Appropriate timing of blood sampling for blood gas analysis in the ventilated rabbit

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Abstract
Background: Arterial and venous blood gas analyses (BGAs) are essential to evaluate devices that measure biological oxygenation. The appropriate timing of blood sampling for BGA after respiratory rate (RR) change in animal experiments has not been reported. This study investigated the appropriate timing of blood sampling for BGA in ventilated rabbits and whether venous samples are an alternative to arterial samples.

Materials and methods: Under general anesthesia, 14 rabbits (body weight, 3.02 ± 0.09 kg) were ventilated and their RR was changed (40/min, 30/min, and 20/min). Blood was sampled through cervical arterial and venous catheters. Experiment 1: in seven rabbits, arterial BGA was measured at 0, 0.5, 1, 2, 3, 5, 10, 15, and 20 min after the RR change. Experiment 2: in seven different rabbits, simultaneous arterial and venous BGA were measured at 0, 2, 5, 10, 15, and 20 min after the RR change.

Results: Oxygen partial pressure (PO2) and saturation (SO2) of the arterial blood stabilized 0.5 min after the RR changed. In venous BGA, no index stabilized during observation. The arterial and venous values of the carbon dioxide partial pressure (PCO2) and pH had significant correlations (arterial PCO2 = 0.9316 × venous PCO2−4.4425 [r = 0.9178]; arterial pH = 1.0835 × venous pH−0.5795 [r = 0.9453]).

Conclusions: In ventilated rabbits, arterial PO2 and SO2 stabilized in 0.5 min. No venous value stabilized after the RR change. Only the PCO2 and pH of venous samples may be an alternative to arterial samples under the defined formula.

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Introduction
The development of new medical devices has driven the progress of intensive medicine. Medical monitoring devices such as the electrocardiograph and pulse oximeter are broadly used to assess a patient’s condition noninvasively and to decide whether medical treatments are needed. In clinical settings, the pulse oximeter is widely used. This

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simple device monitors oxygen saturation in arterial blood and provides information about the respiratory state. However, some studies show limitations in the accuracy of the pulse oximeter, and blood gas analysis (BGA) is recognized as the most reliable index for respiratory condition.

Pulse oximeter and real-time monitoring of oxygenation using similar technology (i.e., near-infrared spectroscopy [NIRS]) are also used in intensive care for neonates with congenital or peculiar diseases. Relative to the blood gas analyzer, these devices sometimes cause improper readings because of hemodynamic collapse due to hypothermia, hypoxia, or acute anemia; skin color; and brightness around the sensor. A dramatic change in circulation occurs in neonates because of hypothermia, hypoxia, or subsequent oxygen therapy. In these specific conditions, the accuracy of a pulse oximeter and NIRS may be unreliable. To assess accuracy of these devices, in vivo experiments are an important step to prove the utility of these devices, although experiments in humans are sometimes unacceptable for ethical reasons. Therefore, animal experiments remain important.

BGAs in experimental animals are essential to improve oxygenation monitoring systems; however, no study exists concerning the appropriate timing after the change in the settings of a ventilator. In several studies that used pulse oximetry in rabbits, arterial blood gas was sampled at 2 min after the setting of artificial ventilation. The investigators confirmed that the values of the pulse oximeter were stabilized at this point, but they did not confirm that the values of arterial blood gas were stabilized.

In human arterial BGA, the time to reach oxygenation equilibrium after changing respiratory conditions is reportedly 7-20 min. In general, cardiac output is a linear factor of oxygen delivery in tissues. Edwards et al. report that the average cardiac output of adult rabbits weighing 2.0 kg is 350 mL/min. The average human cardiac output in male adults weighing 70 kg is 5 L/min. Rabbits have 2.39 times larger cardiac output per kilogram of body weight. Therefore, we set a hypothesis that the time to reach oxygenation equilibrium in rabbits are 3-8.4 min because of the difference of cardiac output between human and rabbits.

Venous blood gas seemed to be an important factor to discuss oxygenation and oxygen consumption; however, the behavior of venous blood oxygenation is unclear. The effect of arterial and venous blood gas values of tissue oxygen saturation, as measured by NIRS in medical practice, is controversial. Arterial and venous BGA is essential for further research. To the best of our knowledge, there has been no study on simultaneous arterial and venous gas analysis.

In this study, we therefore used rabbits, which have a similar body weight as a human neonate and are widely used as models of acute respiratory disease and validation of pulse oximeters. The aim of the study was to investigate the appropriate timing of blood sampling for BGA in a ventilated rabbit model and to investigate whether venous samples are an alternative to arterial samples.

### Materials and methods

#### Ethical approval

All animal experiments were approved by the Animal Experimentation Committee at the National Defense Medical College (Tokorozawa, Saitama, Japan). The approval number is 13091.

#### Animal preparation

Fourteen adult 15-wk to 21-wk-old female Japanese White rabbits weighing 3.02 ± 0.089 kg (mean ± standard deviation) were used for the experiment. The animals were purchased from a laboratory animal supplier (Kitayama Labes Co, Ltd, Nagano, Japan) and housed singly in a cage in our animal facility more than 1 wk before the experiments to acclimate them to the environment. Constant room temperature (25°C) and humidity (50% ± 5%) were maintained with 12 h of light-dark cycle. They had ad libitum access to pellet food and water.

#### Anesthesia and monitoring

Anesthesia was induced by an intramuscular injection of mixture of 35 mg/kg ketamine (Ketalar; Daiichi Sankyo Co, Ltd, Tokyo, Japan) and 5 mg/kg xylazine (Sederac; Nippon Zenyaku Kogyo Co, Ltd, Fukushima, Japan) in the gluteal region. Figure 1 shows the experimental setup. After the eyelash reflex and pinch reflex disappeared, the rabbits were placed supine on a heating table (KN303-B; Natsume Seisakusho Co, Ltd, Tokyo, Japan), and the hair in the neck and left hindlimb were shaved. All surgical procedures were performed under sterile conditions. The rabbits were monitored by a pulse oximeter, a noninvasive manometer, and an electrocardiograph with three needle-type subcutaneous electrodes (Life Scope BSM-5192; Nihon Kohden Corp, Tokyo, Japan). The subcutaneous electrodes were placed at the right and left upper chest and left upper abdomen. A lead II electrocardiogram monitored the animals throughout the experiment. Blood pressure in the right lower thigh was noninvasively measured every 2.5 min by the manometer (Life Scope BSM-5192; Nihon Kohden Corp). The rabbits’ bodies were covered by a heating blanket (Hemothermic Monitor K020917; Harvard Apparatus, Holliston, MA) and their rectal temperature was maintained at 39.5°C ± 1.0°C.

A 23-gauge venous catheter (Surflo, SR-OT2419C; Terumo, Tokyo, Japan) was inserted in the left auricular vein, and physiological saline (Otsuka Pharmaceutical Co, Ltd, Tokushima, Japan) was infused at a rate of 6 mL/kg/h. An L-shaped incision was created in the neck region. A midline incision was made through the jaw to the jugular notch of the sternum and extended 3-cm horizontally to the right. A tracheotomy was performed 1-cm dorsal from the cricoid and a tracheal tube with a 4-mm internal diameter (RUSCH Safety Clear; Teleflex Medical OEM, Gurnee, IL) and a 5.3-mm external diameter was cut to 11 cm length and distal 4 cm of tube was inserted into trachea (Fig. 2A). Tracheal tube was connected to...
Y-Luer adapter (73-4121, Harvard Apparatus, Holloston, MA). Y-Luer adapter was connected to a ventilator (Volume Control 155-7058; Harvard Apparatus, Holliston, MA) via silicon tubes. All rabbits were artificially ventilated with room air at a respiratory rate (RR) of 40 per min, which was calculated by formula (1),\(^{26,27}\) assuming that the weight was 3.0 kg at the start of the experiments. The tidal volume was calculated by formula (2).\(^{26,27}\)

\[
\text{Respiration rate/min} = 53.5 \times (\text{body weight})^{-0.26} \quad (1)
\]

\[
\text{Tidal volume} = 0.0062 \times (\text{body weight})^{1.01} \quad (2)
\]

General anesthesia was maintained by 1.0% of sevoflurane (Sebofuren; Maruishi Pharmaceutical, Osaka, Japan) inhalation,\(^ {28}\) and muscular relaxation was maintained with a bolus intravenous infusion of 0.6 mg/kg rocuronium bromide (Eslax, 50 mg/5.0 mL; MSD Co, Tokyo, Japan). An additional 0.6 mg/kg bolus infusion of rocuronium bromide was added when any sign of spontaneous respiration appeared.\(^ {29}\) The concentration of sevoflurane was maintained within 0.5%-2.0% to maintain sufficient anesthesia\(^ {18}\) and minimum blood pressure (i.e., mean arterial pressure >40 mm Hg or systolic pressure >60 mm Hg\(^ {30}\) by noninvasive monometer or invasive monometer by arterial catheter).

Arterial and venous catheterization

Figure 2B shows the surgical view of catheterization procedure. We exposed the right carotid artery and identified the bifurcation of the right carotid artery and the right brachiocephalic artery. We used a microscope (ocular, ×10; magnification, ×0.8; Stemi 2000-C; Carl Zeiss Industrielle Messtechnik GmbH, Oberkochen, Germany) to incise the right carotid artery and to insert a 22-gauge catheter (Surflo, SR-OT2232C; Terumo, Tokyo, Japan), which connected to a three-way stopcock (R1-L; Top Co, Tokyo, Japan), barotolerance extension tube (S50; Hakko Co, Ltd, Nagano, Japan), barometer transducer (MP333N; Edward Lifesciences Co, Irvine, CA), barometer infuser (Mediquick Plus ME-ACS-223; Terumo, Tokyo, Japan), and a 500-mL saline bottle with 125 units of heparin to avoid coagulation.\(^ {31,32}\) To collect circulating blood, we placed the tip of a catheter at the origin of the right carotid artery from the brachiocephalic artery (i.e., the bifurcation point of the right carotid artery and brachiocephalic artery), as shown in Figure 2B.

We then exposed the right jugular vein running laterally and inserted a 20-gauge catheter (Surflo, SR-OT2032C; Terumo, Tokyo Japan) so that its tip was at the junction of the right jugular and subclavian vein, as shown in Figure 2B. The catheter and the tube were connected to the infusion bottle.
Sample collection and experiment protocol

Because the volume of samples necessary for a single BGA was 0.15 mL, we sampled the volume of 0.3 mL to allow for reanalysis. The total dead space of the catheter and three-way stopcock was 0.23 mL. We collected the samples, as follows: a volume of 0.5 mL (approximately twice the dead space) was removed; 0.3 mL of blood in a 1-mL heparin-rinsed syringe was sampled to measure the data. After sampling, 0.5 mL of blood in the “dead space” was retransfused, and 0.3 mL of heparinized saline was additionally infused to prevent coagulation. Michael et al. recommend that blood sampling should be limited to 15% of total blood volume for 24 h to prevent a rapid change in the physiological conditions. Because they reported that the total blood volume of rabbits was 56 mL/kg body weight, we calculated the limit of sampling as 25.2 mL/d for rabbits weighing 3 kg.

All BGAs were examined by a portable blood gas analyzer (i-STAT; Abbott Point of Care Inc, Princeton, NJ) with a cartridge (CG4+; Abbott Point of Care Inc, Princeton, NJ). The i-STAT gas analyzer is able to measure the partial pressure of oxygen (PO$_2$), partial pressure of carbon dioxide (PCO$_2$), and pH of the whole blood. The oxygen saturation (SO$_2$) concentration...
of bicarbonate ions (HCO₃⁻) and the base excess of extracellular fluid (BEecf) were calculated by formulas (3-5).

\[
\text{SaO}_2 = 100 \times \left( X^3 + 150X/(X^3 + 150X + 23400) \right)
\]

\[
X = \text{PO}_2 \times 10^{(0.48\text{pH} - 7.4) - 0.0013(\text{HCO}_3^- - 25)}
\] (3)

\[
\text{Log(HCO}_3^-) = \text{pH} + \text{log(}\text{PCO}_2) - 7.608
\] (4)

\[
\text{BEecf} = (\text{HCO}_3^-) - 24.8 + 16.2(\text{pH} - 7.40)
\] (5)

We set two experiments to perform step-by-step analysis. In experiment 1, we aimed to study appropriate timing of arterial blood sampling. In experiment 2, we aimed to study appropriate timing of venous blood sampling and to study correlation between arterial and venous blood gas. To fit two goals, we separated two experiments to avoid excess blood loss.

**Experiment 1: arterial BGA after RR change**

We changed the RR of the ventilator in seven rabbits, as indicated in Figure 3A. We defined steps 1-4 as follows. In steps 1 and 2, the RR phases were decreased from 40 per min to 30 per min and from 30 per min to 20 per min, respectively. Steps 3 and 4 were the recovery of phases from 20 per min to 30 per min and from 30 per min to 40 per min, respectively. We observed each step for 25 min.

According to previous reports in intensive care patients, the time to stabilization occurred 7-20 min after changing the respiratory condition. Therefore, we set the observation time after intubation to 20 min with RR of 40 per min. The point at the time of 20 min observation was defined as “time 0,” and blood sampling was started at this point. Table 1 summarizes the data of BGA of time 0 of step 1. Single arterial blood samples were collected at times 0.5, 1, 2, 3, 5, 10, 15, and 20 min after RR change in each step, and the BGA was examined (Fig. 3A). We used the value of 20 min in just the previous step as a substitute for the value of time 0. The total sample points were 33, and the total sample volume was 9.9 mL.

**Experiment 2: arterial and venous BGA after the RR change**

We changed the RR as described in experiment 1 and performed a simultaneous sampling of the arterial and venous...
blood. Figure 3B shows the protocol and sample points of experiment 2. Using seven rabbits, we collected 147 points of simultaneous sampling of the arterial and venous blood gas (i.e., 294 samples in total, which included 147 arterial samples and 147 venous samples; 42 samples per rabbit). After 20 min of observation at an RR of 40 per min, we measured the BGA and value as time 0. The arterial and venous blood gases were measured at 2, 5, 10, 15, and 20 min for each step. The difference of sample timing from experiment 1 is due to technical limitation of the procedure. The total sample points were 21, and total sample volume was 12.6 mL. The BGA were performed as soon as possible after sampling.

### Statistical analysis

In experiments 1 and 2, one-way repeated measures analysis of variance (i.e., one-way analysis of variance) was performed. The Tukey honest significant difference test was performed as a multiple comparison. We considered a “stable condition” by following the criteria: (1) a significant difference between the value at time 0 of each step and the value of the concerned point and (2) no significant difference between the values of the concerned point and the values of all subsequent points. In experiment 2, we additionally used regression analysis to assess the correlation of the arterial and venous data.

Statistical analyses were performed using commercial statistical software (Windows Excel Office 2013, Microsoft Co, Redmond, WA; and JMP Pro software, version 11.2.0, SAS Institute, Ltd, Cary, NC). Values of $P < 0.05$ were regarded as significant. Values are expressed as the mean ± standard deviation.

### Results

Fourteen rabbits were used in this study. Seven rabbits were used for arterial BGA (i.e., experiment 1), and seven rabbits were used for arterial and venous BGA (i.e., experiment 2). No rabbit died during the experiments. The data of arterial BGA at the starting point (i.e., RR = 40) are summarized in Table 1. The body weight of the 14 rabbits was 3.02 ± 0.09 kg. The results of experiments 1 and 2 are shown in Figures 4-6.

#### Experiment 1: arterial BGA after RR change

Figure 4 presents the arterial PO$_2$ (PaO$_2$), pH, PCO$_2$ (PaCO$_2$), and SO$_2$ (SaO$_2$) after the RR change. The time to stabilization in each item after the RR change are summarized in Table 2. The PaO$_2$ and SaO$_2$ became stable in 0.5 min for all steps.

The PaO$_2$ decreased from 94.8 ± 7.2 mm Hg to 84 ± 16.2 mm Hg in 0.5 min ($P = 0.0030$ for time 0.5 min versus time 0) in step 1. After 0.5 min, the values were statistically the same. In step 2, the PaO$_2$ similarly decreased from 83.4 ± 8.9 mm Hg to 66.3 ± 6.9 mm Hg ($P < 0.0001$) in 0.5 min and became stable after 0.5 min. In step 3, the PaO$_2$ increased from 65.9 ± 9.2 mm Hg to 81.3 ± 10.3 mm Hg ($P < 0.0001$) in 0.5 min. In step 4, the PaO$_2$ increased from 82.1 ± 5.9 mm Hg to 98.1 ± 10.3 mm Hg ($P < 0.0001$) in 0.5 min.

In step 1, the SaO$_2$ decreased from 98.0% ± 0.6%, and the stable point was 96.0% ± 2.3% in 0.5 min ($P = 0.0124$). In step 2, the SaO$_2$ decreased from 96.1% ± 1.5% to 91.6% ± 3.1% ($P < 0.0001$) in 0.5 min and became stable. In steps 3 and 4, the SaO$_2$ increased from 90.3% ± 4.1% to 94.9% ± 2.0% ($P < 0.0001$) and from 95.7% ± 1.1% to 98.1% ± 0.7% ($P = 0.0001$), respectively, and became stable after 0.5 min.

The PaCO$_2$ also became stable within 3 min for all steps: it stabilized by 2 min in step 1, 1 min in step 2, 3 min in step 3, and 0.5 min in step 4. In step 1, the PaCO$_2$ increased from 29.1 ± 3.3 mm Hg to 32.1 ± 5.7 mm Hg ($P = 0.0006$) in 2 min and then became stable. In step 2, the PaCO$_2$ increased from 32.9 ± 4.9 mm Hg to 38.7 ± 6.3 mm Hg ($P = 0.0031$) in 1 min, and then became stable. In step 3, the PaCO$_2$ decreased from 40.3 ± 5.0 mm Hg to 40.4 ± 4.6 mm Hg ($P = 0.0103$) in 3 min. In step 4, the PaCO$_2$ decreased from 32.6 ± 3.6 mm Hg to 27.4 ± 0.2 mm Hg ($P = 0.0002$) in 0.5 min.

The pH became stable within 1 min in step 1, in 3 min in step 2, and in 1 min in step 3. In step 4, the pH did not become stable. In step 1, the pH decreased from 7.45 ± 0.05 to 7.41 ± 0.05 ($P = 0.0233$) in 1 min and then became stable. In step 2, the pH decreased from 7.40 ± 0.04 to 7.35 ± 0.05 ($P < 0.0001$) in 3 min and then became stable. In step 3, the pH also decreased from 7.34 ± 0.05 to 7.36 ± 0.04 ($P = 0.0409$) in 1 min. In step 4, the pH was 7.38 ± 0.04 at time 0, and thereafter it showed no statistically significant change.

#### Experiment 2: arterial and venous BGA after RR change

Figure 5 shows the arterial and venous PO$_2$, pH, PCO$_2$, and SO$_2$ values. The venous oxygenation values changed with a smaller range compared with the arterial values. Table 2 summarizes the time to the stabilization of the venous and arterial PO$_2$, PCO$_2$, pH, and SO$_2$ after the RR change. The results of the venous BGA were not stable in most steps. With regard to the venous PO$_2$ (PvO$_2$) compared with the value of time 0, we could find no significant differences thereafter.

In step 1, the PvO$_2$ was 28.1 ± 4.8 mm Hg at time 0. The venous SO$_2$ (SvO$_2$) had a significant difference between time 0 and sporadic point, but it did not become stable. The SvO$_2$ moved irregularly between 49.4% ± 15.1% and 65.7% ± 7.6%.

The PvCO$_2$ became stable within 2 min in step 1 and within 15 min in step 2. The PvCO$_2$ increased from 37.3 ± 8.2 mm Hg

### Table 1 – Baseline of arterial blood gas analysis obtained after 20-min artificial ventilation with RR of 40 per min (mean ± standard deviation) n = 14.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PaO$_2$ (mm Hg)</td>
<td>90.5 ± 7.12</td>
<td>30.36 ± 5.97</td>
</tr>
<tr>
<td>PaCO$_2$ (mm Hg)</td>
<td>7.47 ± 0.093</td>
<td>97.7 ± 0.73</td>
</tr>
<tr>
<td>pH</td>
<td>22.3 ± 4.06</td>
<td>-1.57 ± 4.26</td>
</tr>
</tbody>
</table>

BEecf = base excess of extracellular fluid; HCO$_3$ = bicarbonate ions; PaCO$_2$ = arterial carbon dioxide partial pressure; PaO$_2$ = arterial oxygen partial pressure; RR = respiratory rate; SaO$_2$ = arterial oxygen saturation.
In only step 1, the pH became stable within 5 min. The pH changed from $7.44 \pm 0.11$ to $7.39 \pm 0.09$ ($P = 0.0031$) in 5 min and then became stable. In step 2, the pH did not become stable at any point after 5 min in statistical multiple comparison. In steps 3 and 4, the pH did not change statistically.
from 7.30 ± 0.08 (time 0). In step 4, the pH also did not change statistically from 7.29 ± 0.10 (time 0).

The arterial and venous BGA, PCO₂, and pH changed in parallel. The correlation of the arterial and venous PCO₂ and the correlation of the arterial and venous pH are represented by the following equations (Fig. 6):

Arterial PCO₂ = 0.9316 × venous PCO₂ – 4.4425
Arterial pH = 1.0835 × venous pH – 0.5795

The correlation coefficients (R) were 0.9178 and 0.9453, respectively.
Discussion

In the arterial BGA (i.e., experiment 1), the \( \text{PaO}_2 \) became stable in 0.5 min for all steps. The \( \text{PaCO}_2 \) became stable in less than 3 min for all steps. The \( \text{SaO}_2 \) (which was calculated with the \( \text{PaO}_2 \), \( \text{PaCO}_2 \), and pH) also became stable in 0.5 min for all steps. The \( \text{BEecf} \) and \( \text{HCO}_3^-/\text{CO}_2^- \) did not become stable for all steps.

In experiment 2, we performed simultaneous sampling of the arterial and venous blood. The value of the venous sample did not become stable for most steps. The results of the arterial BGA in experiment 2 corresponded with the results of experiment 1. The value of the venous blood was different from that of the arterial blood, although we found significant positive correlations between the arterial and venous \( \text{PCO}_2 \) and pH (\( r = 0.9178 \) and \( r = 0.9453 \), respectively). The results of arterial BGA (i.e., experiment 1) showed that the \( \text{PaO}_2 \) and \( \text{SaO}_2 \) became stable in 0.5 min.

In experiment 1, the \( \text{PaO}_2 \) and \( \text{SaO}_2 \) became stable in shorter time than our hypothesis. We set the hypothesis based on the oxygenation equilibrium time in human and the cardiac output per kilogram body weight in rabbits. The oxygenation equilibrium time was obtained from patients in intensive care units with malfunctioning lung in some reports\(^{15,16}\) and we used animal with healthy lung. The time to stabilization that we obtained in this study might be shorter because our experiments were performed using healthy animal. Sasse et al.\(^{36}\) described three main factors that could affect the equilibrium reaching time: (1) lung-blood interface of reflect ventilation-to-perfusion matching, (2) air movement into and out of the lungs, and (3) the mechanical transport of blood to the peripheral arteries. In our study, we used normal rabbits with the same body weight and ventilated them with a fixed tidal volume. Among these three factors, the lung interface and air movement were fixed throughout the experiment, and only the mechanical transport of blood to the peripheral arteries could affect the results.

In general, oxygen delivery in tissues is represented by the following equation:

\[
\text{Oxygen delivery (DO}_2) = \text{cardiac output} \times \text{arterial oxygen content}
\]

Rabbits have 2.39 times larger cardiac output per kilogram of body weight. Moreover, rabbits have a smaller distance between the heart and peripheral tissues and smaller vessel diameters than humans. Rabbits may have a more effective blood transport system in comparison to humans. This factor may be why the time to become stable was more than seven times shorter in rabbits.

Rabbits are widely used for experiments on the analysis of respiratory function such as validation of a pulse oximeter\(^{12-14,20}\) because the hemoglobin of rabbits is optically similar to that of humans.\(^ {12,13}\) In several studies on BGA in rabbits,\(^ {12-14,20}\) investigators confirmed that the value of pulse oximeter are stabilized within 2 min. Therefore, 2 min are used for the timing of blood sampling. However, no rationale for 2 min was described, and therefore, the time of 2 min has been conventional. In our study, we determined that, after changing the RR, the time that the \( \text{PaO}_2 \) and \( \text{SaO}_2 \) became stable was 0.5 min for all steps.

The \( \text{PaCO}_2 \) became stable within 3 min, whereas the \( \text{PaO}_2 \) became stable in 0.5 min. The \( \text{PaO}_2 \) became stable in 0.5 min for all steps (Table 2), although the \( \text{PaCO}_2 \) did not mirror the

### Table 2 – Time required to be stable in blood gas analysis (BGA) after changing respiratory rate (RR).

<table>
<thead>
<tr>
<th>Respiratory rate (per min, step)</th>
<th>40–30</th>
<th>30–20</th>
<th>20–30</th>
<th>30–40</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{PaO}_2 )</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( \text{PaCO}_2 )</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>pH</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>Unstable</td>
</tr>
<tr>
<td>( \text{SaO}_2 )</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( \text{PvO}_2 )</td>
<td>Unstable</td>
<td>Unstable</td>
<td>Unstable</td>
<td>Unstable</td>
</tr>
<tr>
<td>( \text{PvCO}_2 )</td>
<td>2</td>
<td>15</td>
<td>Unstable</td>
<td>Unstable</td>
</tr>
<tr>
<td>pH</td>
<td>5</td>
<td>Unstable</td>
<td>Unstable</td>
<td>Unstable</td>
</tr>
<tr>
<td>( \text{SvO}_2 )</td>
<td>Unstable</td>
<td>15</td>
<td>Unstable</td>
<td>Unstable</td>
</tr>
</tbody>
</table>

\( \text{PaCO}_2 \) = arterial carbon dioxide partial pressure; \( \text{PaO}_2 \) = arterial oxygen partial pressure; \( \text{PvCO}_2 \) = venous carbon dioxide partial pressure; \( \text{PvO}_2 \) = venous oxygen partial pressure; \( \text{SaO}_2 \) = arterial oxygen saturation; \( \text{SvO}_2 \) = venous SO2.

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Fig. 6 – Correlation between arterial and venous parameters (experiment 2). (A) Correlation between the arterial \( \text{PCO}_2 \) and venous \( \text{PCO}_2 \). (B) Correlation between the arterial pH and venous pH. (Color version of figure is available online.)
changes in the PaO2. The PaCO2 changed less than 10 mm Hg, whereas the PaO2 changed more than 15 mm Hg (Fig. 4 and Table 2). The PaCO2 may shift slower and range less than PaO2. Ivanov and Nunn38 reported that, after a rapid change in the tidal volume, the PaCO2 moved slower than PaO2. Our results showed the same trend. Slonim and Hamilton39 also reported that the change in PaCO2 did not mirror that of PaO2 because CO2 is generated in the process of metabolism. In our experiment, the PCO2 gradually increased and increased further at the RR of 20 per min (step 2). In step 3, when we increased the RR to 30 per min, the PaCO2 gradually decreased.

In the experiment 2, the value of venous BGA did not become stable in most steps. The pH and PvO2 showed no change from time 0 for all steps. This result indicated that the oxygenation indexes, including PO2, SO2, and PCO2 of venous blood, showed only a small change, even if the RR was reduced from 40 per min to 20 per min.

There was no correlation between the arterial and venous PO2 and the arterial and venous SO2 in our study. Korner et al.40 report that acute hypoxia causes temporal tachycardia and subsequent bradycardia, and vasoconstriction raises blood pressure. This physiological response decreases cardiac output in hypoxia. Calbet et al.41 and Mortola et al.42 have shown that in acute hypoxia, oxygen delivery to the peripheral tissues decreased and the extraction of oxygen in the peripheral tissues increased little. Therefore, the consumption of oxygen in the tissues declined in hypoxia. In hypoxia, the dissociation curves of hemoglobin shifts to the right and the oxygen affinity of blood increases. This response maintains the effective oxygen delivery in low oxygen pressure.44

Oxygen consumption by tissues (VO2) is defined by the following equation.

\[
\text{Cardiac output} = \frac{\text{VO2}}{\text{arterial oxygen content} - \text{venous oxygen content}}
\]

Oxygen Content in blood = \(1.34 \times \text{hemoglobin} \times \frac{\text{SaO2}}{100} + 0.003 \times \text{PaO2}\)  

Substitute (8) for (7),

\[
\text{VO2} = \text{cardiac output} \times 1.34 \times \text{hemoglobin} \times \frac{\text{SaO2} - \text{SvO2}}{100} + 0.003 \times (\text{PaO2} - \text{PvO2})
\]

Oxygen consumption is reflected by the gradient of arterial and venous value such as PO2 and SO2.

In our study, the gradient of the arterial and venous values such as PO2 and SO2 were the largest at the RR of 40 per min, and the differences were the smallest at the RR of 20 per min.

The VO2 in Equation (9) decreased in hypoxia because the cardiac output and SaO2–SvO2 decreased. Calbet et al.41 and Mortola et al.42 described the decrease in oxygen consumption in hypoxic tissues and their findings supported these results.

To evaluate new medical devices such as NIRS that measure tissue oxygen saturation, the weighted average of oxygen saturation or partial pressure of an artery and vein in the same area are usually used as the standard.9,44 In many studies,43–45 the cervical artery and vein (which we used) are used as the sampling points to monitor cerebral oxygenation. The arterial blood oxygen saturation and partial oxygen pressure were used as the most reliable indexes for tissue oxygenation. The normal range of oxygenation indexes of arterial blood is relatively narrow, and the normal range is established, although the range of venous blood is much broader and the normal range is generally not established. This finding may be because the venous blood oxygenation is affected by many factors such as peripheral circulation, arterial blood oxygenation, and tissue metabolism; there are also considerable individual differences.45 Watzman et al.20 showed that the distribution of arterial and venous blood was different in different tissues. Thus, the normal range of oxygen saturation in each tissue has not been established and the difference from individual baseline should be assessed.46 In our study, the individual difference of PvO2 and SvO2 were much larger than those of the artery. Additional studies should be performed to reveal the meanings and mechanisms of venous oxygenation. We sampled venous blood from the cervical vein, although the values of venous blood analysis were affected by the sample site, even if anatomically using the same central vein.47 It should be important to define the exact site of blood sampling to evaluate the tissue oxygenation.

In experiment 2, the base-acid balance such as the pH and PCO2 level showed a significant correlation between the arterial and venous values. Some studies48–55 evaluated the indexes of acid-base balance, which includes the PCO2, pH, HCO3, and BEecf of the central venous blood, as a substitute for arterial BGA in shock patients. The results have been controversial; some studies48–50 report that the venous value is an alternative to the arterial value, whereas some studies51,52 report that the venous value is not to be considered or is to be considered as an alternative to the arterial value only in the normal condition.53,54 In our study, although the venous pH and PCO2 were different from the arterial values, a significant correlation was detected in the hypoxic condition.

Our experimental setting has several limitations. The first limitation was that we changed the RR at only three levels: 40 per min, 30 per min, and 20 per min. Thus, rabbits were exposed to relatively mild hypoxia. Therefore, we cannot discuss severe hypoxia. The second limitation was the limit of the observation time. We observed the rabbits for a maximum of 30 min for each step, after changing the RR; therefore, we
did not confirm whether the stability was maintained for a longer period.

Several studies evaluated the equilibrium reaching time in intensive care patients with respiratory distress syndrome, and the most investigators changed the positive end-expiratory pressure or inspire-oxygen concentration. There are few studies about RR changes in our best knowledge. Baumgardner et al. monitored PaO$_2$ in RR change using rabbits as a lung injury model, although they did not make reference to equilibrium time. We controlled the RR to produce hypoxia condition because RR is an important and the simplest ventilator setting. In our study, after changing the RR, the arterial blood gas value became stable within 0.5 min and remained stable until 20 min. In addition, the venous blood gas value did not become stable after changing the RR. We believe that our results establish a rationale for further experiments.

Conclusions

We found that the arterial blood gas value became stable in 0.5 min after changing the RR, but the venous blood gas value did not become stable during 20 min. We also found that the indexes of the acid-base balance such as the pH and the arterial and venous PCO$_2$ showed different values but moved in parallel with significant correlation. Under limited conditions, the venous blood value may be used as an alternative to the arterial blood value only in defined equations.

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Disclosure

Conflicts of interest: None.

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