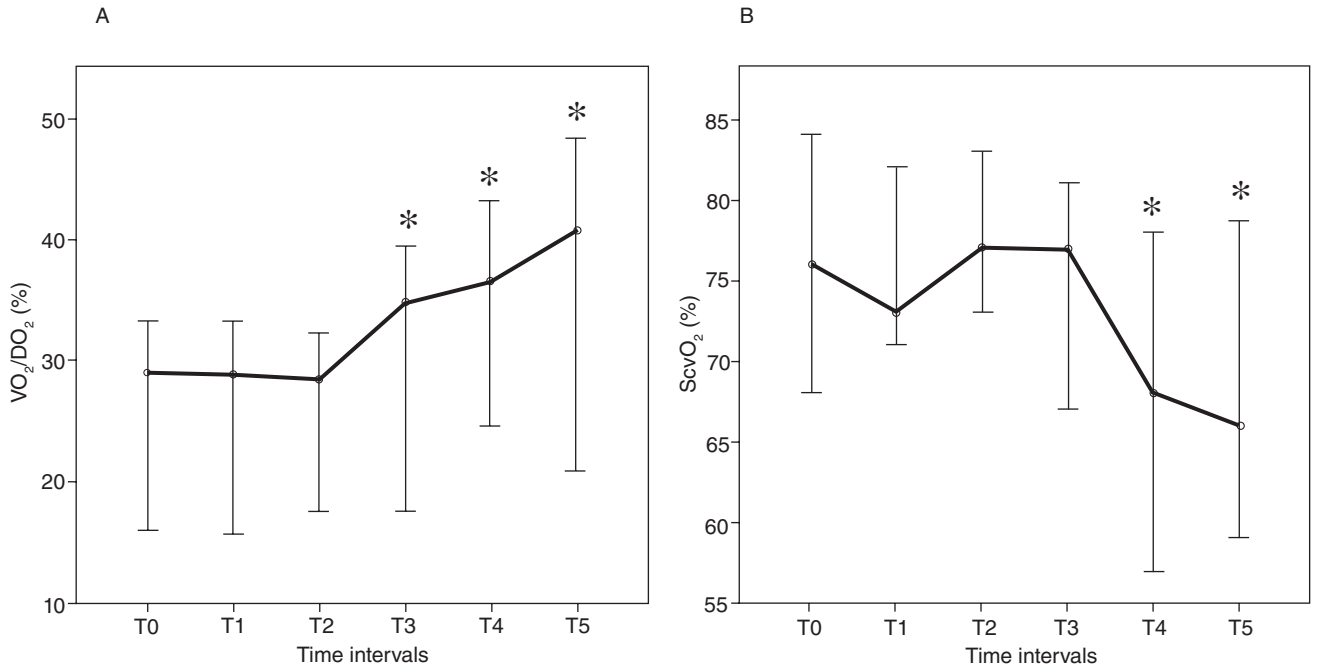

*Fig. 2. Changes of VO_2/DO_2 (A) and $ScvO_2$ (B) over time (T_0–T_5). Data are presented as median (interquartile range). *P < 0.05 compared with T_0.*

excess blood administration.[6,7] In our study in isovolemic anemia, we found that $ScvO_2$ is a sensitive indicator of oxygen balance and may thus serve as a rational guide to therapy in this all too common problem.

Oxygen balance and $ScvO_2$

In certain clinical conditions, an $ScvO_2$ value of ~70% has been used as a goal to therapeutic intervention in attempts at improving DO_2.[15–18] In one study in septic patients goal-directed therapy

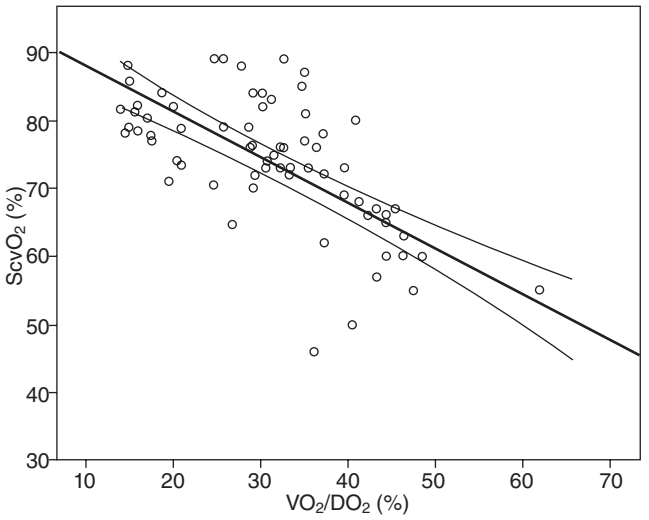

Fig. 3. Correlation between VO_2/DO_2 and $ScvO_2$. Data are presented as scatter with a linear regression line and its mean's 95% confidence interval. VO_2/DO_2: oxygen extraction, $ScvO_2$: central venous oxygen saturation.

according to $ScvO_2$ values, saw an absolute 16% reduction in in-hospital mortality as compared with conventional therapy.[15] Two recent studies have also demonstrated that a low $ScvO_2$ predicts peri- and post-operative morbidity and complications in high-risk surgery.[16,17] It may also serve as a useful tool to assist the weaning process in mechanically ventilated patients.[18]

In our experiment, despite a continuous and significant drop in Hb levels, the value of VO_2/DO_2 increased significantly only from T_3, and exceeded the physiologic threshold of 30%. The change in $ScvO_2$ displayed a similar pattern as VO_2/DO_2 and fell below 70% only at T_4. If we translate that into clinical practice, the reduced Hb concentrations could have indicated blood transfusion from T_2; however, we found no evidence of impaired VO_2/DO_2 until the Hb was well below the current recommended transfusion threshold.

About two decades ago it was found during hemorrhage in animal and human experimental models that $ScvO_2$ may be useful for the identification of patients with occult or ongoing clinically significant blood loss.[19,20] In a prospective human interventional study it was found that in acute isovolemic anemia of Hb 50 g/l (3.1 mmol/l) in conscious healthy resting humans did not produce evidence of inadequate systemic DO_2 and oxygen imbalance was accompanied by a significant drop in SvO_2.[21] These results were reinforced by a retrospective analysis of a prospective observational study in which $ScvO_2$ was found to be a good indicator of transfusion.[22] Our results give further evidence that anemia-induced change in oxygen balance can be monitored by $ScvO_2$.

Hemodynamic effects of isovolaemic anemia

Our goal of maintaining isovolemia was achieved as parameters of preload and central blood volume, such as GEDI, ITBI, CVP did not change throughout the experiment. A significant increase in cardiac index was found, caused by increased HR, as SV did not change significantly over time. This increase was in agreement with the hemodynamic changes of a recent study by Krantz et al. with a similar experimental setting, but with a different hypothesis.[23] Myocardial contractility as indicated by dPmx values, also increased significantly, most likely in response to isovolemic anemia. Although we did not measure catecholamine levels, a theoretical explanation could be that the bleeding caused an enhanced stress response resulting in the observed positive inotropic effect.[24,25]

Limitations of the study

One of the possible limitations of our study is that the length of the preparation of the animals may have been too long, which resulted in persistent tachycardia and increased levels of lactate from baseline to the end of the experiment. The steady-state periods may also have been relatively short, although, the same time intervals have been used previously.[26] Another concern might be the type of fluid replacement, as one cannot exclude the possibility that the use of different types of colloid solutions would affect these results. Furthermore, these data were obtained in anesthetized animals, and may not be the same in conscious animals. Finally, our results cannot be applied directly in those clinical conditions where other confounding factors are present, which may affect $ScvO_2$, such as severe sepsis, septic shock, multiple trauma, etc.

Conclusions

In conclusion, our results show that $ScvO_2$ reflects changes in oxygen extraction in isovolemic anemia. Furthermore in isovolemic anemia, $ScvO_2$ better identifies the point when compensatory mechanisms fail and DO_2 begins to decline, as compared with the Hb concentration alone. These findings are in accord with the idea that compensatory changes in CO and other parameters of DO_2 make Hb concentration alone a less sensitive marker of oxygen

balance and for the need for therapeutic intervention to increase DO_2. $ScvO_2$ could therefore be important in helping to avoid hypoxia and tissue injury under such circumstances, while helping to more accurately guide blood transfusions. This remains to be confirmed in the clinical setting.

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Conflicts of interest: The authors have no conflicts of interest.

References

1. Vallet B, Tavernier B, Lund N. Assessment of tissue oxygenation in the critically ill. Eur J Anaesthesiol 2000; 17: 221–29.
2. Reinhardt K, Bloos F. The value of venous oximetry. Curr Opin Crit Care 2005; 11: 259–63.
3. Gattinoni L, Chiumello D. Anemia in the intensive care unit: how big is the problem? Transfus Altern Transfus Med 2002; 4: 118–20.
4. Corwin HL, Parsonnet KC, Gettinger A. RBC transfusion in the ICU. Is there a reason. Chest 1995; 108: 767–71.
5. Vincent JL, Baron JF, Reinhart K, Gattinoni L, Thijs L, Webb A, Meier-Hellmann A, Nollet G, Peres-Bota D, ABC Investigators. Anemia and blood transfusion in critically ill patients. JAMA 2002; 288: 1499–507.
6. Blood Observational Study Investigators of ANZICS-Clinical Trials Group, Westbrook A, Pettilä V, Nichol A, Bailey MJ, Syres G, Murray L, Bellomo R, Wood E, Phillips LE, Street A, French C, Orford N, Santamaria J, Cooper DJ. Transfusion practice and guidelines in Australian and New Zealand intensive care units. Intensive Care Med 2010; 36: 1138–46.
7. Vallet B, Robin E, Lebuffe G. Venous oxygen saturation as a physiologic transfusion trigger. Crit Care 2010; 14: 213.
8. Vincent JL, Piagnerelli M. Transfusion in the intensive care unit. Crit Care Med 2006; 34: S96–S101.
9. Hébert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, Tweddale M, Schweitzer I, Yetisir E. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. N Engl J Med 1999; 340: 409–17.
10. Galvin I, Ferguson ND. Acute lung injury in the ICU: focus on prevention. In: Vincent JL ed. Annual update in intensive care and emergency medicine 2011. Berlin: Springer Science+Business Media LLC, 2011: 117–28.
11. Maddirala S, Khan A. Optimizing hemodynamic support in septic shock using central and mixed venous oxygen saturation. Crit Care Clin 2010; 26: 323–33.
12. Vallet B, Futier E. Perioperative oxygen therapy and oxygen utilization. Curr Opin Crit Care 2010; 16: 359–64.
13. Phillips CP, Vinecore K, Hagg DS, Sawai RS, Differding JA, Watters JM, Schreiber MA. Resuscitation of haemorrhagic shock with normal saline vs. lactated Ringer's: effects on oxygenation, extravascular lung water and haemodynamics. Crit Care 2009; 13: R30.
14. Saugel B, Umgelter A, Schuster T, Phillip V, Schmid RM, Huber W. Transpulmonary thermodilution using femoralindicator injection: a prospective trial in patients with a femoral and a jugular central venous catheter. Crit Care 2010; 14: R95.
15. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M, Early Goal-Directed Therapy Collaborative Group. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med 2001; 345: 1368–77.
16. Pearse R, Dawson D, Fawcett J, Rhodes A, Grounds RM, Bennett ED. Changes in central venous saturation after major surgery, and association with outcome. Crit Care 2005; 9: R694–99.
17. Collaborative Study Group on Perioperative $ScvO_2$ Monitoring. Multicentre study on peri- and postoperative central venous oxygen saturation in high-risk surgical patients. Crit Care 2006; 10: R158.
18. Teixeira C, da Silva NB, Savi A, Vieira SR, Nasi LA, Friedman G, Oliveira RP, Cremonese RV, Tonietto TF, Bressel MA, Maccari JG, Wickert R, Borges LG. Central venous saturation is a predictor of reintubation in difficult-to-wean patients. Crit Care Med 2010; 38: 491–96.
19. Scalea TM, Holman M, Fuortes M, Baron BJ, Phillips TF, Goldstein AS, Sclafani SJ, Shaftan GW. Central venous – blood oxygen saturation – an early, accurate measurement of volume during hemorrhage. J Trauma 1988; 28: 725–32.
20. Scalea TM, Hartnett RW, Duncan AO, Atweh NA, Phillips TF, Sclafani SJ, Fuortes M, Shaftan GW. Central venous oxygen-saturation: a useful clinical tool in trauma patients. J Trauma 1990; 30: 1539–43.
21. Weiskopf RB, Viele MK, Feiner J, Kelley S, Lieberman J, Noorani M, Leung JM, Fisher DM, Murray WR, Toy P, Moore MA. Human cardiovascular and metabolic response to acute, severe isovolemic anemia. JAMA 1998; 279: 217–21.
22. Adamczyk S, Robin E, Barreau O, Fleyfel M, Tavernier B, Lebuffe G, Vallet B. Contribution of central venous oxygen saturation in postoperative blood transfusion decision. Ann Fr Anesth Reanim 2009; 28: 522–30.
23. Krantz T, Warberg J, Secher NH. Venous oxygen saturation during normovolemic haemodilution in the pig. Acta Anaesthesiol Scand 2005; 49: 1149–56.
24. Nagy S, Nagy A, Adamicza A, Szabo I, Tarnoky K, Traub A. Histamine level changes in the plasma and tissues in hemorrhagic shock. Circ Shock 1986; 18: 227–39.
25. Adamicza A, Tarnoky K, Nagy A, Nagy S. The effect of anaesthesia on the haemodynamic and sympathoadrenal responses of the dog in experimental haemorrhagic shock. Acta Physiol Hung 1985; 65: 239–54.
26. Meletti JFA, Módolo NSP. Hemorrhagic shock hemodynamic and metabolic behavior: experimental study in dogs. Rev Bras Anestesiol 2003; 53: 623–32.

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Research Article

Central Venous-to-Arterial CO_2 Gap Is a Useful Parameter in Monitoring Hypovolemia-Caused Altered Oxygen Balance: Animal Study

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Monitoring hypovolemia is an everyday challenge in critical care, with no consensus on the best indicator or what is the clinically relevant level of hypovolemia. The aim of this experiment was to determine how central venous oxygen saturation ($ScvO_2$) and central venous-to-arterial carbon dioxide difference (CO_2 gap) reflect hypovolemia-caused changes in the balance of oxygen delivery and consumption. Anesthetized, ventilated Vietnamese minipigs ($n = 10$) were given a bolus followed by a continuous infusion of furosemide. At baseline and then in five stages hemodynamic, microcirculatory measurements and blood gas analysis were performed. Oxygen extraction increased significantly, which was accompanied by a significant drop in $ScvO_2$ and a significant increase in CO_2 gap. There was a significant negative correlation between oxygen extraction and $ScvO_2$ and significant positive correlation between oxygen extraction and CO_2 gap. Taking $ScvO_2 < 73\%$ and CO_2 gap >6 mmHg values together to predict an oxygen extraction >30%, the positive predictive value is 100%; negative predicted value is 72%. Microcirculatory parameters, capillary perfusion rate and red blood cell velocity, decreased significantly over time. Similar changes were not observed in the sham group. Our data suggest that $ScvO_2 < 73\%$ and CO_2 gap >6 mmHg can be complementary tools in detecting hypovolemia-caused imbalance of oxygen extraction.

1. Introduction

Diagnosing hypovolemia is an everyday challenge in critical care. Clinicians utilize a large array of tools from simple clinical signs to invasive hemodynamic measurements, but a universally accepted gold standard remains elusive [1]. Although diagnosis may prove difficult, early recognition of hypovolemia is of utmost importance. By the time macrohemodynamic changes manifest, the microcirculation may already be damaged [2]. Furthermore, fluid therapy is a double-edged sword: on the one hand fluid resuscitation can save lives, but on the other hand a cumulative positive fluid balance is an independent factor for mortality [3, 4]. Deciding on the level of monitoring (noninvasive, "less" invasive, invasive) and which parameter to monitor in order to keep the critically ill patient normovolemic remains uncertain.

Central venous oxygen saturation ($ScvO_2$), an easily obtained parameter via the central venous catheter already *in situ* in most critically ill patients, is often used as a marker of the balance between oxygen delivery (DO_2) and consumption (VO_2). The main factors, which influence $ScvO_2$, are hemoglobin (Hb), arterial oxygen saturation (SaO_2), cardiac output (CO), and VO_2. Theoretically if Hb, SaO_2, and VO_2 are kept constant, the value of $ScvO_2$ should reflect the change in CO. Recent studies have translated theory into practice and demonstrated that $ScvO_2$ may be a good marker for assessing fluid responsiveness [5, 6].

The normal value of $ScvO_2$ varies between 73 and 82%. It is slightly higher than mixed-venous oxygen saturation (SvO_2) and is considered a reasonable surrogate marker in the clinical setting [7].

Changes in $ScvO_2$ reflect systemic oxygen uptake but may be falsely positive (>70%) in regional hypoxia [8]. Under these conditions the central venous-to-arterial CO_2 difference (CO_2 gap) has been proposed as an alternative [8–10]. The physiological value of CO_2 gap is <5 mmHg, but this may be higher in low-flow states [8, 9]. However, it remains unclear how and whether the CO_2 gap changes in hypovolemia.

Therefore, the aim of our hypovolemic animal model was to investigate the association between $ScvO_2$, CO_2 gap, microcirculatory blood flow and hypovolemia-caused altered VO_2/DO_2.

2. Methods

The study protocol was approved by the local Ethics Committee and the Institutional Animal Care and Use Committee at the University of Szeged, and the study was conducted in the research laboratory of the Institute of Surgical Research in a manner that does not inflict unnecessary pain or discomfort upon the animal.

2.1. Animals and Instrumentation. Vietnamese minipigs (n = 15) weighing 28 ± 4 kg underwent a 24 hr fast preoperatively but with free access to water. Anesthesia was induced by intramuscular injection of a mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) and maintained with a continuous infusion of propofol (6 mg/kg/hr i.v.), while analgesia was maintained with nalbuphine (0.1 mg/kg). A tracheal tube was inserted and the animals' lungs were ventilated mechanically. The tidal volume was set at 10 mL/kg, and the respiratory rate was adjusted to maintain the end-tidal carbon dioxide and partial pressure of arterial carbon dioxide in the range of 35–45 mmHg and the arterial pH between 7.35 and 7.45. The adequacy of the depth of anesthesia was assessed by monitoring the jaw tone. After induction of anesthesia, the right jugular vein and the right femoral artery and vein were dissected and catheterized. Tonometric probes and catheters were placed simultaneously into the stomach and the small bowel. A suprapubic urinary catheter was also inserted to monitor urine output. Animals were kept warm (35 ± 1°C) by an external warming device.

For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter (PiCCO, PULSION Medical Systems SE, Munich, Germany) was placed in the femoral artery, and a pulmonary artery catheter (PV2057 VoLEF Catheter, PULSION Medical Systems SE, Munich, Germany) was placed in the femoral vein. The latter was also used to draw mixed venous blood gas samples from which the VO_2 was calculated. The femoral artery served as the site for arterial blood gas sampling and the central venous line was used for taking central venous blood gas samples and for the injection of cold saline boluses for the thermodilution measurements.

For continuous noninvasive visualization of the microcirculation in the sublingual region an intravital orthogonal polarization spectral (OPS) imaging technique (Cytoscan A/R, Cytometrics, Philadelphia, PA, USA) was used [2, 11]. A 10x objective was introduced onto the sublingual serosa, and microscopic images were recorded with an S-VHS video recorder (Panasonic AG-TL 700, Osaka, Japan).

For the tonometry special probes (Tonosoft Medical-Technical and R&G Ltd.) were used and monitoring was performed with a Sidestream Microcap Handheld Capnograph (Oridion Medical Ltd., Jerusalem, Israel) instrument [12].

To assess further biochemical changes in the microcirculation, plasma big-endothelin-1 (BigET) levels were determined. BigET is a 38 amino acid containing protein, the precursor of endothelin-1, which becomes elevated in tissue hypoxia [13].

2.2. Hemodynamic Measurements. Cardiac output (CO), global end-diastolic volume index (GEDI), stroke volume (SV), heart rate (HR), and mean arterial pressure (MAP) were measured by transpulmonary thermodilution and pulse contour analysis at baseline and at the end of each interval. Detailed description of transpulmonary thermodilution and pulse contour analysis is provided elsewhere [14, 15]. All hemodynamic parameters were indexed for body surface area or bodyweight. The average of three measurements following 10 mL bolus injections of ice-cold 0.9% saline was recorded. Central venous pressure (CVP) was measured via the central venous catheter at the same times as the other hemodynamic variables.

Arterial, central venous, and mixed venous blood gas samples (Cobas b 221, Roche Ltd., Basel, Switzerland) were drawn and analyzed by cooximetry simultaneously at baseline and at the end of each cycle.

2.3. Monitoring the Microcirculation. Microcirculatory evaluation of the sublingual region was performed offline by frame-to-frame analysis of the videotaped images. Capillary red blood cell velocity (RBCV) and capillary perfusion rate (CPR) were determined in three separate fields using a computer-assisted image analysis system (IVM Pictron, Budapest, Hungary). All OPS measurements were performed by one investigator.

Gastric and small bowel changes in partial pressure of carbon dioxide (ΔPCO_2) were calculated by subtracting tonometric PCO_2 from arterial PCO_2 (gastric-arterial: Ga-PCO_2; bowel-arterial: Ba-PCO_2) [12].

For measurements of BigET, blood samples of 2 mL were drawn from the jugular vein into chilled polypropylene tubes containing EDTA (1 mg/mL). The samples were centrifuged at 1200 g for 10 min at 4°C. The plasma samples were then collected and stored at −70°C until assay.

2.4. Experimental Protocol. At baseline (T_0) hemodynamic, microcirculatory and blood gas parameters were recorded. Hypovolemia was induced via a bolus followed by a continuous infusion of furosemide (5 mg/kg and 5 mg/kg/2 h, resp.) in a group of 10 animals—hypovolemic group (HG). After the administration of bolus furosemide measurements were recorded in five stages with 20-minute interval between each measurement (T_1–T_5). When the preload parameter (GEDI) decreased by >20% its baseline value, OPS imaging and BigET sampling were performed, which were repeated only at the end of the experiment. There were 5 anaesthetised,

Table 1: Hemodynamic changes.

		T_0	T_1	T_2	T_3	T_4	T_5
GEDI (mL/m^2)	HG	349 ± 51	293 ± 53$^{*\ddagger\#}$	257 ± 47$^{*\ddagger\#}$	246 ± 53$^{*\#}$	233 ± 42$^{*\#}$	223 ± 31$^{*\#}$
	SG	350 ± 26	387 ± 43	360 ± 22	365 ± 76	356 ± 51	349 ± 46
CVP (mmHg)	HG	7 ± 4	4 ± 3$^{*\ddagger\#}$	4 ± 3$^{*\#}$	4 ± 3$^{*\ddagger\#}$	3 ± 3$^{*\#}$	4 ± 3$^{*\#}$
	SG	8 ± 1	7 ± 1	7 ± 1	7 ± 2	7 ± 1	7 ± 2
MAP (mmHg)	HG	121 ± 15	107 ± 17*‡	94 ± 19*‡	86 ± 15*‡	85 ± 15*	83 ± 17*
	SG	120 ± 13	121 ± 14	117 ± 15	113 ± 16	121 ± 24	111 ± 25
HR (1/beats)	HG	78 ± 14	83 ± 15*‡	93 ± 17*	102 ± 19*	130 ± 28*‡	142 ± 28$^{*\ddagger\#}$
	SG	74 ± 12	75 ± 12	74 ± 9	78 ± 10	83 ± 16	80 ± 9
CI (L/min/m^2)	HG	2.30 ± 0.35	1.78 ± 0.31*‡	1.54 ± 0.32*‡	1.46 ± 0.35*‡	1.52 ± 0.41*	1.58 ± 0.36*
	SG	2.38 ± 0.50	2.65 ± 0.65	2.42 ± 0.48	2.38 ± 0.76	2.32 ± 0.50	2.27 ± 0.27
SVI (mL/m^2)	HG	29 ± 5	19 ± 2$^{*\ddagger\#}$	15 ± 4$^{*\#}$	14 ± 4$^{*\#}$	12 ± 4$^{*\#}$	12 ± 3$^{*\#}$
	SG	33 ± 4	34 ± 6	33 ± 3	32 ± 6	31 ± 8	28 ± 5

GEDI: global end-diastolic volume index; CVP: central venous pressure; MAP: mean arterial pressure; HR: heart rate; CI: cardiac index; SVI: stroke volume index. T_0: baseline measurement; T_1–T_5: five intervals. $^{*}p < .05$ as compared to T_1; $^{\ddagger}p < .05$ as compared to the previous value; RM ANOVA; $^{\#}p < .05$ HG versus SG; Mann-Whitney U-test with Bonferroni correction.

ventilated animals in the sham group (SG), who did not receive any furosemide, but maintenance infusion of lactated Ringer (4 mL/kg/h) and hemodynamic, microcirculatory and blood gas parameters were recorded in the same fashion as described previously. At the end of the experiment all animals were humanely euthanized.

2.5. Data Analysis and Statistics. Data are reported as means ± standard deviations unless indicated otherwise. For testing normal distribution the Kolmogorov-Smirnov test was used. Changes in all parameters throughout the experiment were tested by repeated measures analysis of variance (RM ANOVA), and the number of degrees of freedom was adjusted to Greenhouse-Geisser epsilon when needed. Mann-Whitney U-test with Bonferroni correction was used for between-groups analysis. For pairwise comparisons Pearson's correlation was used. To evaluate the performance of $ScvO_2$, CO_2 gap and microcirculatory parameters in detecting altered oxygen extraction with a threshold of 30%, receiver operating characteristics (ROC) curve analysis was performed, and sensitivity, specificity, positive predictive (PPV), and negative predictive values (NPV) were also determined. Post hoc calculation showed a power of 83% with an effect of 36% decrease in GEDI for a sample size of 10 and $\alpha = 0.05$. For statistical analysis SPSS version 18.0 for Windows (SPSS, Chicago, IL, USA) was used and $p < .05$ was considered statistically significant.

3. Results

3.1. Hemodynamic Effects of Hypovolemia. Urine output in the hypovolemic group following the bolus and the onset of infusion was 176 ± 160 mL at T_1, which increased to 647 ± 231 mL at T_5. In contrast, in the sham group, urine output was 74 ± 74 mL at T_1 and had increased to 325 ± 175 mL by T_5. All other hemodynamic data are summarized in Table 1. Preload, as indicated by GEDI, decreased significantly after each phase in the hypovolemic group compared to baseline and dropped by 36% of its baseline value by the end of the experiment. The change of the other macrohemodynamic variables followed a similar pattern. When comparing the sham versus hypovolemic animals, variables differed between the two groups from T_1, but significant differences over time continued only for GEDI, CVP and SVI. In the sham group there were no significant changes over time throughout the experiment.

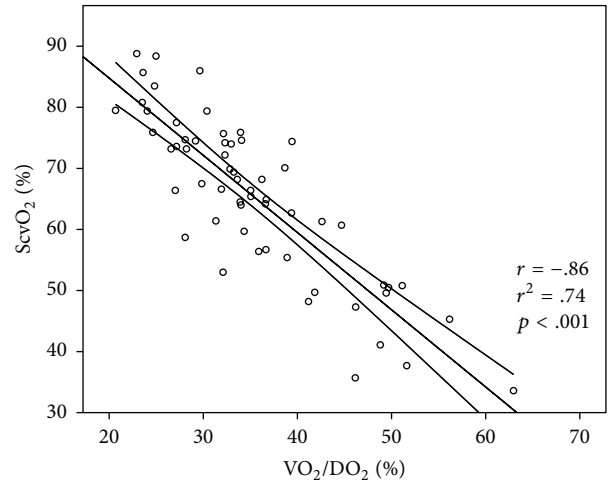

Figure 1: Correlation between VO_2/DO_2 and $ScvO_2$. Data are presented as scatter with a linear regression line and its mean 95% confidence interval. VO_2/DO_2: oxygen extraction, $ScvO_2$: central venous oxygen saturation.

3.2. Effects on Oxygen Balance. Variables related to oxygen balance are listed in Table 2. In the hypovolemic group DO_2 fell significantly from T_1 and remained so for the rest of the experiment. The VO_2/DO_2 increased significantly over 30% from T_1, while $ScvO_2$ and CO_2 gap followed this change only after T_2. Lactate changed significantly from T_3. There were no significant changes in the sham group throughout the experiment.

In the hypovolemic group there was a significant correlation between VO_2/DO_2 and $ScvO_2$ and CO_2 gap (Figures 1

Table 2: Changes of oxygen balance.

		T_0	T_1	T_2	T_3	T_4	T_5
SaO_2 (%)	HG	94 ± 6	95 ± 5	95 ± 2	96 ± 2	94 ± 3	94 ± 2
	SG	95 ± 5	94 ± 8	96 ± 5	94 ± 8	95 ± 5	94 ± 7
Hb (g/L)	HG	122 ± 9	129 ± 13	132 ± 7*	134 ± 7*‡	137 ± 7*‡	138 ± 8*
	SG	119 ± 10	115 ± 9	110 ± 6	108 ± 11	111 ± 9	102 ± 10
DO_2I (mL/min/m^2)	HG	361 ± 39	302 ± 52*‡	268 ± 47*‡	258 ± 55*	269 ± 65*	284 ± 55*
	SG	365 ± 78	396 ± 88	346 ± 73	337 ± 67	324 ± 55	298 ± 36
VO_2/DO_2 (%)	HG	28 ± 5	31 ± 6*‡	34 ± 7*‡	39 ± 9*‡	41 ± 9*	40 ± 10*
	SG	25 ± 4	25 ± 6	26 ± 7	29 ± 5	27 ± 6	30 ± 7
$ScvO_2$ (%)	HG	74 ± 10	71 ± 10	67 ± 11*‡	64 ± 14*	59 ± 13*‡	57 ± 14*
	SG	77 ± 8	76 ± 10	76 ± 9	75 ± 11	73 ± 14	73 ± 12
CO_2-gap (mmHg)	HG	4.3 ± 2.3	7.5 ± 3.3	7.1 ± 2.6*	8.3 ± 2.8*	7.3 ± 2.9*	10.1 ± 5.5$^{*\#}$
	SG	4.1 ± 2.4	3.5 ± 1.9	4.5 ± 1.3	3.9 ± 2.9	4.6 ± 1.9	4.4 ± 1.9
Lactate (mmol/L)	HG	3.8 ± 1.4	3.9 ± 1.3	4.3 ± 0.9	4.7 ± 0.9*‡	5.1 ± 1.2*‡	5.3 ± 1.5*
	SG	3.8 ± 0.9	3.9 ± 1.2	4.2 ± 1.8	4.6 ± 2.1	4.7 ± 2.7	4.9 ± 3.2
VO_2I (mL/min/m^2)	HG	98 ± 11	93 ± 16	88 ± 9	96 ± 7‡	104 ± 10‡	109 ± 16
	SG	88 ± 16	96 ± 6	85 ± 12	96 ± 8	85 ± 12	88 ± 14

SaO_2: arterial oxygen saturation; Hb: hemoglobin; DO_2: oxygen delivery; VO_2/DO_2: oxygen extraction ratio; $ScvO_2$: central venous oxygen saturation; CO_2 gap: venous-to-arterial carbon dioxide difference; VO_2: oxygen consumption. T_0: baseline measurement; T_1–T_5: five intervals. $^{*}p < .05$ as compared to T_1; $^{‡}p < .05$ as compared to the previous value; RM ANOVA; $^{\#}p < .05$ HG versus SG; Mann-Whitney U-test with Bonferroni correction.

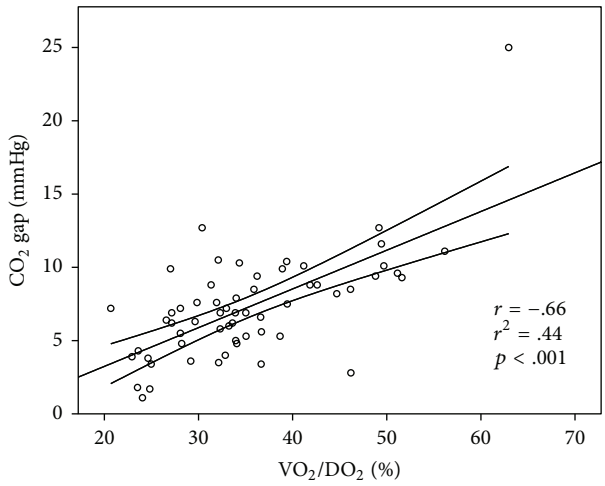

Figure 2: Correlation between VO_2/DO_2 and CO_2 gap. Data are presented as scatter with a linear regression line and its mean 95% confidence interval. VO_2/DO_2: oxygen extraction; CO_2 gap: central venous-to-arterial carbon dioxide difference.

and 2). Lactate also showed a significant, but weak correlation with VO_2/DO_2 ($r = .38$, $r^2 = .14$; $p < .05$).

With receiver-operator characteristic (ROC) curves for $ScvO_2$, CO_2 gap and lactate to detect a VO_2/DO_2 >30%, the area under the curves (AUC) was significant for $ScvO_2$, CO_2 gap (AUC ± SE = 0.887 ± 0.046; 0.783 ± 0.062; $p < .05$, resp.) while lactate did not reach statistical significance. The cut-off values to give the best sensitivity and specificity for $ScvO_2$ and CO_2 gap were 73% and 6.5 mmHg, respectively. Sensitivity, specificity, positive predictive, and negative predictive values for $ScvO_2$ and CO_2 gap are summarized in Table 3. Taking $ScvO_2$ and CO_2 gap values together to predict a VO_2/DO_2 >30%, the false positive and false negative values were reduced.

Table 3: Complementation of $ScvO_2$ with CO_2 gap.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
$ScvO_2$ ≤ 73%	78	83	91	63
CO_2 gap > 6 mm Hg	71	72	85	52
$ScvO_2$ + CO_2 gap (≤73%) (>6 mm Hg)	58	100	100	72

$ScvO_2$: central venous oxygen saturation; CO_2 gap: venous-to-arterial carbon dioxide difference; PPV: positive redictive value; NPV: negative predictive value.

3.3. Effects on Microcirculation. Variables related to microcirculation are listed in Table 4. In the hypovolemic group tonometry increased significantly only when measured in the intestines. Capillary perfusion rate and red blood cell velocity gradually and significantly decreased over time. This change was accompanied by a significant increase in BigET levels. In contrast, the sham group BigET decreased significantly by T_5, while the other parameters remained unchanged. There was a significant difference in capillary perfusion rate, red blood cell velocity and BigET between the two groups.

The ROC curves for predicting VO_2/DO_2 >30% proved to be significant for capillary perfusion rate and red blood cell velocity in the hypovolemic group. The area under curve did not differ significantly between these two parameters (AUC ± SE = 0.848 ± 0.084; 0.848 ± 0.092; $p < .05$, resp.).

The correlation between $ScvO_2$ and the microcirculatory parameters proved to be significant apart from Ga-PCO_2

Table 4: Changes in microcirculation.

		T_0	T_1	T_2	T_3	T_4	T_5
Ba-PCO_2 (mmHg)	HG	24 ± 8	35 ± 16*‡	35 ± 17	36 ± 13*	33 ± 13*	37 ± 16*
	SG	19 ± 5	22 ± 9	20 ± 6	22 ± 11	22 ± 10	20 ± 8
Ga-PCO_2 (mmHg)	HG	40 ± 12	43 ± 14	42 ± 14	39 ± 14	36 ± 10	37 ± 11
	SG	36 ± 22	37 ± 24	34 ± 21	32 ± 21	34 ± 20	32 ± 18
CPR (%)	HG	82 ± 15	—	53 ± 11$^{*‡\#}$	—	—	45 ± 16$^{*‡\#}$
	SG	91 ± 50		81 ± 80			83 ± 70
RBCV (μm/s)	HG	887 ± 141	—	509 ± 120$^{*‡\#}$	—	—	463 ± 209$^{*\#}$
	SG	1054 ± 141		848 ± 194			963 ± 51
BigET (fmol/mL)	HG	1.44 ± 0.53	—	1.97 ± 0.84*‡	—	—	2.29 ± 0.89$^{*\#}$
	SG	1.36 ± 0.93		1.49 ± 1.27			0.98 ± 0.92*

Ba-PCO_2: small bowel-to-arterial carbon dioxide difference; Ga-PCO_2: gastric-to-arterial carbon dioxide difference; CPR: capillary perfusion rate; RBCV: red blood cell velocity; BigET: big endothelin. T_0: baseline measurement; T_1–T_5: five intervals. $^{*}p < .05$ as compared to T_1, $^{‡}p < .05$ as compared to the previous value, GLM repeated measures ANOVA; $^{\#}p < .05$ HG versus SG, Mann-Whitney U-test with Bonferroni correction.

($ScvO_2$-Ba-PCO_2, -CPR, -RBCV, -BigET: $r = -.38$, $r^2 = .15$; $r = .49$, $r^2 = .24$; $r = .40$, $r^2 = .16$; $r = -.47$, $r^2 = .23$; $p < .05$, resp.). CO_2 gap showed significant correlations with Ba-PCO_2 and CPR ($r = .48$, $r^2 = .23$; $r = -.51$, $r^2 = .26$; $p < .05$, resp.).

4. Discussion

The main finding of our study is that it provides further evidence that low or decreasing $ScvO_2$, as well as high or increasing CO_2-gap can reflect changes and may be complementary in global oxygen balance and altered microcirculatory blood flow in hypovolemia.

4.1. Hemodynamic Changes. Our goal of achieving hypovolemia was reached as global end diastolic index, central venous pressure and stroke volume decreased significantly throughout the experiment, which resulted in a significant drop in cardiac index in the hypovolemia group. This decrease was notable until T_3. Due to this change VO_2/DO_2 increased significantly from T_1.

4.2. CO_2 Gap and $ScvO_2$ in Hypovolemia. Up until now, there has been consensus neither on the most accurate hemodynamic marker of hypovolemia nor on the endpoints for optimal fluid therapy [1, 16, 17]. Many recent studies have suggested that fluid therapy should be based on dynamic (such as cardiac output, pulse pressure variation and stroke volume variation) rather than static hemodynamic variables (such as CVP, pulmonary artery occlusion pressure), because they are better predictors of fluid responsiveness in ICU patients. However, pulse pressure variation and stroke volume variation are limited to patients who are fully ventilated and have no arrhythmias [18, 19]. Although it is not strictly a hemodynamic variable, in certain clinical conditions an $ScvO_2$ value of ~70% has been used as a therapeutic endpoint to improve oxygen delivery [3, 7, 20, 21]. In a recent study it was found that a change in $ScvO_2$ is a reliable parameter to define fluid responsiveness at the bedside in critically ill patients [5]. Similar results have been reported in two other studies demonstrating a close relationship between $ScvO_2$ and cardiac index [6, 22]. However, one has to bear in mind that fluid resuscitation reduces hemoglobin levels, which may result in no change or decrease in $ScvO_2$ therefore, the above may only hold true for hypovolemic patients with low $ScvO_2$.

In our experiment the change in VO_2/DO_2, which increased significantly from T_1, was accompanied by a fall in $ScvO_2$, which is in accordance with previous findings. The change in VO_2/DO_2 was also accompanied by an increase in CO_2 gap from T_2. There is some evidence that CO_2 gap increases in certain low flow states [8, 9]. The pathophysiology of increased CO_2 gap may be due to the CO_2 stagnation phenomenon. When cardiac output decreases, blood flow is slow and the washout is impaired; therefore, more CO_2 is accumulated in the tissues, and as CO_2 diffuses easily and quickly the CO_2 gap increases [23].

$ScvO_2$ showed very good sensitivity and specificity with a threshold of 73% for determining VO_2/DO_2 >30%, which was further improved when CO_2 gap >6.5 mmHg was added, leading to less false negative and false positive results. The $ScvO_2$ and CO_2 gap showed a significant and strong negative correlation. It is also important to note that lactate showed a significant but substantially weaker correlation to VO_2/DO_2 as compared to $ScvO_2$ or CO_2 gap. This highlights the limitation of lactate levels as therapeutic endpoint for resuscitation. This finding is in accordance with previously published data showing the limitation of lactate levels as therapeutic endpoint for resuscitation [24].

In cases when due to microcirculatory and/or mitochondrial defects oxygen uptake is insufficient, $ScvO_2$ may be elevated (i.e. false negative). Previous studies have suggested that under such circumstances the increased value of CO_2 gap (>5 mmHg) may help the clinician in detecting inadequate DO_2 to tissues [8–10]. Our results lend further support to this theory. Furthermore, adding the CO_2 gap to $ScvO_2$ for identifying VO_2/DO_2 >30%, there was an improvement in specificity, positive predictive, and negative predictive values.

We are not aware of any studies that have tailored hemodynamic support based on or supported by changes

in CO_2 gap; therefore, its clinical relevance remains unclear. However, our data clearly shows, and to our knowledge this is the first experiment to show that, an altered VO_2/DO_2 caused by hypovolemia is reflected by an increase in CO_2 gap. Therefore its value may be an important alarm signal for the clinician and would help decision making at the bedside, especially when considering a fluid challenge or where commencing advanced hemodynamic monitoring is concerned.

4.3. Microcirculation. In any shock like states the microcirculation plays a vital role, as the most devastating effects of oxygen debt occur here in the cells [2, 22]. It has been demonstrated that microcirculatory disturbances can occur not only in cases of severe hypovolemic shock, but also in cases of a moderate hypovolemia without severe hypotension in human patients [25]. Recently, Bartels et al. evaluated the alteration of the sublingual microcirculation in response to controlled, central hypovolemia using sidestream dark field imaging in human subjects with intact autoregulation. They confirmed that despite adequate compensation of hypovolemia it can still be associated with decreased microcirculatory response, consequently with decreased oxygen delivery to the tissues [26].

In a prospective observational study in patients with septic shock it was found that the capillary perfusion rate was different in survivors (in whom it was increased) as compared to nonsurvivors. Moreover, it was the only factor to differentiate survivors and patients dying of multiple-system organ failure after the shock had resolved [2]. In accordance with these results we saw a gradual and significant decrease in both capillary perfusion rate and red blood cell velocity over time. There was also a very good area under curve when defining VO_2/DO_2 >30% and good correlation with $ScvO_2$ and CO_2 gap. All these changes were observed only in the hypovolemic, but not in the sham group.

According to the microcirculatory parameters measured in this experiment, the inflicted hypovolemia resulted in significant changes to the microcirculation. Tonometry showed a significant increase in Ba-PCO_2 due to hypovolemia, indicating decreased blood flow in the intestines. This is in accordance with previously published reports [27]. In contrast, there was no significant change in the Ga-PCO_2. Regarding the importance and the value of gastric mucosal pH is controversial and its routine use has declined in intensive care over the last decades [28–30]. The difference between Ga-PCO_2 and Ba-PCO_2 is an interesting observation and also difficult to explain. However monitoring both has already been suggested, in order to give a small additional value in the diagnosis of possible mismatch in splanchnic perfusion [31, 32].

There was also a significant correlation between Ba-PCO_2 both with $ScvO_2$ and CO_2 gap.

Little is known about BigET, but there is some evidence that ET-1 reflects tissue hypoxia [33]. However, in contrast to the insignificant change in BigET found in healthy volunteers suffering from acute hypoxia, our results showed a significant difference of BigET levels between the hypovolemic and the sham groups, which indicates that there is an effect of hypovolemia-induced tissue hypoxia on BigET levels [34].

4.4. Limitations of the Study. One of the possible limitations of our study is the long preparation period which might have resulted in the slightly elevated lactate levels although this was observed in both groups. Its clinical impact may be limited as a decreased $ScvO_2$ and elevated CO_2 gap may be influenced by several factors other than hypovolemia, including heart failure, severe sepsis/septic shock, and multiple trauma; thus, these results can only be applied when these conditions are unlikely to be present, for example, in postoperative critical care. Furthermore, these data were obtained in anesthetized animals and may not be the same in conscious human subjects. Finally, as there are no gold standards for hypovolemic animal experiments, therefore one cannot exclude that the choice of furosemide-caused hypovolemia may not be the most appropriate model. The disadvantage of this model is that it does not replicate real life clinical diseases. Traumatic hypovolemia and hypovolemia associated with sepsis are associated with profound microcirculatory changes which become superimposed on the changes following hypovolemia. This is particularly important in patients with sepsis, where $ScvO_2$ is known to be a poor marker of tissue oxygenation. Indeed, in patients with sepsis, a high rather than a low $ScvO_2$ is predictive of mortality.

5. Conclusion

Our results have shown that in addition to central venous oxygen saturation ($ScvO_2$), central venous-to-arterial CO_2 difference (CO_2 gap) may also be used as a simple, but valuable indicator of hypovolemia-caused imbalance of oxygen extraction (VO_2/DO_2). Further clinical studies have to validate its clinical merits in indicating and tailoring hemodynamic support.

Conflict of Interests

The authors declare no conflict of interests.

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References

[1] A. Perner, "Diagnosing hypovolemia in the critically ill," *Critical Care Medicine*, vol. 37, no. 9, pp. 2674–2675, 2009.

[2] Y. Sakr, M. J. Dubois, D. De Backer, J. Creteur, and J. L. Vincent, "Persistent-microcirculatory alterations are associated with organ failure and death in patients with septic shock," *Critical Care Medicine*, vol. 32, no. 9, pp. 1825–1831, 2004.

[3] E. Rivers, B. Nguyen, S. Havstad et al., "Early goal-directed therapy in the treatment of severe sepsis and septic shock," *The New England Journal of Medicine*, vol. 345, no. 19, pp. 1368–1377, 2001.

[4] Y. Sakr, J.-L. Vincent, K. Reinhart et al., "High tidal volume and positive fluid balance are associated with worse outcome in acute lung injury," *Chest*, vol. 128, no. 5, pp. 3098–3108, 2005.

[5] R. Giraud, N. Siegenthaler, A. Gayet-Ageron, C. Combescure, J.-A. Romand, and K. Bendjelid, "$ScvO_2$ as a marker to define fluid responsiveness," *Journal of Trauma*, vol. 70, no. 4, pp. 802–807, 2011.

[6] T. Krantz, J. Warberg, and N. H. Secher, "Venous oxygen saturation during normovolaemic haemodilution in the pig," *Acta Anaesthesiologica Scandinavica*, vol. 49, no. 8, pp. 1149–1156, 2005.

[7] Collaborative Study Group on Perioperative ScvO2 Monitoring, "Multicentre study on peri- and postoperative central venous oxygen saturation in high-risk surgical patients," *Critical Care*, vol. 10, no. 6, 2006.

[8] F. Vallée, B. Vallet, O. Mathe et al., "Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock?" *Intensive Care Medicine*, vol. 34, no. 12, pp. 2218–2225, 2008.

[9] E. Futier, E. Robin, M. Jabaudon et al., "Central venous O_2 saturation and venous-to-arterial CO_2 difference as complementary tools for goal-directed therapy during high-risk surgery," *Critical Care*, vol. 14, no. 5, article R193, 2010.

[10] B. Vallet and G. Lebuffe, "How to titrate vasopressors against fluid loading in septic shock," *Advances in Sepsis*, vol. 6, no. 2, pp. 34–40, 2007.

[11] W. Groner, J. W. Winkelman, A. G. Harris et al., "Orthogonal polarization spectral imaging: a new method for study of the microcirculation," *Nature Medicine*, vol. 5, no. 10, pp. 1209–1213, 1999.

[12] D. Boda, J. Kaszaki, and G. Tálosi, "A new simple tool for tonometric determination of the PCO_2 in the gastrointestinal tract: *in vitro* and *in vivo* validation studies," *European Journal of Anaesthesiology*, vol. 23, no. 8, pp. 680–685, 2006.

[13] S. Kourembanas, P. A. Marsden, L. P. McQuillan, and D. V. Faller, "Hypoxia induces endothelin gene expression and secretion in cultured human endothelium," *Journal of Clinical Investigation*, vol. 88, no. 3, pp. 1054–1057, 1991.

[14] C. R. Phillips, K. Vinecore, D. S. Hagg et al., "Resuscitation of haemorrhagic shock with normal saline vs. lactated Ringer's: effects on oxygenation, extravascular lung water and haemodynamics," *Critical Care*, vol. 13, no. 2, article R30, 2009.

[15] B. Saugel, A. Umgelter, T. Schuster, V. Phillip, R. M. Schmid, and W. Huber, "Transpulmonary thermodilution using femoral indicator injection: a prospective trial in patients with a femoral and a jugular central venous catheter," *Critical Care*, vol. 14, no. 3, article R95, 2010.

[16] J. M. P. Barros, P. Do Nascimento Jr., J. L. P. Marinello et al., "The effects of 6% hydroxyethyl starch-hypertonic saline in resuscitation of dogs with hemorrhagic shock," *Anesthesia and Analgesia*, vol. 112, no. 2, pp. 395–404, 2011.

[17] B. Vallet, "Intravascular volume expansion: which surrogate markers could help the clinician to assess improved tissue perfusion?" *Anesthesia and Analgesia*, vol. 112, no. 2, pp. 258–259, 2011.

[18] F. Michard and J.-L. Teboul, "Predicting fluid responsiveness in ICU patients: a critical analysis of the evidence," *Chest*, vol. 121, no. 6, pp. 2000–2008, 2002.

[19] X. Monnet, D. Osman, C. Ridel, B. Lamia, C. Richard, and J.-L. Teboul, "Predicting volume responsiveness by using the end-expiratory occlusion in mechanically ventilated intensive care unit patients," *Critical Care Medicine*, vol. 37, no. 3, pp. 951–956, 2009.

[20] R. Pearse, D. Dawson, J. Fawcett, A. Rhodes, R. M. Grounds, and E. D. Bennett, "Changes in central venous saturation after major surgery, and association with outcome," *Critical Care*, vol. 9, no. 6, pp. R694–R699, 2005.

[21] C. Teixeira, N. B. Da Silva, A. Savi et al., "Central venous saturation is a predictor of reintubation in difficult-to-wean patients," *Critical Care Medicine*, vol. 38, no. 2, pp. 491–496, 2010.

[22] O. J. Liakopoulos, J. K. Ho, A. Yezbick et al., "An experimental and clinical evaluation of a novel central venous catheter with integrated oximetry for pediatric patients undergoing cardiac surgery," *Anesthesia and Analgesia*, vol. 105, no. 6, pp. 1598–1604, 2007.

[23] B. Vallet, J.-L. Teboul, S. Cain, and S. Curtis, "Venoarterial CO_2 difference during regional ischemic or hypoxic hypoxia," *Journal of Applied Physiology*, vol. 89, no. 4, pp. 1317–1321, 2000.

[24] T. C. Jansen, J. Van Bommel, and J. Bakker, "Blood lactate monitoring in critically ill patients: a systematic health technology assessment," *Critical Care Medicine*, vol. 37, no. 10, pp. 2827–2839, 2009.

[25] K. R. Ward, M. H. Tiba, K. L. Ryan et al., "Oxygen transport characterization of a human model of progressive hemorrhage," *Resuscitation*, vol. 81, no. 8, pp. 987–993, 2010.

[26] S. A. Bartels, R. Bezemer, D. M. J. Milstein et al., "The microcirculatory response to compensated hypovolemia in a lower body negative pressure model," *Microvascular Research*, vol. 82, no. 3, pp. 374–380, 2011.

[27] K. R. Walley, B. P. Friesen, M. F. Humer, and P. T. Phang, "Small bowel tonometry is more accurate than gastric tonometry in detecting gut ischemia," *Journal of Applied Physiology*, vol. 85, no. 5, pp. 1770–1777, 1998.

[28] J. Creteur, D. De Backer, and J.-L. Vincent, "Does gastric tonometry monitor splanchnic perfusion?" *Critical Care Medicine*, vol. 27, no. 11, pp. 2480–2484, 1999.

[29] F. Palizas, A. Dubin, T. Regueira et al., "Gastric tonometry versus cardiac index as resuscitation goals in septic shock: a multicenter, randomized, controlled trial," *Critical Care*, vol. 13, no. 2, article R44, 2009.

[30] L. A. Steiner, S. Staender, C. C. Sieber, and K. Skarvan, "Effects of simulated hypovolaemia on haemodynamics, left ventricular function, mesenteric blood flow and gastric PCO_2," *Acta Anaesthesiologica Scandinavica*, vol. 51, no. 2, pp. 143–150, 2007.

[31] J. A. Otte, A. B. Huisman, R. H. Geelkerken, and J. J. Kolkman, "Jejunal tonometry for the diagnosis of gastrointestinal ischemia. Feasibility, normal values and comparison of jejunal with gastric tonometry exercise testing," *European Journal of Gastroenterology and Hepatology*, vol. 20, no. 1, pp. 62–67, 2008.

[32] A. Thorén, S. M. Jakob, R. Pradl, M. Elam, S.-E. Ricksten, and J. Takala, "Jejunal and gastric mucosal perfusion versus splanchnic blood flow and metabolism: an observational study on postcardiac surgical patients," *Critical Care Medicine*, vol. 28, no. 11, pp. 3649–3654, 2000.

[33] H. Lal, Q. Yu, K. Ivor Williams, and B. Woodward, "Hypoxia augments conversion of big-endothelin-1 and endothelin ET_B receptor-mediated actions in rat lungs," *European Journal of Pharmacology*, vol. 402, no. 1-2, pp. 101–110, 2000.

[34] T. Lenz, M. Nadansky, J. Gossmann, G. Oremek, and H. Geiger, "Exhaustive exercise-induced tissue hypoxia does not change endothelin and big endothelin plasma levels in normal volunteers," *American Journal of Hypertension*, vol. 11, no. 8 I, pp. 1028–1031, 1998.

III.

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Central Venous-To-Arterial CO_2-Gap May Increase in Severe Isovolemic Anemia

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Abstract

Despite blood transfusions are administered to restore adequate tissue oxygenation, transfusion guidelines consider only hemoglobin as trigger value, which gives little information about the balance between oxygen delivery and consumption. Central venous oxygen saturation is an alternative, however its changes reflect systemic metabolism and fail to detect regional hypoxia. A complementary parameter to $ScvO_2$ may be central venous-to-arterial carbon dioxide difference (CO_2-gap). Our aim was to investigate the change of alternative transfusion trigger values in experimental isovolemic anemia. After splenectomy, anesthetized Vietnamese mini pigs (n = 13, weight range: 18–30 kg) underwent controlled bleeding in five stages (T_1–T_5). During each stage approximately 10% of the estimated starting total blood volume was removed and immediately replaced with an equal volume of colloid. Hemodynamic measurements and blood gas analysis were then performed. Each stage of bleeding resulted in a significant fall in hemoglobin, the O_2-extraction increased significantly from T_3 and $ScvO_2$ showed a similar pattern and dropped below the physiological threshold of 70% at T_4. By T_4 CO_2-gap increased significantly and well correlated with VO_2/DO_2 and $ScvO_2$. To our knowledge, this is the first study to show that anemia caused altered oxygen extraction may have an effect on CO_2-gap.

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Introduction

Transfusion of red blood cells is an everyday practice in critical care with the primary aim of restoring adequate tissue oxygenation. Transfusion guidelines consider certain levels of hemoglobin as transfusion trigger [1,2], which on its own gives little information if any about the balance between oxygen delivery (DO_2) and consumption (VO_2). Hence, there is a clear need for additional physiologic transfusion trigger values. One of the potentially useful physiological parameters is the central venous oxygen saturation ($ScvO_2$), which has been shown to be a potential physiologic transfusion trigger in hemodynamically stable but anemic patients [3]. Its normal value is around 70–75% and it is the product of the VO_2 and DO_2 relationship. Low $ScvO_2$ usually indicates inadequate DO_2, but higher than physiological values may be difficult to interpret as these can indicate reduced oxygen consumption, but may also mean inappropriate oxygen uptake [4,5]. Under these circumstances additional parameters are needed.

Central venous-to-arterial carbon dioxide difference (CO_2-gap) may be one of the potential alternatives to complement $ScvO_2$. Under physiological circumstances its value is less than 6 mmHg [6,7]. Transport of carbon dioxide in blood ensues in three forms: dissolved in plasma, as bicarbonate ion and bound to hemoglobin. The CO_2-gap may be higher during anaerobic respiration when lactic acid has to be buffered by bicarbonate or under aerobic respiration in poorly perfused tissues when flow stagnation results in an accumulation of CO_2 [8,9,10]. From previous experiments it seems that increased CO_2-gap during ischemia is related to decreased blood flow and impaired CO_2 washout rather than to hypoxemia [10]. Whether anemia caused tissue hypoxemia is reflected in changes of the CO_2-gap has not been investigated before.

Another additional parameter may be the central venous-to-arterial pCO_2 difference divided by the difference of the arterio-venous oxygen content, $P(v\text{-}a)CO_2/C(a\text{-}v)O_2$, which is considered to give information about tissue oxygenation. It was found in a retrospective study that this ratio reflected the occurrence of anaerobic metabolism better than other oxygen-, or CO_2-derived parameters [11].

Our aim was to investigate how CO_2-gap and $P(v\text{-}a)CO_2/C(a\text{-}v)O_2$ change during experimental isovolemic anemia.

Materials and Methods

The study protocol was approved by the local ethics committee at the University of Szeged and the study was carried out in the research laboratory of the Institute of Surgical Research. The current experiment complements our previously published data on the relationship of $ScvO_2$ and isovolemic anemia [12]. Vietnamese mini pigs (n = 13) weighing 24±3 kg were anaesthetized and mechanically ventilated in pressure control mode. Anesthesia was induced with an intramuscular injection of a mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) and maintained with a continuous infusion of propofol (6 mg/kg/h i.v.). The tidal volume

Table 1. Hemodynamic effects of isovolemic anemia. These data have been published earlier [12].

	T_0	T_1	T_2	T_3	T_4	T_5
Hb (g/L)	125(113–134)	102(90–109)*#	79(73–93)*#	68(60–76)*#	59(53–67)*#	49(43–55)*#
HR (beats/min)	125(91–135)	119(100–138)*	123(102–146)*	129(110–159) *	139(118–179) *	147(131–177)*
MAP (mm Hg)	91(79–105)	89(79–101)	83(75–98)*	82(68–90)*	72(59–85)*	72(63–86)*
CVP (mm Hg)	6(5–8)	8(5–9)	7(4–9)	7(5–9)	7(5–9)	7(3–10)
CI (L/min/m^2)	2.6(2.3–2.8)	3.3(2.7–3.6)*#	3.6(2.9–3.8)*#	3.6(3.3–4.1)*	3.5(3.2–4.0)*	3.9(3.6–4.1)*
GEDI (mL/m^2)	270 (243–284)	271 (245–320)	276 (248–298)	274 (236–305)	268 (227–302)	261 (232–298)
ELWI (mL/kg)	9 (9–10)	10 (10–10)	9 (9–10)	10 (9–10)	10 (9–10)	10 (9–11)
dPmx (mm Hg/s)	540(485–790)	700(540–985)*	800(570–1075)*	810(540–1480)*	880(560–1360)*	975(562–1275)*

Hb- Hemoglobin, HR- Heart rate, MAP- Mean arterial pressure, CVP- Central venous pressure, CI- Cardiac index, GEDI- Global end-diastolic volume index, ELWI- extravascular lung water index, dPmx- Index of left ventricular contractility. T_0- Baseline measurement, T_1-T_5- Five intervals of bleeding.
*$p<.05$ compared with T_0; #$p<.05$ compared with previous; GLM repeated measures ANOVA.
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was set at 13±2 ml/kg and the respiratory rate was adjusted to maintain the end-tidal carbon dioxide and the partial pressure of arterial carbon dioxide in the range of 35–45 mmHg and the arterial pH between 7.35 and 7.45. The adequacy of the depth of anesthesia was assessed by monitoring the jaw tone. After the initiation of anesthesia, the right carotid artery and jugular vein and the right femoral artery and vein were dissected and catheterized. The animals underwent suprapubic urinary catheter placement and laparotomy for splenectomy. Splenectomy in swine hemorrhage models are performed because of the distensibility of the spleen and the resultant variation in the amounts of sequestered blood [13]. The core temperature was maintained at 37±1°C through use of an external warming device.

For invasive hemodynamic monitoring, a transpulmonary thermodilution catheter (PiCCO, PULSION Medical Systems AG, Munich, Germany) was placed in the femoral artery and a pulmonary artery catheter (PV2057 VoLEF Catheter, PULSION Medical Systems AG) by pressure tracings via the femoral vein. The latter was also used to draw mixed venous blood gas samples. The femoral artery served as the site of arterial blood gas samples and the central venous line was used for central venous blood gas sampling and for the injection of cold saline boluses for

Table 2. Descriptives (Median±IQR).

	Time intervals					
	T_0	T_1	T_2	T_3	T_4	T_5
$_{cv}CO_2$-gap (mmHg)	5.0(2.6–8.5)	6.0(3.1–7.0)	5.0(3.5–5.5)	5.4(4.4–7.0)	8.0(4.3–8.5)*	6.3(5.9–11.0)*
$_{v}CO_2$-gap (mmHg)	5.5(4.0–9.0)	6.5(4.5–7.8)	6.5(5.1–7.0)	5.5(3.7–6.0)	5.4(5.0–8.0)	6.2(5.5–8.0)
$P_{cv}CO_2/C_{(a\text{-}cv)}O_2$	2.01(1.42–2.23)	2.27(1.76–3.34)	2.67(1.71–2.85)	2.59(1.50–4.47)	3.30(2.89–3.74)*	3.93(2.55–5.11)*
$P_vCO_2/C_{(a\text{-}v)}O_2$	1.57(0.77–1.99)	1.69(0.91–2.00)	1.71(1.36–1.99)	1.61(0.96–2.17)	2.14(1.58–2.23)	2.30(1.93–3.56)*
$ScvO_2$ (%)#	76(69–83)	73(72–82)	77(75–83)	77(68–81)	68(61–76)*	66(60–76)*
SvO_2 (%)#	68 (64–77)	67 (64–77)	68 (63–79)	64 (58–76)	62 (55–72)*	58 (52–72)*
DO_2 (ml/min/m^2) #	431 (362–474)	438 (323–524)	378 (302–412)*	344 (252–376)*	284 (236–333)*	247 (216–292)*
VO_2 (ml/min/m^2) #	119 (82–139)	130 (77–151)	93 (66–136)	113 (67–141)	98 (72–120)*	105 (70–120)*
VO_2/DO_2 (%)#	29(18–33)	29(17–33)	29(18–32)	35(21–40)*	37(26–43)*	41(27–47)*
ERO_2 (%)#	19(13–26)	19(14–24)	20(14–22)	21(16–28)	30(22–37)*	32(21–39)*
Lactate (mmol/L) #	4.5 (3.2–5.3)	4.2 (3.0–5.1)	5.0 (3.2–6.0)	4.1 (2.9–6.0)	4.2 (2.9–6.5)	4.0 (3.0–6.4)
vLactate (mmol/L)	4.6(3.7–5.3)	4.3(3.3–5.3)	4.4(3.1–5.4)	4.4(2.8–5.2)	4.4(3.0–5.2)	4.1(3.0–6.4)
cvLactate (mmol/L)	4.5(3.5–5.5)§	3.9(3.4–5.4)§	4.2(3.3–6.3)§	4.1(3.1–5.6)§	3.9(2.9–5.7)§	3.9(3.0–6.4)§
$PaCO_2$ (mmHg) #	39(35–44)	38(35–45)	37(34–45)	39(34–46)	37(34–42)	38(35–41)
PaO_2 (mmHg) #	76(66–80)	75(72–80)	76(73–80)	77(72–82)	79(75–85)	81(77–90)

$_{cv}CO_2$-gap: central venous-to-arterial carbon dioxide difference; $_{v}CO_2$-gap: mixed venous-to-arterial carbon dioxide difference; $P_{(cv\text{-}a)}CO_2/C_{(a\text{-}cv)}O_2$: the central venous-to-arterial pCO_2 difference divided by the difference of the arterio-venous oxygen content; $P_{(v\text{-}a)}CO_2/C_{(a\text{-}v)}O_2$: the mixed venous-to-arterial pCO_2 difference divided by the difference of the arterio-venous oxygen content; $ScvO_2$: central venous oxygen saturation; SvO_2: mixed venous oxygen saturation; DO_2: oxygen delivery; VO_2: oxygen consumption; VO_2/DO_2: oxygen extraction ratio; ERO_2: simplified oxygen extraction ratio; $PaCO_2$: arterial partial pressure of carbon dioxide; PaO_2: arterial partial pressure of oxygen * $p<.05$ as compared to baseline, § $p<.05$ significant difference between mixed venous and central venous blood with Friedman and Wilcoxon tests, # Data published earlier [12].
doi:10.1371/journal.pone.0105148.t002

thermodilution measurements. Central venous catheter was positioned by using guidewire attached intracavital ECG. During the experiment blood was drained from the catheter in the right carotid artery, which was also used to replace the blood loss with the same amount of colloid, in order to avoid a sudden increase in right ventricular preload.

At baseline (T_0) hemodynamic and blood gas parameters were recorded, and heparin sulfate (200 IU/kg) was administered through the central venous line. Isovolemic anemia was achieved in five intervals (T_1–T_5). During each interval 10% of the estimated total blood volume was withdrawn over a 5- to 10-min period. Hemodynamic parameters were recorded and the amount of blood drained was immediately replaced by an equal volume of colloid (hydroxyethyl starch 130 kDa/0.4, 6%, Voluven, Fresenius, Germany). To achieve a steady state, the animals were allowed to rest for 10 min between intervals. At the end of each cycle, hemodynamic and blood gas parameters were measured. At the end of the experiment the animals were humanely euthanized.

Arterial, central venous, and mixed venous blood gas samples (Cobas b 221, Roche Ltd., Basel, Switzerland) were drawn and analyzed by cooximetry simultaneously at baseline and at the end of each cycle. From these parameters the oxygen delivery (DO_2), oxygen consumption (VO_2), oxygen extraction ratio (VO_2/DO_2) and the simplified oxygen extraction ratio (ERO_2) were calculated according to standard formulae:

$$DO_2 = SV * HR * [Hb * 1.34 * SaO_2 + (0.003 * PaO_2)]$$
$$VO_2 = CO * [CaO_2 - (Hb * 1.34 * SvO_2 + (0.003 * PvO_2))]$$
$$ERO_2 = (SaO_2 - ScvO_2)/SaO_2$$

Central venous-to-arterial CO_2-gap ($_{cv}CO_2$-gap), mixed venous-to-arterial CO_2-gap ($_{v}CO_2$-gap), the $P_{(cv-a)}CO_2/C_{(a-cv)}O_2$ and $P_{(v-a)}CO_2/C_{(a-v)}O_2$ were also calculated from the arterial, central venous and mixed venous blood gas samples.

These were calculated according to standard formulae:

$$cvCO_2\text{-gap} = PcvCO_2 - PaCO_2$$
$$vCO_2\text{-gap} = PvCO_2 - PaCO_2$$
$$P(cv-a)CO_2/C(a-cv)O_2 \text{ratio} = (PcvCO_2 - PaCO_2)/(CaO_2 - CcvO_2)$$
$$P(v-a)CO_2/C(a-v)O_2 \text{ratio} = (PvCO_2 - PaCO_2)/(CaO_2 - CvO_2)$$
$$CaO_2 = (1.34 * SaO_2 * Hb) + (0.003 * PaO_2)$$
$$CcvO_2 = (1.34 * ScvO_2 * Hb) + (0.003 * PcvO_2)$$
$$CvO_2 = (1.34 * SvO_2 * Hb) + (0.003 * PvO_2)$$

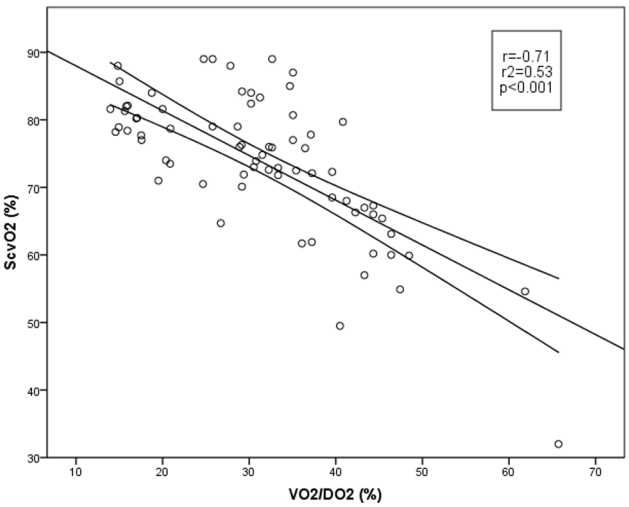

Figure 1. The association between VO_2/DO_2 and $ScvO_2$. VO_2/DO_2: oxygen extraction ratio; $ScvO_2$: central venous oxygen saturation.
doi:10.1371/journal.pone.0105148.g001

Analysis

Data are reported as median±standard deviation unless indicated otherwise. For testing normal distribution the Kolmogorov-Smirnov test was used. Changes in all parameters throughout the experiment were tested by Friedman test and repeated measures analysis of variance (RM ANOVA), and the number of degrees of freedom was adjusted to Greenhouse-Geisser epsilon when needed. For pairwise comparisons Pearson's correlation was used. To evaluate the performance in detecting altered oxygen extraction of >30% (considered as the "physiological threshold"), receiver operating characteristics (ROC) curve analysis was performed. Post-hoc calculation showed a power of 86% with an effect of 25% increase in VO_2/DO_2, for a sample size of 13 and $\alpha = 0.05$. For statistical analysis SPSS version 20.0 for Windows (SPSS, Chicago, IL, USA) was used and $p<0.05$ was considered statistically significant.

Results

All 13 animals survived the study. The bleeding caused a gradual decrease in hemoglobin level after each phase and by the end of the experiment it had fallen by 61% of the baseline value. The hemodynamic parameters are summarized in Table 1. The SaO_2 remained in the normal range throughout the experiment. DO_2 fell significantly from T_2, VO_2 at T_4, VO_2/DO_2 increased significantly from T_3, and exceeded the physiologic threshold of 30% (Table 2). The change in $ScvO_2$ displayed a similar pattern as VO_2/DO_2 and changed significantly and also fell below 70% only at T_4. There was strong negative correlation between VO_2/DO_2 and $ScvO_2$ (Fig. 1).

The CO_2-gap was calculated for both, central venous ($_{cv}CO_2$-gap) and mixed venous blood ($_{v}CO_2$-gap). By T_4 $_{cv}CO_2$-gap increased significantly, however $_{v}CO_2$-gap did not change. The correlations of VO_2/DO_2 and $ScvO_2$ were significant with $_{cv}CO_2$-gap, while there were only weak correlations with $_{v}CO_2$-gap (Fig. 2).

$P_{(cv-a)}CO_2/C_{(a-cv)}O_2$ increased by T_4 and $P_{(v-a)}CO_2/C_{(a-v)}O_2$ by T_5. The correlations of VO_2/DO_2 and $ScvO_2$ were significant with $P_{(cv-a)}CO_2/C_{(a-cv)}O_2$, but it was found to be weak between $P_{(v-a)}CO_2/C_{(a-v)}O_2$ and VO_2/DO_2, and there was no significant correlation with $ScvO_2$ (Fig. 3).

ROC analysis revealed the same tendency as the correlation. With 30% taken as the physiologic threshold for VO_2/DO_2, the area under the curve (AUC), its standard error and that of the 95% confidence interval were >0.5 only for $_{cv}CO_2$-gap, $P_{(cv-a)}CO_2/C_{(a-cv)}O_2$ ratio, $ScvO_2$ (Table 3.)

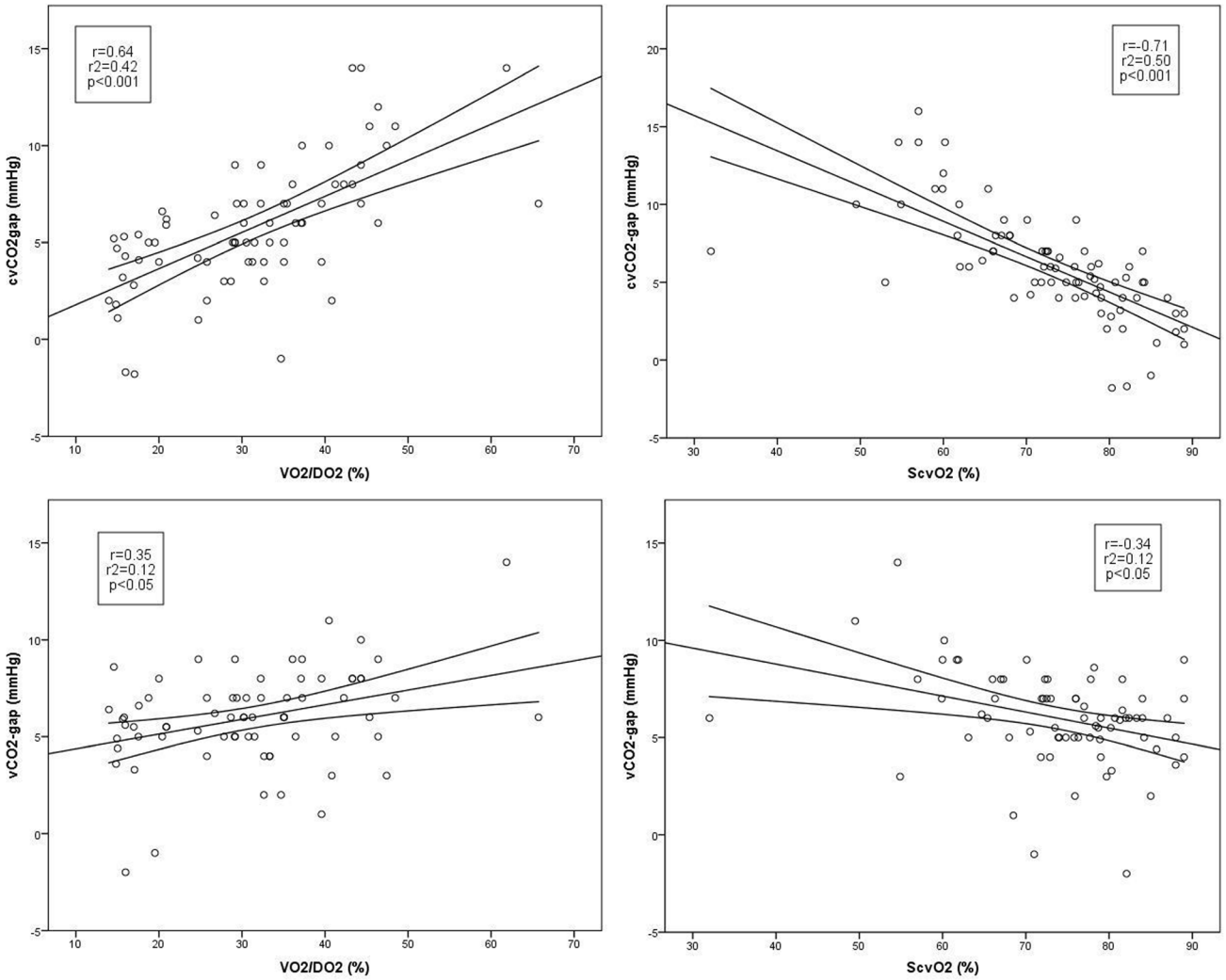

Figure 2. Correlation between oxygen balance parameters and CO_2-gap. $_{cv}CO_2$-gap and VO_2/DO_2 and $ScvO_2$ (on the left); $_{v}CO_2$-gap and VO_2/DO_2 and $ScvO_2$ (on the right). $cvCO_2$-gap: central venous-to-arterial carbon dioxide difference; VO_2/DO_2: oxygen extraction ratio; $ScvO_2$: central venous oxygen saturation; vCO_2-gap: mixed venous-to-arterial carbon dioxide difference.
doi:10.1371/journal.pone.0105148.g002

Linear regression revealed a significant relationship between $ScvO_2$ ($r = 0.71$, $r^2 = 0.50$, $p < .001$) and VO_2/DO_2. This relationship became significantly stronger when $_{cv}CO_2$-gap was added to $ScvO_2$ ($r = 0.74$, $r^2 = 0.54$, $p = .015$). According to the Pratt's importance coefficient, $ScvO_2$ was responsible for this increase in 63% and $_{cv}CO_2$-gap in 37%.

Discussion

Our results in this isovolemic anemia animal model show that besides $ScvO_2$, both central venous-to-arterial CO_2-gap and the $P_{(cv\text{-}a)}CO_2/C_{(a\text{-}cv)}O_2$ correlated well with changes in anemia caused increase in VO_2/DO_2. Furthermore, mixed venous blood driven indices, such as $_{v}CO_2$-gap and $P_{(v\text{-}a)}CO_2/C_{(a\text{-}v)}O_2$ failed to indicate changes in oxygen extraction. When oxygen extraction ratio started to increase (from T_3) it was followed by a decrease of $ScvO_2$ and an increase of $_{cv}CO_2$-gap and $P_{(cv\text{-}a)}CO_2/C_{(a\text{-}cv)}O_2$, and both performed well in the ROC analysis, with the $_{cv}CO_2$-gap's AUC being marginally better. In addition, in our experiment neither $_{v}CO_2$-gap nor $P_{(v\text{-}a)}CO_2/C_{(a\text{-}v)}O_2$ or lactate could detect the increase in $VO_2/DO_2 > 30\%$ as revealed by ROC analysis.

An interesting finding of our experiment is that despite isovolemia was maintained as indicated by the stable global end diastolic volume index values and there were in fact increasing cardiac output and stroke volume, we observed a rise in $_{cv}CO_2$-gap. This observation seemingly contradicts previously published results to some extent. The occurrence of increased CO_2-gap has fundamentally been explained by the CO_2 stagnation phenomenon [5]. This was based on the finding that there was inverse correlation between CO_2-gap and cardiac index during non-septic and septic low flow states [5,9,10]. Moreover, it was also found that the amount of CO_2 produced is negligible when anaerobic respiration is present and CO_2-gap therefore cannot serve as a marker of tissue hypoxia [10]. The paramount study on this theory by Vallet et al. used an isolated hind limb model and reached hypoxia either by decreasing flow or decreasing arterial oxygen content [10]. They found that occurrence of an increased CO_2-gap during ischemia was related to decreased blood flow and impaired carbon dioxide washout; moreover, dysoxia *per se* was

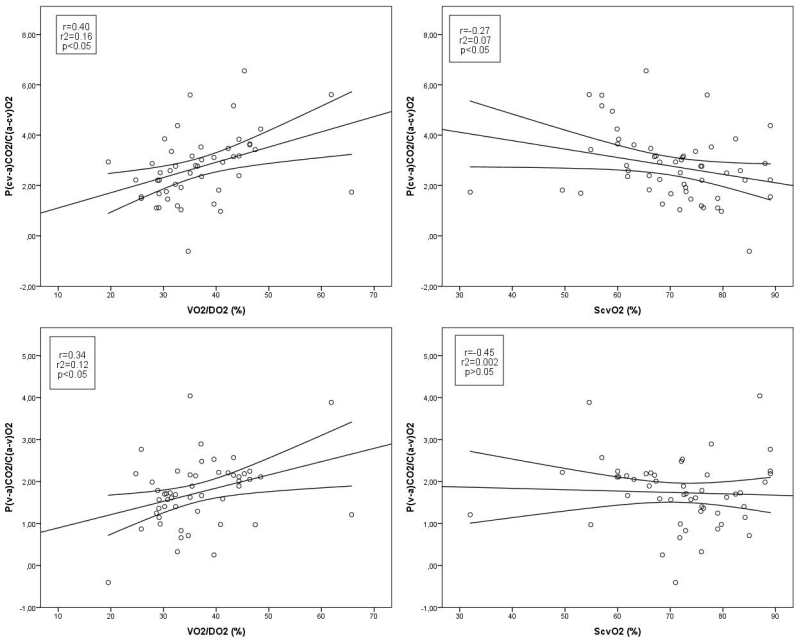

Figure 3. Correlation between tissue oxygenation and oxygen balance parameters. $P_{(cv-a)}CO_2/C_{(a-cv)}O_2$ and VO_2/DO_2 and $ScvO_2$ (on the left); $P_{(v-a)}CO_2/C_{(a-v)}O_2$ and VO_2/DO_2 and $ScvO_2$ (on the right).$P_{(cv-a)}CO_2/C_{(a-cv)}O_2$: the central venous-to-arterial pCO_2 difference divided by the difference of the arterio-venous oxygen content; VO_2/DO_2: oxygen extraction ratio; $ScvO_2$: central venous oxygen saturation; $P_{(v-a)}CO_2/C_{(a-v)}O_2$: the mixed venous-to-arterial pCO_2 difference divided by the difference of the arterio-venous oxygen content.
doi:10.1371/journal.pone.0105148.g003

not sufficient to increase CO_2-gap. However, the latter could also be due to the Haldane's effect. As the carbon dioxide dissociation curve is influenced by the saturation of hemoglobin with oxygen, the lower the saturation of hemoglobin with oxygen, the higher the saturation of hemoglobin with carbon dioxide for a given carbon dioxide partial pressure is [14]. In our experiment arterial oxygen saturation and PaO_2 remained in the normal range and did not change over time, hence the CO_2 dissociation curve was not influenced by low saturation of hemoglobin with oxygen.

Nevertheless, anemia resulted in increased VO_2/DO_2 above the baseline and also above the physiological 30% after the 3rd bleeding event, which was followed by the significant decrease of SvO_2 and $ScvO_2$. (It is important to note that there is mathematical coupling between VO_2 and SvO_2, which is not the case considering $ScvO_2$). The most interesting finding of the current study is the increase of $_{cv}CO_2$-gap during the last two stages of the experiment, without any change in the $_{v}CO_2$-gap. One of the possible reasons for this difference is that due to isovolemia cardiac output was maintained to avoid low flow in the systemic circulation, which is also reinforced by the unchanged lactate levels. Therefore when CO_2 was measured in the mixed venous blood it was unchanged and within the normal range

Table 3. ROC analysis for determining VO_2/DO_2>30%.

Test Result Variable(s)	Area	Std. Error	Sig.	95% CI	
$_{cv}CO_2$-gap	,769	,078	,007	,617	,921
$_{v}CO_2$-gap	,553	,097	,598	,363	,742
$P_{(cv-a)}CO_2/C_{(a-cv)}O_2$ ratio	,742	,070	,016	,604	,879
$P_{(v-a)}CO_2/C_{(a-v)}O_2$ ratio	,641	,096	,157	,453	,829
$ScvO_2$	,768	,056	,000	,657	,879
SvO_2	,986	,010	,000	,967	1,000
Lactate	,517	,078	,867	,363	,670

$_{cv}CO_2$-gap: central venous-to-arterial carbon dioxide difference;
$_{v}CO_2$-gap: mixed venous-to-arterial carbon dioxide difference;
$P_{(cv-a)}CO_2/C_{(a-cv)}O_2$: the central venous-to-arterial pCO_2 difference divided by the difference of the arterio-venous oxygen content;
$P_{(v-a)}CO_2/C_{(a-v)}O_2$: the mixed venous-to-arterial pCO_2 difference divided by the difference of the arterio-venous oxygen content;
$ScvO_2$: central venous oxygen saturation; SvO_2: mixed venous oxygen saturation.
doi:10.1371/journal.pone.0105148.t003

almost throughout. As central venous blood driven variables mostly reflect blood flow and metabolism of the brain [15], our hypothesis is that anemia reached such a degree by T_4 that it caused tissue hypoxia and consecutive anaerobic respiration with CO_2 production. However, due to the low hemoglobin levels the Haldane effect could not take effect, hence there was a significant increase in central venous pCO_2. But these changes in the brain did not have significant effects on the systemic level, to be picked up in mixed venous blood. As anemia has greater influence on arterial oxygenation than hypoxemia [16], this might explain the observed increase in $_{cv}CO_2$-gap. This is further reinforced by the $P_{(cv\text{-}a)}CO_2/C_{(a\text{-}cv)}O_2$ results. Both the $P_{(cv\text{-}a)}CO_2/C_{(a\text{-}cv)}O_2$ and the $P_{(v\text{-}a)}CO_2/C_{(a\text{-}v)}O_2$ increased at T_4 and T_5, but there was a more pronounced change in central venous as compared to mixed venous blood, which is also reflected in the results of the ROC analysis. We also measured mixed venous and central venous lactate levels and found that central venous lactate was significantly lower than in the mixed venous blood, which might give further proof to this hypothesis [17,18]. In a previous animal experiment by Hare *et al*, it was found that hemodilutional isovolemic anemia led to cerebral hypoxia, and they also reported a gradual increase in the jugular venous pCO_2 with a CO_2-gap of 2.9 to 7.8 mmHg (mean) 60 minutes after hemodilution in the traumatic brain injured animals [19]. Although this finding was not discussed in the article, as the authors mainly focused on oxygenation, but nevertheless this is in accord with our results and gives some support to our hypothesis.

There is increasing evidence that untreated anemia can be associated with a worse outcome and increased mortality, while transfusion may cause various infectious and non-infectious adverse effects [20,21]. $_{cv}CO_2$-gap may be an additional quantitative parameter, beyond Hb and $ScvO_2$, that would give information on anemia related altered oxygen extraction and hence the need for blood administration. $_{cv}CO_2$-gap is a choice of plausible alternatives as it can be easily obtained via the central venous and arterial catheters already *in situ* in most critically ill patients and no additional invasive device is needed; moreover we found that mixed venous blood driven indices failed to indicate changes in oxygen extraction.

There are several limitations of our study. As the experiment was not designed to measure the effects of isovolemic anemia specifically on the brain, our hypothesis cannot be supported by specific measurements, such as regional cerebral blood flow, cerebral tissue oxygen and carbon dioxide tension. Furthermore, splenectomy and the length of the preparation of the animals may have been too long, which resulted in increased levels of lactate from baseline to the end of the experiment. The steady-state periods may also have been relatively short, although, the same time intervals have been used previously [22]. Another concern might be the type of fluid replacement, as one cannot exclude the possibility that the use of different types of colloid or crystalloid solutions would affect these results.

Conclusions

To our knowledge, this is the first study to show that anemia caused altered oxygen extraction may have an effect on $_{cv}CO_2$-gap and $P_{(cv\text{-}a)}CO_2/C_{(a\text{-}cv)}O_2$ that cannot be detected from mixed venous blood. The clinical relevance of this finding has to be further tested in both experimental and clinical studies.

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Author Contributions

Conceived and designed the experiments: ZM SK. Performed the experiments: JK DÉ GD SK. Analyzed the data: SK. Contributed reagents/materials/analysis tools: JK DÉ. Wrote the paper: ZM SK.

References

1. Retter A, Wyncoll D, Pearse R, Carson D, McKechnie S, et al. British Committee for Standards in Haematology (2013) Guidelines on the management of anaemia and red cell transfusion in adult critically ill patients. Br J Haematol 160(4):445–464.
2. Blood Observational Study Investigators of ANZICS-Clinical Trials Group, Westbrook A, Pettilä V, Nichol A, Bailey MJ, Syres G, et al. (2010) Transfusion practice and guidelines in Australian and New Zealand intensive care units. Intensive Care Med 36: 1138–46.
3. Adamczyk S, Robin E, Barreau O, Fleyfel M, Tavernier B, et al. (2009) Contribution of central venous oxygen saturation in postoperative blood transfusion decision. Ann Fr Anesth Reanim 28: 522–30.
4. Vallet B, Robin E, Lebuffe G (2010) Venous oxygen saturation as a physiologic transfusion trigger. Critical Care 14:213.
5. Vallée F, Vallet B, Mathe O, Parraquette J, Mari A, et al. (2008) Central venous-to-arterial carbon dioxide difference: an additional target for goal-directed therapy in septic shock? Intensive Care Med 34:2218–2225.
6. Geers C, Gros G (2000) Carbon dioxide transport and carbonic anhydrase in blood and muscle. Physiol Rev 80:681–715.
7. Guyton AC, Hall JE (2006) Transport of Oxygen and Carbon Dioxide in Blood and Tissue Fluids. In: Guyton AC, Hall JE, editors. Textbook of Medical Physiology. Eleventh Edition. Philadelphia, Elsevier Saunders, pp. 502–513.
8. Schlichtig R, Bowles SA (1994) Distinguishing between aerobic and anaerobic appearance of dissolved CO_2 in intestine during low flow. J Appl Physiol 76: 2443–2451.
9. Lamia B, Monnet X, Teboul JL (2006) Meaning of arterio-venous PCO_2 difference in circulatory shock. Minerva Anestesiol 72:597–604.
10. Vallet B, Teboul JL, Cain S, Curtis S (2000) Venoarterial CO_2 difference during regional ischemic or hypoxic hypoxia. J Appl Physiol 89:1317–1321.
11. Mekontso-Dessap A, Castelain V, Anguel N, Bahloul M, Schauvliege F, et al. (2002) Combination of venoarterial PCO_2 difference with arteriovenous O_2 content difference to detect anaerobic metabolism in patients. Intensive Care Med 28:272–277.
12. Kocsi S, Demeter G, Fogas J, Érces D, Kaszaki J, et al. (2012) Central venous oxygen saturation is a good indicator of altered oxygen balance in isovolemic anemia. ACTA Anaesthesiol Scand 56: 291–297.
13. Phillips CP, Vinecore K, Hagg DS, Sawai RS, Differding JA, et al. (2009) Resuscitation of haemorrhagic shock with normal saline vs. lactated Ringer's: effects on oxygenation, extravascular lung water and haemodynamics. Critical Care 13:R30.
14. West JB (1990) Gas transport to the periphery. In: West JB. Baltimore MD, editor, Respiratory Physiology: The Essentials (4th ed.), Williams and Wilkins, pp. 69–85.
15. Maddirala S, Khan A (2010) Optimizing hemodynamic support in septic shock using central and mixed venous oxygen saturation. Crit Care Clin 26: 323–333.
16. Marino PL (2014) Systemic Oxygenation. In: Marino PL, editor, The ICU Book (4th ed.) Wolters Kluwer Health/Lippincot Williams and Wilkins, pp. 171–192.
17. Jalloh I, Helmy A, Shannon RJ, Gallagher CN, Menon DK, et al. (2013) Lactate uptake by the injured human brain: evidence from an arteriovenous gradient and cerebral microdialysis study. J Neurotrauma 30(24): 2031–2037.
18. Gallagher CN, Carpenter KL, Grice P, Howe DJ, Mason A, et al. (2009) The human brain utilizes lactate via the tricarboxylic acid cycle: a 13C-labelled microdialysis and high-resolution nuclear magnetic resonance study. Brain 132(Pt10): 2839–2849.
19. Hare GM, Mazer CD, Hutchison JS, McLaren AT, Liu E, et al. (2007) Severe hemodilutional anemia increases cerebral tissue injury following acute neurotrauma. J Appl Physiol 103: 1021–9.
20. Vincent JL, Piagnerelli M (2006) Transfusion in the intensive care unit. Crit Care Med 34: S96–S101.
21. Galvin I, Ferguson ND (2011) Acute lung injury in the ICU: focus on prevention. In: Vincent JL, editor. Annual update in intensive care and emergency medicine. Berlin: Springer Science+Business Media LLC. pp. 117–28.
22. Meletti JFA, Módolo NSP (2003) Hemorrhagic Shock Hemodynamic and Metabolic Behavior: Experimental Study in Dogs. Revista Brasielira de Anestesiologia 53: 623–632.