Ratio of venous-to-arterial PCO2 to arteriovenous oxygen content difference during regional ischemic or hypoxic hypoxia

Jihad Mallat, Benoit Vallet

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Ratio of venous-to-arterial PCO_2 to arteriovenous oxygen content difference during regional ischemic or hypoxic hypoxia

Jihad Mallat[1,2,3]✉ & Benoit Vallet[4]

The purpose of the study was to evaluate the behavior of the venous-to-arterial CO_2 tension difference (ΔPCO_2) over the arterial-to-venous oxygen content difference (ΔO_2) ratio ($\Delta PCO_2/\Delta O_2$) and the difference between venous-to-arterial CO_2 content calculated with the Douglas' equation (ΔCCO_{2D}) over ΔO_2 ratio ($\Delta CCO_{2D}/\Delta O_2$) and their abilities to reflect the occurrence of anaerobic metabolism in two experimental models of tissue hypoxia: ischemic hypoxia (IH) and hypoxic hypoxia (HH). We also aimed to assess the influence of metabolic acidosis and Haldane effects on the PCO_2/CO_2 content relationship. In a vascularly isolated, innervated dog hindlimb perfused with a pump-membrane oxygenator system, the oxygen delivery (DO_2) was lowered in a stepwise manner to decrease it beyond critical DO_2 (DO_{2crit}) by lowering either arterial PO_2 (HH-model) or flow (IH-model). Twelve anesthetized and mechanically ventilated dogs were studied, 6 in each model. Limb DO_2, oxygen consumption ($\dot{V}O_2$), $\Delta PCO_2/\Delta O_2$, and $\Delta CCO_{2D}/\Delta O_2$ were obtained every 15 min. Beyond DO_{2crit}, $\dot{V}O_2$ decreased, indicating dysoxia. $\Delta PCO_2/\Delta O_2$, and $\Delta CCO_{2D}/\Delta O_2$ increased significantly only after reaching DO_{2crit} in both models. At DO_{2crit}, $\Delta PCO_2/\Delta O_2$ was significantly higher in the HH-model than in the IH-model (1.82 ± 0.09 vs. 1.39 ± 0.06, $p = 0.002$). At DO_{2crit}, $\Delta CCO_{2D}/\Delta O_2$ was not significantly different between the two groups (0.87 ± 0.05 for IH vs. 1.01 ± 0.06 for HH, $p = 0.09$). Below DO_{2crit}, we observed a discrepancy between the behavior of the two indices. In both models, $\Delta PCO_2/\Delta O_2$ continued to increase significantly (higher in the HH-model), whereas $\Delta CCO_{2D}/\Delta O_2$ tended to decrease to become not significantly different from its baseline in the IH-model. Metabolic acidosis significantly influenced the PCO_2/CO_2 content relationship, but not the Haldane effect. $\Delta PCO_2/\Delta O_2$ was able to depict the occurrence of anaerobic metabolism in both tissue hypoxia models. However, at very low DO_2 values, $\Delta PCO_2/\Delta O_2$ did not only reflect the ongoing anaerobic metabolism; it was confounded by the effects of metabolic acidosis on the CO_2–hemoglobin dissociation curve, and then it should be interpreted with caution.

Abbreviations

CO_2	Carbon dioxide
VO_2	Oxygen consumption
VCO_2	Carbon dioxide production
DO_2	Oxygen delivery
RQ	Respiratory quotient
ΔPCO_2	Venous-to-arterial carbon dioxide tension difference
CCO_2	CO_2 content
ΔCCO_2	Venous-to-arterial carbon dioxide content difference
$CCvCO_2$	Venous CO_2 content
$CCaCO_2$	Arterial CO_2 content
ΔO_2	Arterial-to-venous oxygen content difference
$PaCO_2$	Partial arterial carbon dioxide tension

[1]Department of Critical Care Medicine, Critical Care Institute, Cleveland Clinic Abu Dhabi, Al Maryah Island, Abu Dhabi, UAE. [2]Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH, USA. [3]Normandy University, UNICAEN, ED 497, Caen, France. [4]EA2694, University of Lille, Lille, France. ✉email: mallatjihad@gmail.com

$PvCO_2$	Partial venous carbon dioxide tension
SvO_2	Venous oxygen saturation
SaO_2	Arterial oxygen saturation
PaO_2	Partial arterial oxygen tension
PvO_2	Partial venous oxygen tension
Hb	Hemoglobin

In a landmark study, Vallet et al. demonstrated the determinant role of blood flow in the tissue hypoxia-induced increased venous-to-arterial CO_2 tension difference (ΔPCO_2)[1]. Their data supported the hypothesis that increases in the venous PCO_2 are primarily a function of changes in regional blood flow, independently of the degree of hypoxia. Gutierrez G has confirmed this conclusion in a mathematical model of tissue-to-blood CO_2 exchange during hypoxia[2]. In these previous publications, the behavior of ΔPCO_2 over the arterial-to-venous oxygen content difference (ΔO_2) ratio ($\Delta PCO_2/\Delta O_2$), and the difference between venous-to-arterial CO_2 content (ΔCCO_2) over ΔO_2 ratio ($\Delta CCO_2/\Delta O_2$) in a model of progressive tissue hypoxia generated by reducing either flow [ischemic hypoxia (IH)] or arterial oxygen tension [hypoxic hypoxia (HH)], were not investigated[1,2].

Several clinical studies[3–7] have shown that $\Delta PCO_2/\Delta O_2$ ratio, taken as a surrogate of respiratory quotient (RQ), was associated with elevated lactate levels and oxygen supply dependency considered, in those studies, as indices of global anaerobic metabolism in critically ill patients with tissue hypoperfusion. However, in an experimental study, Dubin et al. found that $\Delta PCO_2/\Delta O_2$ ratio was a poor indicator of anaerobic metabolism in the hemodilution model of tissue hypoxia, where anemia was associated with preserved blood flow[8]. Similarly, other authors suggested that $\Delta PCO_2/\Delta O_2$ ratio might not rise during tissue hypoxia conditions when associated with normal/high blood flow because venous blood flow seemed to guarantee a sufficient clearance of CO_2 generated by the anaerobic metabolism[9]. Thus, it is unclear if the $\Delta PCO_2/\Delta O_2$ ratio would be able to depict the presence of anaerobic metabolism in patients with maintained blood flow (cardiac output).

Furthermore, one estimates that the $\Delta PCO_2/\Delta O_2$ ratio might be affected by other factors than anaerobic metabolism by influencing the relationship between CO_2 content (CCO_2) and PCO_2. Indeed, metabolic acidosis can change the PCO_2/CCO_2 relationship so that PCO_2 is higher for a given CCO_2. Low oxygen saturation, by promoting more CO_2 binding to hemoglobin (Haldane effect), increases the CCO_2 for a given PCO_2[10]. It is not completely clear to what extent these factors would impact the PCO_2/CCO_2 relationship and influence the $\Delta PCO_2/\Delta O_2$ ratio. Answering this question would help to define the applicability of this ratio in different clinical situations.

Therefore, we used, in secondary analysis, the original study published by Vallet et al.[1] with the aim to assess the behavior of $\Delta PCO_2/\Delta O_2$ ratio, $\Delta CCO_2/\Delta O_2$ ratio, and their components in the regional model of progressive tissue hypoxia generated by IH or HH[1]. We also investigated the metabolic acidosis (pH) and Haldane effects on the PCO_2/CCO_2 relationship. Since the flow was maintained unchanged in the HH model, we hypothesized that $\Delta PCO_2/\Delta O_2$ and $\Delta CCO_2/\Delta O_2$ ratios might not be able to detect the occurrence of anaerobic metabolism as the sustained blood flow would be sufficient to wash out the CO_2 generated by hypoxic cells in that model.

Methods

Animal preparation. The original study was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee. The study is reported in accordance with the ARRIVE guidelines. All experiments were performed in accordance with relevant guidelines and regulations. Twelve dogs of either sex and mixed breed were used[1]. All animals were anesthetized with intravenous 30 mg/kg of sodium phenobarbital and mechanically ventilated with a Harvard animal respirator at 10 breaths/min. Lamps suspended above the operating table were used to maintain core temperature near 37 °C. Tidal volume was varied to maintain systemic arterial PCO_2 between 30 and 35 mmHg. The ventilator setting was kept unchanged during the rest of the experiment. A 20 mg of succinylcholine chloride was given intramuscularly and a continuous infusion (0.1 mg/mL/min) was begun. Anesthesia depth was checked regularly by vigorous toe pinching, and additional anesthetic was given if systemic blood pressure and heart rate responded.

Catheters were inserted into the pulmonary artery (through the internal jugular vein) and common carotid artery for continuous measurements of vascular pressures and blood sampling. Arterial inflow (Q) and venous outflow from the left hindlimb were isolated, as previously described[1,11] (Supplemental Digital Content 1, Appendix). A roller occlusive pump directed blood flow from the right hindlimb femoral artery to the femoral artery of the vascularly isolated left hindlimb. A sampling port and pressure transducer were placed in this circuit proximal to the limb. A membrane oxygenator (model 0800-2A, Sci Med) was interposed in the perfusion circuit. A gas flow mixer (model GF-3, Cameron Instruments) supplied O_2, N_2, and CO_2 to the oxygenator, as needed, to produce normoxia or hypoxia with normocapnia in the blood supply to the hindlimb. A water bath warmed the oxygenator so that perfusion to the isolated hindlimb was at 37 °C after heat loss through the tubing.

Measurements. Blood samples from the carotid, femoral, and pulmonary arteries and femoral vein were obtained simultaneously. Blood gas tensions and pH were measured in an acid–base analyzer (ABL-30, Radiometer, Westlake, OH) at 37 °C and later corrected to esophageal temperature at the time of sampling. Oxygen saturation was measured with a co-oximeter calibrated for dog blood (IL-282, Instrumentation Lab, Lexington, MA). Arterial oxygen content was calculated as CaO_2 (mL) = 1.34 × Hb (g/dL) × SaO_2 + 0.0031 × PaO_2 (mmHg), where SaO_2 is the oxygen saturation of arterial blood, Hb the hemoglobin concentration, and PaO_2 the arterial oxygen tension. Hindlimb venous oxygen content was calculated as CvO_2 (mL) = 1.34 × Hb (g/dL) × SvO_2 + 0.0031 × PvO_2 (mmHg), where PvO_2 is the hindlimb venous oxygen tension, and SvO_2 is the hindlimb venous oxygen saturation. ΔO_2 was calculated as $CaO_2 - CvO_2$. Hindlimb VO_2 ($\dot{V}O_2$)was calculated as the product of Q (leg blood flow) and ΔO_2. Hindlimb oxygen delivery (DO_2) was calculated by using the formula: DO_2 (mL/min) = $CaO_2 \times Q \times 10$. Hindlimb oxygen extraction (OE) was defined as: OE = $\dot{V}O_2/DO_2$.

ΔPCO_2 was calculated as the difference between the hindlimb venous carbon dioxide tension ($PvCO_2$) and hindlimb arterial PCO_2 ($PaCO_2$). In the original study, the hindlimb difference between venous-to-arterial CO_2 content ($CvCO_2 - CaCO_2$) was calculated with the McHardy equation (as proposed by Neviere et al.[12]): $\Delta CCO_2 = 11.02 \times [(PvCO_2)^{0.396} - (PaCO_2)^{0.396}] - (15 - Hb) \times 0.015 \times (PvCO_2 - PaCO_2) - (95 - SaO_2) \times 0.064$. However, the most used equation to calculate the blood CO_2 content is the Douglas equation[13], which includes pH:

$$\begin{aligned}&\text{Blood } CO_{2D} \text{ content [blood Douglas } CCO_2 \text{ (mL)]}\\&\quad = \text{Plasma } CCO_2 \times [1-0.0289 \times (Hb)/(3.352-0.456 \times SO_2) \times (8.142-pH)]\end{aligned}$$

where plasma $CCO_2 = 2.226 \times S \times \text{plasma } PCO_2 \times (1 + 10^{pH-pK'})$, CCO_2 is CO_2 content, SO_2 is oxygen saturation, S is the plasma CO_2 solubility coefficient, and pK′ is the apparent pK.

S and pK′ were calculated as follow:

$$S = 0.0307 + [0.00057 \times (37-T)] + [0.00002 \times (37-T)^2]$$

and

$$pK' = 6.086 + [0.042 \times (7.4-pH)] + ((38-T) \times \{0.00472 + [0.00139 \times (7.4-pH)]\})$$

where T is the temperature expressed as °C.

The difference between venous-to-arterial CCO_2 calculated with the Douglas equation was: $\Delta CCO_{2D} = CvCO_{2D} - CaCO_{2D}$.

To investigate the metabolic acidosis and Haldane effects on the PCO_2/CCO_2 relationship, default (Def) values of blood CCO_2 were calculated with the Douglas's equation by using only the resting values of pH and SvO_2 for each dog as following: DefpH-ΔCCO_{2D} = DefpH-$CvCO_{2D}$ − DefpH-$CaCO_{2D}$, and DefSvO_2-ΔCCO_{2D} = DefSvO_2-$CvCO_{2D}$ − DefSvO_2-$CaCO_{2D}$.

Leg blood flow, DO_2, and $\dot{V}O_2$ were reported per kilogram of muscle mass.

We also calculated the hindlimb $\Delta PCO_2/\Delta O_2$, $\Delta CCO_2/\Delta O_2$, and $\Delta CCO_{2D}/\Delta O_2$ ratios.

Experimental protocol. The experimental model was already described previously[1]. After all pressures and flows were stable for at least 30 min, the experiment began with a 30-min control period, during which measurements were obtained every 15 min. In the progressive ischemic hypoxia (IH) group, Q was then decreased every 15 min to produce Q values of ~ 60, 45, 40, 30, 20, 15, and 10 mL/kg/min. In the hypoxic hypoxia (HH) group, Q was set at 60 mL/kg/min and limb DO_2 was reduced by decreasing arterial PO_2 from 100 to ~ 15 mmHg (i.e., CaO_2 of 17 to 2 mL O2/100 mL) in eight steps at 15-min intervals. A flow rate of 60 mL/kg/min was chosen for progressive hypoxia because it is within the range of resting blood flow to normal skeletal muscle and for the practical reason that a moderate flow was necessary to achieve the desired low PO_2 values using the membrane oxygenator. Oxygen and CO_2-derived variables were determined every 15 min, 13 min after the change in hindlimb arterial flow or PO_2.

For each experiment, regression lines were fitted to the delivery independent and dependent portions of the delivery-uptake curve using a dual-line, least squares method[14]. The intercept of these two lines defined the critical DO_2 (DO_2crit), that is, the delivery at which $\dot{V}O_2$ began to fall with any further decline in DO_2.

Statistical analysis. All data are expressed as mean ± SEM after assessed for normality using the Kolmogorov–Smirnov test.

Comparisons of data within and between groups were performed using a mixed ANOVA. Post-hoc paired and unpaired *t* tests were used, as appropriate, for one-time comparisons. The Bonferroni method was used to adjust for multiple comparisons.

Statistical analysis was performed using GraphPad Prism 6.0 software for windows (San Diego, California, USA). $p < 0.006$ and $p < 0.007$ were considered statistically significant for the between-group and within-group (with the baseline) comparisons, respectively. All reported *p* values are two-sided.

Results

Systemic hemodynamics and oxygen-derived variables remain unchanged throughout the protocol with no differences between the IH and HH models (Supplemental Digital Content 2, Table S1).

In both groups, the $\dot{V}O_2/DO_2$ graph depicts the typical biphasic relationship (Supplemental Digital Content 3, Figure S1). There was no statistically significant difference between the mean DO_{2Crit} in the HH and IH models (6.9 ± 0.6 vs. 6.0 ± 0.5 mL/kg/min, $p = 0.28$, respectively). SvO_2 at DO_{2Crit} was not statistically different between the two groups (25 ± 1.7% in HH vs. 26 ± 1.5% in IH, $p = 0.66$). However, for the lower DO_2 values, SvO_2 was significantly higher in the IH model than in the HH group (Supplemental Digital Content 4, Figure S2). EO_2 at DO_{2Crit} was significantly higher in the IH group than in the HH model (74 ± 2% vs. 60 ± 4%, $p = 0.01$) and increased continuously and similarly in both groups (Supplemental Digital Content 5, Figure S3). ΔPCO_2 risen significantly in the IH model and did not change in the HH model (Supplemental Digital Content 6, Figure S4).

Time course of venous-to-arterial CCO_2 difference. ΔCCO_2 calculated with the McHardy equation increased progressively along with the decrease in DO_2 in the IH group but remained unchanged and even significantly decreased at the lowest DO_2 value on the HH group (Fig. 1A). At DO_{2Crit}, ΔCCO_2 was significantly

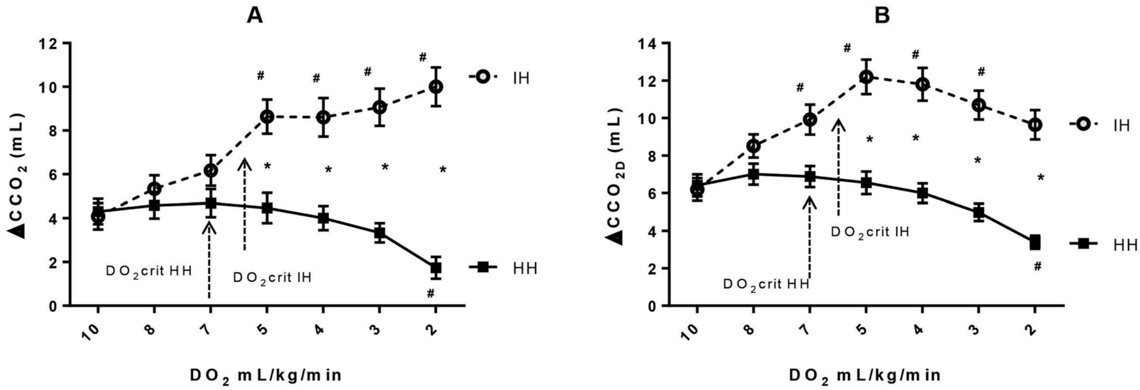

Figure 1. Hindlimb venous-to-arterial CO_2 content difference (ΔCCO_2) calculated with McHardy equation (**A**) and with Douglas equation (ΔCCO_{2D}) (**B**) as a function of hindlimb oxygen delivery (DO_2) for ischemic hypoxia model (IH) and hypoxic hypoxia model (HH). $^{*}p<0.006$ vs. HH, $^{\#}p<0.007$ vs. baseline, mixed ANOVA.

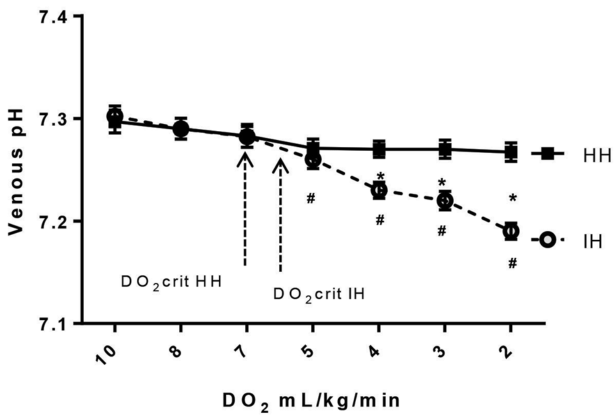

Figure 2. Hindlimb venous pH as a function of hindlimb oxygen delivery (DO_2) for ischemic hypoxia model (IH) and hypoxic hypoxia model (HH). $^{*}p<0.006$ vs. HH, $^{\#}p<0.007$ vs. baseline, mixed ANOVA.

higher in the IH group than in the HH group (7.5 ± 0.66 vs. 4.6 ± 0.5 mL, $p=0.006$, respectively), and it was significantly different from the baseline only in the IH group ($p=0.0023$).

ΔCCO_{2D} calculated with the Douglas equation, in the IH group, increased with the decrease in DO_2 down to DO_{2crit}. However, beyond DO_{2crit}, ΔCCO_{2D} started to decrease with the further decline in DO_2 to become not significantly different from its baseline value at the lowest value of DO_2 (Fig. 1B). In the HH group, ΔCCO_{2D} had the same pattern as ΔCCO_2 calculated with the McHardy equation (Fig. 1A,B), which remained unchanged in parallel with the decreases in DO_2 to become significantly lower than its baseline ($p<0.001$) only at the end of the experiment. At DO_{2crit}, ΔCCO_{2D} was greater in the IH group compared to the HH group (11.0 ± 0.88 vs. 7.0 ± 0.56 mL, $p=0.003$, respectively), and it was significantly higher than its baseline value ($p<0.001$) only in the IH group (Fig. 1B).

pH and Haldane effects on the PCO_2/CCO_2 relationship. Hindlimb venous pH (pHv) remained unchanged with the decline in DO_2 down to DO_{2crit} in both groups (Fig. 2). However, beyond DO_{2crit}, pHv decreased significantly only in the IH group and remained stable in the HH group (Fig. 2).

The venous CCO_2 calculated, with the Douglas equation, by acknowledging the changes in pHv ($CvCO_{2D}$) increased first with the rise in $PvCO_2$, but then after, it stabilized despite further increases in $PvCO_2$, due to the fall in pHv. Eventually, despite the continuously increasing $PvCO_2$, $CvCO_{2D}$ decreased due to the marked decline in pHv (Fig. 3). On the contrary, there was almost a linear increase in DefpH-$CvCO_{2D}$ (without accounting for the changes in pHv) with the increase in $PvCO_2$ (Fig. 4). Also, DefpH-ΔCCO_{2D} increased linearly with the decreases in DO_2 in the IH group, while it remained unchanged in the HH group (Supplemental Digital Content 7, Figure S5).

The relationship between $PvCO_2$ and CCO_2 calculated without accounting for the changes in SvO_2 was the same as that if we acknowledged the variations in SvO_2 (Supplemental Digital Content 8, Figure S6).

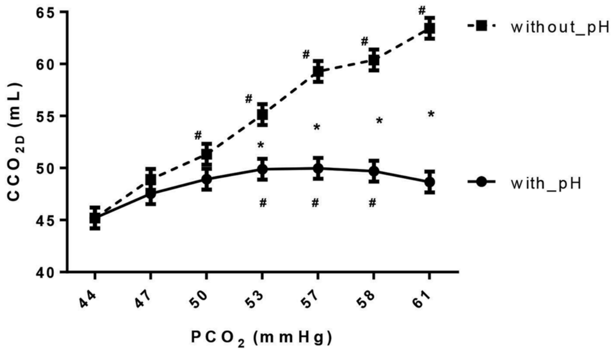

Figure 3. Hindlimb venous CO_2 content (CCO_2) as a function of hindlimb venous PCO_2 for CCO_2 calculated with accounting for pH changes (with_pH) and without accounting for pH changes (without_pH) using Douglas equation (CCO_{2D}). *$p < 0.006$ vs. HH, #$p < 0.007$ vs. baseline, mixed ANOVA.

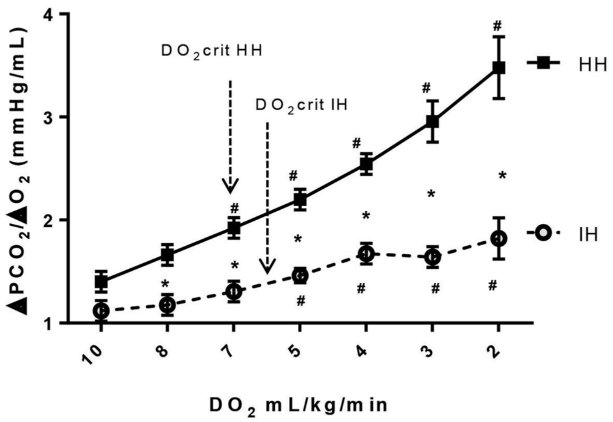

Figure 4. Hindlimb venous-to-arterial PCO_2 difference (ΔPCO_2) over the arterial-to-venous O_2 difference (ΔO_2) ratio ($\Delta PCO_2/\Delta O_2$) as a function of hindlimb oxygen delivery (DO_2) for ischemic hypoxia model (IH) and hypoxic hypoxia model (HH). At DO_2crit, $\Delta PCO_2/\Delta O_2$ was significantly higher in HH model (1.82 ± 0.09) than IH model (1.39 ± 0.06). *$p < 0.006$ vs. HH, #$p < 0.007$ vs. baseline, mixed ANOVA.

Time course of $\Delta PCO_2/\Delta O_2$, $\Delta CCO_2/\Delta O_2$, and $\Delta CCO_{2D}/\Delta O_2$ ratios. ΔO_2 increased significantly in the IH and decreased in the HH in parallel with the decreases in DO_2 (Supplemental Digital Content 9, Figure S7).

At DO_{2crit}, $\Delta PCO_2/\Delta O_2$ ratio was significantly higher in the HH group than in the IH group (1.82 ± 0.09 mmHg/mL vs. 1.39 ± 0.06 mmHg/mL, $p = 0.002$, respectively). In both groups, $\Delta PCO_2/\Delta O_2$ ratio increased significantly only after reaching DO_{2crit} (Fig. 4). Also, the increase in $\Delta PCO_2/\Delta O_2$ ratio was significantly higher in the HH than in the IH group.

$\Delta CCO_2/\Delta O_2$ ratio increased after DO_{2crit} was reached in both groups, with a trend to decrease by the end of the experiment in the HH group (Supplemental Digital Content 10, Figure S8). At DO_{2crit}, there was no significant difference between the two groups (IH: 0.59 ± 0.02 vs. HH: 0.67 ± 0.03, $p = 0.05$).

In both groups, $\Delta CCO_{2D}/\Delta O_2$ ratio increased significantly after reaching DO_{2crit}. However, in the HH group, at lower values of DO_2, $\Delta CCO_{2D}/\Delta O_2$ ratio started to decline but remained significantly higher than its baseline value. In the IH group, beyond DO_{2crit}, $\Delta CCO_{2D}/\Delta O_2$ ratio begun to decrease at a higher value of DO_2 than in the HH group, to become not significantly different from its baseline value at the end of the experiment (Fig. 5). At DO_{2crit}, $\Delta CCO_{2D}/\Delta O_2$ was not significantly different between the two groups (0.87 ± 0.05 for IH vs. 1.01 ± 0.06 for HH, $p = 0.09$).

In both groups, DefpH-$\Delta CCO_{2D}/\Delta O_2$ (without accounting for pH changes) increased similarly and linearly in parallel with the decrease in DO_2 (Supplemental Digital Content 11, Figure S9). The increase in DefpH-$\Delta CCO_{2D}/\Delta O_2$ in IH occurred before reaching DO_{2crit}.

Discussion

The main findings of our study were that: (1) in both groups, $\Delta PCO_2/\Delta O_2$ as well as $\Delta CCO_2/\Delta O_2$, and $\Delta CCO_{2D}/\Delta O_2$ increases significantly in parallel with the decreases in DO_2 only after reaching DO_{2crit}; (2) beyond DO_{2crit}, the time course of $\Delta PCO_2/\Delta O_2$ ratio was different from that of $\Delta CCO_{2D}/\Delta O_2$ or $\Delta CCO_2/\Delta O_2$ ratio, in both

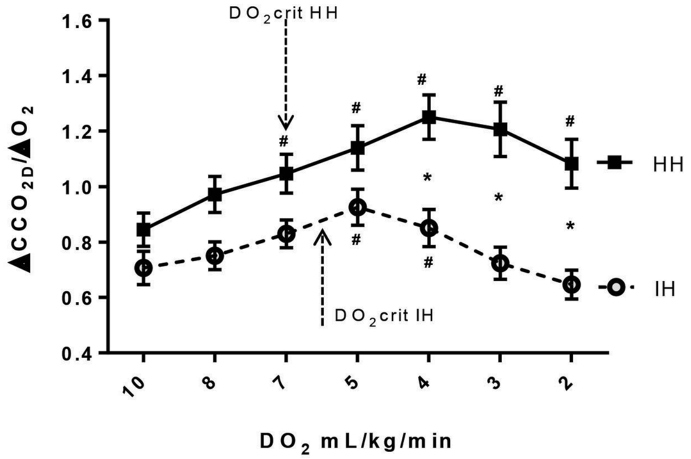

Figure 5. Hindlimb venous-to-arterial CO_2 content difference calculated with Douglas equation (ΔCCO_{2D}) over the arterial-to-venous O_2 difference (ΔO_2) ratio ($\Delta CCO_{2D}/\Delta O_2$) as a function of hindlimb oxygen delivery (DO_2) for ischemic hypoxia model (IH) and hypoxic hypoxia model (HH). At DO_2crit, there was no significantly difference between HH model (1.01 ± 0.06) and IH model (0.87 ± 0.05). $^*p < 0.006$ vs. HH, $^\#p < 0.007$ vs. baseline, mixed ANOVA.

groups; (3) metabolic acidosis, but not Haldane effect influenced significantly the PCO_2/CCO_2 relationship explaining the discrepancy between ΔPCO_2 and ΔCCO_{2D}; (4) the method of CCO_2 calculation had a considerable impact on the results and yielded different conclusions.

Anaerobic metabolism occurrence is usually due to cellular hypoxia[15]. Whenever oxygen delivery decreases relative to demand, and the compensatory mechanism is exhausted, extra-mitochondrial anaerobic glycolysis occurs, and lactic acidosis develops[16]. We aimed to investigate if $\Delta PCO_2/\Delta O_2$ and $\Delta CCO_{2D}/\Delta O_2$ could reflect the development of anaerobic metabolism in two regional models of tissue hypoxia: IH, where the oxygen delivery progressively decreased by decreasing the blood flow, and HH, where the blood flow was maintained unchanged, and the oxygen delivery was reduced by decreasing the arterial oxygen content.

In experimental conditions of tissue hypoxia, the drop in VO_2 leads to decreased total VCO_2 generation, mainly related to the decrease in aerobic CO_2 production. However, under situations of hypoxia, tissue CO_2 increases as hydrogen ions generated by anaerobic sources of energy (hydrolysis of high-energy phosphates) are buffering by bicarbonate existing in the cells (anaerobic CO_2 production)[17]. Therefore, VCO_2 being reduced less than VO_2, the RQ (VCO_2/VO_2) should increase. Accordingly, the increase in RQ has been shown to be a useful marker of global tissue hypoxia[18,19]. Indeed, Groeneveld et al.[18] observed, in an experimental model of a graded increase in positive end-expiratory pressure-induced a decrease in cardiac output and oxygen delivery in pigs, that the decline in VCO_2 (by $21 \pm 2\%$) was less than in VO_2 (by $27 \pm 2\%$).

However, airway RQ measurement necessitates a specific monitoring device (indirect calorimetry) that many hospitals might not have. Recently, there has been a growing interest in the $\Delta PCO_2/\Delta O_2$ ratio as a surrogate of the RQ to detect the development of global anaerobic metabolism in critically ill patients[3–7]. Indeed, several studies found an association between increased $\Delta PCO_2/\Delta O_2$ ratio and hyperlactatemia[5] and decreased lactate clearance[6,7], which were taken as markers of anaerobic metabolism activation. We[4] and other authors[3] have also shown that $\Delta PCO_2/\Delta O_2$ ratio had an excellent ability to detect the presence of VO_2/DO_2 dependency phenomenon, better than central venous oxygen saturation and blood lactate levels, in septic shock patients. Recently, Mesquida et al.[20] reported an association between $\Delta PCO_2/\Delta O_2$ ratio and ICU mortality in septic shock patients. In contrast, in other studies, $\Delta PCO_2/\Delta O_2$ was unable to predict hyperlactatemia, poor lactate clearance, or VO_2/DO_2 dependency and was not associated with outcome in septic shock or cardiac surgery patients[9,21–23]. Thus, the relationship between $\Delta PCO_2/\Delta O_2$ and the presence of tissue hypoxia is controversial.

Indeed, the use of $\Delta PCO_2/\Delta O_2$ ratio as a surrogate of RQ supposes that the PCO_2/CCO_2 relationship is quasi-linear, which may be true over the physiological range of PCO_2[24]. However, this relationship can be influenced by the degree of metabolic acidosis[25], hematocrit[26], and oxygen saturation (Haldane effect)[8,27], and it becomes nonlinear if these factors change[28]. Indeed, severe metabolic acidosis, low hematocrit, and high oxygen saturation can increase PCO_2 for a given CCO_2 since less CO_2 is bound to hemoglobin[8]. Thus, ΔPCO_2 and $\Delta PCO_2/\Delta O_2$ ratio might be increased due to several factors unrelated to the blood flow and anaerobic metabolism. We found that metabolic acidosis influenced the PCO_2/CCO_2 relationship significantly. Indeed, when the changes in pHv were ignored, the PCO_2/CCO_2 relationship was almost linear (Fig. 3). However, CCO_2 was not linearly related to PCO_2 when the changes in pH were acknowledged. In fact, PCO_2 and CCO_2 changed in opposite directions as metabolic acid was added to the blood by the hypoxic cells (Fig. 3). That is because metabolic acidosis causes plasma and red blood cell CCO_2 and bicarbonates to decrease[29]. In our study, the Haldane effect did not influence the PCO_2/CCO_2 relationship as the latter was the same, taking into account or not for the changes in SvO_2 (Supplemental Digital Content 8, Figure S6).

Our findings suggest that, in situations with moderate/severe metabolic acidosis, an elevated ΔPCO_2 might not reflect only low or inadequate blood flow but could also be ascribed to modifications of the CO_2–hemoglobin dissociation curve. Our results are in line with previous studies. Indeed, Sun et al.[29] found that, in healthy subjects, during heavy exercise, changes in pH had a significant influence on the PCO_2/CCO_2 relationship with CCO_2 not linearly related to PCO_2 and even varied in opposite directions after the lactic acidosis threshold was reached. However, in that study, changes in SO_2 (Haldane effect) had a minor influence on the PCO_2/CCO_2 relationship. Also, in septic shock patients, Mesquida et al.[20] observed that pH was the only best predictor of the discrepancy found between $\Delta PCO_2/\Delta O_2$ and $\Delta CCO_{2D}/\Delta O_2$; venous oxygen saturation (Haldane effect) had a minimal effect.

We observed that $\Delta PCO_2/\Delta O_2$, and $\Delta CCO_{2D}/\Delta O_2$ significantly increased at DO_{2crit} and not before (Figs. 4 and 5), suggesting that these variables were able to depict the occurrence of oxygen supply dependency (DO_{2crit}) in both IH and HH groups. The increases in these variables were mainly due to the decline in ΔO_2 in the HH group and the rise in ΔPCO_2 and ΔCCO_2 in the IH group induced by the decrease in blood flow. In contrast, in an experimental study of hemodilution model of tissue hypoxia, Dubin et al.[8] found that $\Delta PCO_2/\Delta O_2$ significantly increased before the fall in VO_2 and the sharp increase in RQ (measured by indirect calorimetry), and thus, it was a misleading indicator of anaerobic metabolism. The authors explained this finding by the effects of low hemoglobin on the CO_2–hemoglobin dissociation curve[8]. However, it is hard to compare these results together as the two tissue hypoxia models (HH and hemodilution) are different. Indeed, the effects of anemia on the CO_2–hemoglobin dissociation curve could be different from that of the low oxygen saturation (Haldane effect). Also, the magnitude of the decrease in venous oxygen saturation would be much more pronounced in the HH model, where the flow was maintained constant, than in the hemodilution model, where cardiac output increased by 126%[8]. Beyond DO_{2crit}, we observed a discrepancy between the evolutions of $\Delta PCO_2/\Delta O_2$ and $\Delta CCO_{2D}/\Delta O_2$ in both groups (Figs. 4 and 5). That might be explained by the different behavior of ΔPCO_2 and ΔCCO_{2D} at lower DO_2 values. Indeed, in the IH group, these two variables changed in opposite directions: ΔPCO_2 continued to increase, whereas ΔCCO_{2D} fell caused by metabolic acidosis (decreases in bicarbonate levels). In the HH model, ΔPCO_2 remained unchanged, whereas ΔCCO_{2D} decreased at lower DO_2 values (Fig. 1B and Supplemental Digital Content 6, Figure S4). Therefore, below DO_{2crit}, and at very low DO_2 values, $\Delta PCO_2/\Delta O_2$ ratio is confounded by the changes in the CO_2–hemoglobin curve induced by metabolic acidosis, and it does not reliably reflect the oxygen supply dependency phenomenon and the activation of anaerobic metabolism, especially in the IH tissue hypoxia model. However, in clinical practice, in such cases with very low DO_2, the clinical diagnosis of tissue hypoxia would be obvious without the need for such markers.

It is worth to note that the method of calculation of the difference in CCO_2 matters as the McHardy equation[12], and Douglas equation[13] yielded different findings (Figs. 5 and Supplemental Digital Content 10, Figure S8). However, we think that the Douglas equation is much more used in research papers, and more accurate as it accounts for much more factors such as pH.

There is no reported data, in the literature, on the behavior of $\Delta CCO_{2D}/\Delta O_2$ ratio beyond DO_{2crit} at very low DO_2 values. This ratio tended to decrease in both tissue hypoxia models, even in the presence of anaerobic CO_2 production. It is possible that in case of advanced tissue hypoxia with massive decreases in VO_2, the anaerobic sources of CO_2 becoming much less important than the dramatically decreased aerobic ones leading to a reduction in VCO_2/VO_2 ratio.

We acknowledge several limitations to our study. First, our study was a secondary analysis that is subject to inherent limitations. Second, computation of CCO_2 is subject to an important potential risk of measurement errors due to the number of variables included in the equation[30] that might amplify during the calculation of ΔCCO_{2D}. Nevertheless, $\Delta CCO_{2D}/\Delta O_2$ ratio was already shown to be associated with mortality in septic shock patients[9], suggesting that the influence of measurement errors might be limited.

Conclusions

In both IH and HH regional models of tissue hypoxia, $\Delta PCO_2/\Delta O_2$ and $\Delta CCO_{2D}/\Delta O_2$ ratios both widened significantly only at the beginning of oxygen supply dependency. The hypoxic tissue hypoxia model yielded higher increases in $\Delta PCO_2/\Delta O_2$ than the IH model. At advanced stages of tissue hypoxia (very low DO_2), $\Delta PCO_2/\Delta O_2$ did not only reflect the ongoing anaerobic metabolism, but it was confounded by the effects of metabolic acidosis on the CO_2–hemoglobin dissociation curve, and then it should be interpreted with caution. For clinical practice, in severe metabolic acidosis situations, elevated ΔPCO_2 may not reflect the degree of tissue hypoperfusion. In these cases, calculating the difference in CCO_2 with the Douglas equation is advisable.

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References

1. Vallet, B., Teboul, J. L., Cain, S. & Curtis, S. Venoarterial CO(2) difference during regional ischemic or hypoxic hypoxia. *J. Appl. Physiol.* **89**, 1317–1321 (2000).
2. Gutierrez, G. A mathematical model of tissue-blood carbon dioxide exchange during hypoxia. *Am. J. Respir. Crit. Care Med.* **169**, 525–533 (2004).
3. Monnet, X. *et al.* Lactate and venoarterial carbon dioxide difference/arterial–venous oxygen difference ratio, but not central venous oxygen saturation, predict increase in oxygen consumption in fluid responders. *Crit. Care Med.* **41**, 1412–1420 (2013).
4. Mallat, J. *et al.* Ratios of central venous-to-arterial carbon dioxide content or tension to arteriovenous oxygen content are better markers of global anaerobic metabolism than lactate in septic shock patients. *Ann. Intensive Care* **6**, 10 (2016).
5. Mekontso-Dessap, A. *et al.* Combination of venoarterial PCO_2 difference with arteriovenous O_2 content difference to detect anaerobic metabolism in patients. *Intensive Care Med.* **28**, 272–277 (2002).

6. Mesquida, J. *et al.* Central venous-to-arterial carbon dioxide difference combined with arterial-to-venous oxygen content difference is associated with lactate evolution in the hemodynamic resuscitation process in early septic shock. *Crit. Care* **19**, 126 (2015).
7. He, H. W., Liu, D. W., Long, Y. & Wang, X. T. High central venous-to-arterial CO_2 difference/arterial–central venous O_2 difference ratio is associated with poor lactate clearance in septic patients after resuscitation. *J. Crit. Care* **31**, 76–81 (2016).
8. Dubin, A. *et al.* Venoarterial PCO_2-to-arteriovenous oxygen content difference ratio is a poor surrogate for anaerobic metabolism in hemodilution: An experimental study. *Ann. Intensive Care* **7**, 65 (2017).
9. Ospina-Tascón, G. A. *et al.* Combination of arterial lactate levels and venous–arterial CO_2 to arterial–venous O_2 content difference ratio as markers of resuscitation in patients with septic shock. *Intensive Care Med.* **41**, 796–805 (2015).
10. Teboul, J. L. & Scheeren, T. Understanding the Haldane effect. *Intensive Care Med.* **43**, 91–93 (2016).
11. Cain, S. M. & Chapler, C. K. Oxygen extraction by canine hindlimb during hypoxic hypoxia. *J. Appl. Physiol.* **46**, 1023–1028 (1979).
12. Neviere, R. *et al.* Carbon dioxide rebreathing method of cardiac output measurement during acute respiratory failure in patients with chronic obstructive pulmonary disease. *Crit. Care Med.* **22**, 81–85 (1994).
13. Douglas, A. R., Jones, N. L. & Reed, J. W. Calculation of whole blood CO_2 content. *J. Appl. Physiol.* **65**, 473–477 (1988).
14. Samsel, R. & Schumacker, P. T. Determination of the critical O_2 delivery from experimental data: Sensitivity to error. *J. Appl. Physiol.* **64**, 2074–2082 (1988).
15. Cohen, P. J. The metabolic function of oxygen and biochemical lesions of hypoxia. *Anesthesiology* **37**, 148–177 (1972).
16. Ronco, J. J. *et al.* Identification of the critical oxygen delivery for anaerobic metabolism in critically ill septic and nonseptic humans. *JAMA* **270**, 1724–1730 (1993).
17. Mallat, J., Lemyze, M., Tronchon, L., Vallet, B. & Thevenin, D. Use of venous-to-arterial carbon dioxide tension difference to guide resuscitation therapy in septic shock. *World J. Crit. Care Med.* **5**, 47–56 (2016).
18. Groeneveld, A. B., Vermeij, C. G. & Thijs, L. G. Arterial and mixed venous blood acid-base balance during hypoperfusion with incremental positive end- expiratory pressure in the pig. *Anesth. Analg.* **73**, 576–582 (1991).
19. Cohen, I. L., Sheikh, F. M., Perkins, R. J., Feustel, P. J. & Foster, E. D. Effect of hemorrhagic shock and reperfusion on the respiratory quotient in swine. *Crit. Care Med.* **23**, 545–552 (1995).
20. Mesquida, J. *et al.* Respiratory quotient estimations as additional prognostic tools in early septic shock. *J. Clin. Monit. Comput.* **32**, 1065–1072 (2018).
21. Muller, G. *et al.* Prognostic significance of central venous-to-arterial carbon dioxide difference during the first 24 hours of septic shock in patients with and without impaired cardiac function. *Br. J. Anaesth.* **119**, 239–248 (2017).
22. Abou-Arab, O. *et al.* The ratios of central venous to arterial carbon dioxide content and tension to arteriovenous oxygen content are not associated with overall anaerobic metabolism in postoperative cardiac surgery patients. *PLoS ONE* **13**, e0205950 (2018).
23. Fischer, M. O. *et al.* Assessment of macro- and micro-oxygenation parameters during fractional fluid infusion: A pilot study. *J. Crit. Care* **40**, 91–98 (2017).
24. Cavaliere, F. *et al.* Comparison of two methods to assess blood CO_2 equilibration curve in mechanically ventilated patients. *Respir. Physiol. Neurobiol.* **146**, 77–83 (2005).
25. Jakob, S. M., Groeneveld, A. B. & Teboul, J. L. Venous–arterial CO2 to arterial–venous O_2 difference ratio as a resuscitation target in shock states?. *Intensive Care Med.* **41**, 91–93 (2015).
26. Chiarla, C. *et al.* Significance of hemoglobin concentration in determining blood CO_2 binding capacity in critical illness. *Respir. Physiol. Neurobiol.* **172**, 32–36 (2010).
27. Jakob, S. M., Kosonen, P., Ruokonen, E., Parviainen, I. & Takala, J. The Haldane effect—An alternative explanation for increasing gastric mucosal PCO_2 gradients?. *Br. J. Anaesth.* **83**, 740–746 (1999).
28. Mchardy, G. J. The relationship between the differences in pressure and content of carbon dioxide in arterial and venous blood. *Clin. Sci.* **32**, 299–309 (1967).
29. Sun, X. G., Hansen, J. E., Stringer, W. W., Ting, H. & Wasserman, K. Carbon dioxide pressure-concentration relationship in arterial and mixed venous blood during exercise. *J. Appl. Physiol.* **90**, 1798–1810 (2001).
30. Mallat, J. *et al.* Repeatability of blood gas parameters, PCO_2 gap, and PCO_2 gap to arterial-to-venous oxygen content difference in critically ill adult patients. *Medicine (Baltimore)* **94**, e415 (2015).

Author contributions

J.M., and B.V. designed the study. J.M. conducted statistical analyses. J.M. and B.V. participated in manuscript writing and reviewing. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to J.M.

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