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 macro-hemodynamic 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	$293 \pm 53^{*\ddagger\#}$	$257 \pm 47^{*\ddagger\#}$	$246 \pm 53^{*\#}$	$233 \pm 42^{*\#}$	$223 \pm 31^{*\#}$
	SG	350 ± 26	387 ± 43	360 ± 22	365 ± 76	356 ± 51	349 ± 46
CVP (mmHg)	HG	7 ± 4	$4 \pm 3^{*\ddagger\#}$	$4 \pm 3^{*\#}$	$4 \pm 3^{*\ddagger\#}$	$3 \pm 3^{*\#}$	$4 \pm 3^{*\#}$
	SG	8 ± 1	7 ± 1	7 ± 1	7 ± 2	7 ± 1	7 ± 2
MAP (mmHg)	HG	121 ± 15	$107 \pm 17^{*\ddagger}$	$94 \pm 19^{*\ddagger}$	$86 \pm 15^{*\ddagger}$	$85 \pm 15^{*}$	$83 \pm 17^{*}$
	SG	120 ± 13	121 ± 14	117 ± 15	113 ± 16	121 ± 24	111 ± 25
HR (1/beats)	HG	78 ± 14	$83 \pm 15^{*\ddagger}$	$93 \pm 17^{*}$	$102 \pm 19^{*}$	$130 \pm 28^{*\ddagger}$	$142 \pm 28^{*\ddagger\#}$
	SG	74 ± 12	75 ± 12	74 ± 9	78 ± 10	83 ± 16	80 ± 9
CI (L/min/m^2)	HG	2.30 ± 0.35	$1.78 \pm 0.31^{*\ddagger}$	$1.54 \pm 0.32^{*\ddagger}$	$1.46 \pm 0.35^{*\ddagger}$	$1.52 \pm 0.41^{*}$	$1.58 \pm 0.36^{*}$
	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	$19 \pm 2^{*\ddagger\#}$	$15 \pm 4^{*\#}$	$14 \pm 4^{*\#}$	$12 \pm 4^{*\#}$	$12 \pm 3^{*\#}$
	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
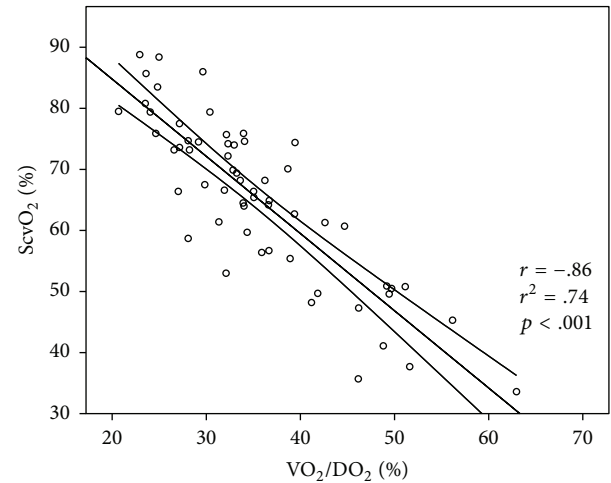

FIGURE 1: Correlation between VO$_2$/DO$_2$ and ScvO$_2$. Data are presented as scatter with a linear regression line and its mean 95% confidence interval. VO$_2$/DO$_2$: oxygen extraction, ScvO$_2$: central venous oxygen saturation.

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.

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.
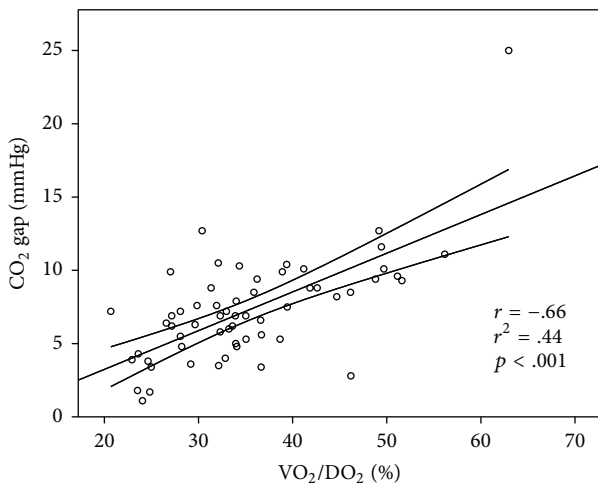

FIGURE 2: Correlation between VO_2/DO_2 and CO_2 gap. Data are presented as scatter with a linear regression line and its mean 95% confidence interval. VO_2/DO_2: oxygen extraction; CO_2 gap: central venous-to-arterial carbon dioxide difference.

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: 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.

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.

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	$35 \pm 16^{*\ddagger}$	35 ± 17	$36 \pm 13^{*}$	$33 \pm 13^{*}$	$37 \pm 16^{*}$
	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	—	$53 \pm 11^{*\ddagger\#}$	—	—	$45 \pm 16^{*\ddagger\#}$
	SG	91 ± 50		81 ± 80			83 ± 70
RBCV (μm/s)	HG	887 ± 141	—	$509 \pm 120^{*\ddagger\#}$	—	—	$463 \pm 209^{*\#}$
	SG	1054 ± 141		848 ± 194			963 ± 51
BigET (fmol/mL)	HG	1.44 ± 0.53	—	$1.97 \pm 0.84^{*\ddagger}$	—	—	$2.29 \pm 0.89^{*\#}$
	SG	1.36 ± 0.93		1.49 ± 1.27			$0.98 \pm 0.92^{*}$

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, $^{\ddagger}p < .05$ 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.

Acknowledgment

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