# Ventilatory response to CO2 with Read's rebreathing method in normal infants

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**Abstract**

Background Methods of evaluating the ventilatory response to CO2 (VRCO2) of the respiratory center include the steady-state and the rebreathing method. Although the rebreathing method can evaluate the respiratory center more in detail, the steady-state method has been mainly performed in infants. The aim of this study was to investigate whether we could perform the VRCO2 with the rebreathing method in normal infants. Methods The subjects were 80 normal infants. The gestational age was 39.9(39.3-40.3)weeks, and the birth body weight was 3,142 (2,851-3,451) grams. We performed the VRCO2 with Read's rebreathing method, measuring the increase in minute volume (MV) in response to the increase in EtCO2 by rebreathing a closed circuit. The value of VRCO2 was calculated as follow: VRCO2 (mL/min/mmHg/kg) = ΔMV / ΔEtCO2 / Body weight. Results We performed the examination without adverse events. The age in days at examination was 3 (2-4), and the examination time was 150±38 seconds. The maximum EtCO2 was 51.1 (50.5-51.9) mmHg. The value of VRCO2 was 34.6 (29.3-42.8). Tidal volume had a greater effect on the increase in MV than respiratory rate (5.4 to 14.3 mL/kg, 44.1 to 55.9 /min, respectively). Conclusion This study suggests that the rebreathing method can evaluate the ventilatory response to high blood CO2 in a short examination time. We conclude that the rebreathing method is useful even in infants. In the future, we plan to measure the VRCO2 of preterm infants, and evaluate the respiratory center of infants in more detail.

Title: Ventilatory response to $CO_2$ with Read's rebreathing method in normal infants

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Keyword: Ventilatory response to $CO_2$, rebreathing method, steady-state method, infants, respiratory center

Running title: $VRCO_2$ with the rebreathing in normal infants

Abstract

Background

Methods of evaluating the ventilatory response to $CO_2$($VRCO_2$) of the respiratory center include the steady-state and the rebreathing method. Although the rebreathing method can evaluate the respiratory center more in detail, the steady-state method has been mainly performed in infants. The aim of this study was to investigate whether we could perform the $VRCO_2$ with the rebreathing method in normal infants.

Methods

The subjects were 80 normal infants. The gestational age was 39.9(39.3-40.3)weeks, and the birth body weight was 3,142 (2,851-3,451) grams. We performed the $VRCO_2$ with Read's rebreathing method, measuring the increase in minute volume (MV) in response to the increase in $EtCO_2$ by rebreathing a closed circuit. The value of $VRCO_2$ was calculated as follow: $VRCO_2$ (mL/min/mmHg/kg) = $\Delta$MV / $\Delta EtCO_2$ / Body weight.

Results

We performed the examination without adverse events. The age in days at examination was 3 (2-4), and the examination time was 150±38 seconds. The maximum $EtCO_2$ was 51.1 (50.5-51.9) mmHg. The value of $VRCO_2$ was 34.6 (29.3-42.8). Tidal volume had a greater effect on the increase in MV than respiratory rate (5.4 to 14.3 mL/kg, 44.1 to 55.9 /min, respectively).

Conclusion

This study suggests that the rebreathing method can evaluate the ventilatory response to high blood $CO_2$ in a short examination time. We conclude that the rebreathing method is useful even in infants. In the future, we plan to measure the $VRCO_2$of preterm infants, and evaluate the respiratory center of infants in more detail.

Introduction:

Respiration is controlled mainly by the central and peripheral chemoreceptors [1)]. The central chemoreceptor located in the respiratory center of the medulla oblongata increases the amount of ventilation in response to pH change due to high blood $CO_2$. The peripheral chemoreceptor in the carotid body responds to hypoxemia for the increase in the amount of ventilation. Respiratory control is mainly regulated by the respiratory center in response to change in blood $CO_2$, because the responsiveness to the change in pH is more sensitive than the change in $PaO_2$. Therefore, chemical respiratory control is evaluated as the ventilatory response to $CO_2$($VRCO_2$), and its evaluation methods include the steady-state and the rebreathing method [2)]. In the steady-state method, subjects breath gas with a fixed $CO_2$ concentration (e.g. 0%, 2%, 4%), and the change in amount of ventilation is evaluated. In the rebreathing method, subjects accumulate blood $CO_2$ by rebreathing a closed circuit, and the amount of ventilation is evaluated continuously at each $CO_2$ concentration. The value of $VRCO_2$which evaluates the responsiveness to $CO_2$ quantitatively is calculated with this formula, $VRCO_2$ (mL/min/mmHg/kg) = $\Delta$Minute volume (MV)/$\Delta EtCO_2$/body weight.

Respiratory control in infants is premature and its prematurity causes respiratory diseases such as apneic attacks. Many preterm infants need oxygen therapy or mechanical ventilation due to prematurity of the respiratory center. Prematurity is also one of the risk factors in brief resolved unexplained events (BRUE)[3)]. Therefore, it is important to evaluate the respiratory center in infants. The $VRCO_2$ was mainly evaluated by steady-state methods in infants so far. Some report low $VRCO_2$ of infants born to smoking and drug abusing mothers [4, 5)]. However, the $VRCO_2$ with the rebreathing method is rarely performed in infants. While the steady-state method evaluates the increase in the amount of ventilation only at several $CO_2$concentrations, the rebreathing method evaluates the amount of ventilation continuously in increasing $CO_2$concentrations with rebreathing [2)]. The rebreathing method can evaluate the $VRCO_2$ in more detail. The aim of this study was to evaluate whether we could perform the $VRCO_2$ with the rebreathing method in normal infants, as a first step in evaluating the respiratory center in more detail.

Methods:

Subjects

The subjects were infants without congenital abnormality and maternal smoking history admitted to the normal newborn room between March and September 2011. Infants in the normal newborn room of our hospital need no medical support such as oxygen, infusion and photo therapy. We performed $SpO_2$ monitoring for 12 hours to infants in the normal newborn room and excluded infants with apneic attacks. The total number of subjects was 80 and we performed $VRCO_2$ with the rebreathing method to each subject. Basic characteristics are shown in Table 1. The gestational age was 39.9(39.3-40.3)weeks, the birth body weight was 3,142 (2,851-3,451) grams, Apgar score at 1 min and 5 min was 8 (8-9) and 9 (9-9) and the rate of caesarean delivery was 22.5%. This study was approved by the ethics committee of our institution. Written informed consent was obtained from the patient's parents.

Ventilatory response to $CO_2$

We performed $VRCO_2$ with the rebreathing method based on Read's method [6] using ARFEL III (Aivison, Tokyo, Japan) (Figure 1). The closed circuit consists of a face mask, EtCO2 sensor (Oridion medical, Jerusalem, Israel), pneumotachograph (Hans Rudolph, Shawnee, Kansas) and a bag filled with 5% $CO_2$and 95% $O_2$. Tidal volume (TV), inspiration time (I), expiration time (E) and $EtCO_2$ were measured with a pneumotachograph and $EtCO_2$ sensor, and these data were transferred to and analyzed by ARFEL III. As in Figure1, we accumulated the subjects' blood $CO_2$ by rebreathing the closed circuit during quiet sleep. Respiratory parameters of each breath were measured until the $EtCO_2$ elevated more than 2% from the beginning of the examination. We terminated the examination when the patient woke up. We monitored $SpO_2$ during the examination and confirmed that the subjects had enough spontaneous breathing to exhaust $CO_2$ after the examination.

The value of $VRCO_2$ was calculated from the relationship between $EtCO_2$ and MV. The formula is as follow: VRCO2 = $\Delta$MV / $\Delta EtCO_2$ / Body Weight. In addition to $VRCO_2$, we investigated TV, inspiratory time/ total respiratory time (I/I+E), and respiratory rate (RR) at the beginning and end of examination. The data were analyzed with JMP® 15 (SAS Institute Inc, Cary, NC, USA). The values are in mean±SD or in median (first quartile – third quartile) according to distribution. Wilcoxon test was used to compare RR, TV and I/E at the beginning and end of examination. P value $< 0.05$ was considered statistically significant.

Results:

We performed the examination without any adverse events in all cases. The age in days at examination was 3 (2-4), and the examination time was 150 ± 38 seconds. The maximum $EtCO_2$ was 51.1 (50.5-51.9) mmHg.

An example of the result is shown in Figure 2. At the beginning of the examination, RR was 44.7 /min, TV was 5.4 mL/kg and I/I+E was 0.47. MV increased in response to the continuous increase of $EtCO_2$ by rebreathing. At the end of the examination, RR was 60.1 /minute, TV was 15.0 mL/kg, I/I+E was 0.44 and $EtCO_2$ was 50.7 mmHg. The value of $VRCO_2$in this case was 36.5 mL/kg/min/mmHg. TV contributed to the increase in MV more than RR.

All data is shown in Table 2. The value of $VRCO_2$ was 34.6 (29.3-42.8) mL/kg/min/mmHg. RR and TV significantly increased through the examination (44.1 to 55.9 /min, P<0.001, 5.4 to 14.3 mL/kg, P<0.001, respectively). The rate of increase was higher in TV than RR. There was no significant change in I/I+E through the examination (0.48 to 0.47, P=0.14).

Discussion:

The aim of this study was to perform the ventilatory response to $CO_2$ with the rebreathing method in normal infants to evaluate the respiratory center. The examination was performed without any adverse events and the value of $VRCO_2$ was 34.6 (29.3-42.8) mL/kg/min/mmHg. The median examination time was 150 seconds and the median maximum $EtCO_2$ was 51.1mmHg. The increase in TV had a greater effect to the increase in MV than that of RR. To the best of our knowledge, this is a study with the largest number of subjects evaluating the $VRCO_2$ with the rebreathing method in normal infants.

The steady-state and rebreathing method are the main methods for evaluating $VRCO_2$. However, some stu-

dies have reported that evaluation with the rebreathing method is more precise and has higher reproducibility than the steady-state method.

Berkenbosch et al. performed the $VRCO_2$ with the steady-state and rebreathing method in 10 males, and reported that the value of $VRCO_2$ with the rebreathing method was 1.85 times higher than that of the steady-state[7]. They described that the difference between $EtCO_2$ and the $CO_2$ partial pressure in the central chemoreceptor in the rebreathing method was less than the difference in the steady-state method, causing the difference in $VRCO_2$ between the 2 methods. In brief, the error between $EtCO_2$ and blood $CO_2$ of the respiratory center was larger in the steady-state method. As a result, the $VRCO_2$ with the steady-state method could be lower than that of the rebreathing method. In the study of Mohan et al., the $VRCO_2$ with the steady-state method was significantly lower than that of the rebreathing method in 5 normal adults[8]. This was because the examination time was longer and the $VRCO_2$ was evaluated at a lower blood $CO_2$ in the steady-state method. The long examination time causes respiratory muscle fatigue, and the increase in the amount of ventilation demanded by the ventilatory response of the respiratory center cannot be generated, which may result in an underestimation of the $VRCO_2$. Hasegawa et al. demonstrated that the tolerance for respiratory load in infants was lower than in adults, and respiratory muscle fatigue was more likely to occur in infants[9]. We think that the short examination time is one of the advantages of the rebreathing method in infants. The increase in $EtCO_2$ with the steady-state method could be insufficient, because the subjects breathe gas with a fixed $CO_2$ concentration repeatedly in the steady-state method. In our study, we increased the maximum $EtCO_2$ to up to 51 mmHg and we were able to evaluate the $VRCO_2$ at a high $CO_2$ concentration.

Mannee et al. performed the $VRCO_2$ with the steady-state and rebreathing method multiple times in 20 normal adults and evaluated the intraclass correlation coefficients (ICC) of the $VRCO_2$ [10]. While the ICC of the rebreathing method was as high as 0.89, the ICC of the steady-state method was 0.56. They found that the $VRCO_2$ with the rebreathing method had higher reproducibility than that of the steady-state method. They assumed that the cause of this difference was due to the discordance between $EtCO_2$ and blood $CO_2$ of the respiratory center in the steady-state method, as in the study of Berkenbosch et al. The high reproducibility is more preferable in infants, because it is difficult for them to follow our orders and to be examined multiple times. This high reproducibility is also an advantage of the rebreathing method.

After focusing on the difference between methods, we focused on the difference in results of the same rebreathing method. The value of $VRCO_2$ ($\Delta MV/\Delta EtCO_2$) in three studies with the rebreathing method in normal adults were 1.2, 4.6, 1.7 L/min/mmHg, respectively [7, 8, 10]. In addition, Akiyama reported the value of $VRCO_2$ in 2 studies, 2.2 for 28 patients[11] and 1.9 for 17 patients [12], respectively with the rebreathing method in normal adults. It was assumed that this difference was due to the different range in $EtCO_2$ evaluated in the examination although the same rebreathing method was adopted. In the future, if we were to evaluate other pathologies using data of this study as reference, we should use the same protocol as of this study. It was difficult to compare our normal infants' data with normal adults' data, because the protocol and equipment were not the same and the data of normal adults' lacked adjustment for body weight or body surface area. However, the data of 17 normal adults reported by Akiyama et al. included respiratory parameters (e.g. RR, TV) during measurement [12], so we compared those parameters with our data. TV and RR at the beginning of examination were 0.27 L and 17.0 /min, and those at the end of examination was 1.53 L and 23.6 /min. The increase in TV was larger than that of RR, which was consistent with our data.

The $VRCO_2$ of the respiratory center has been mainly evaluated with the steady-state method in infants, and the $VRCO_2$ of infants born to smoking and drug abusing mothers has been performed so far [4, 5]. Data of the $VRCO_2$ in normal infants was measured as the control for comparison. Wingkum et al. showed that the $VRCO_2$ in 12 infants of drug abusing mothers was lower in comparison with 12 normal infants[4]. In this study, $EtCO_2$ and MV were measured with the steady-state method in which subjects breathed room air and 4% $CO_2$ gas for 5 minutes each. The maximum $EtCO_2$ was 40.43 mmHg, which was within normal range. The examination time of our study was 2.5 minutes, which was the shortest in all the studies quoted

in this article. Furthermore, the maximum $EtCO_2$ in our study was 51.1 mmHg, so we were able to evaluate the $VRCO_2$ at high blood $CO_2$levels.

Ali et al. compared the $VRCO_2$ among 34 infants born to smoking mothers, 22 infants born to drug abusing mothers and 22 normal infants. They showed that the increase in ventilation was significantly lower in order of infants born to drug abusing mothers, smoking mothers and normal infants [5]. In this examination, MV was measured when breathing 0%, 2%, and 4% $CO_2$ gas, and the $VRCO_2$ was evaluated by the relationship between MV and $F_ICO_2$ rather than $EtCO_2$. The $VRCO_2$ using $F_ICO_2$ could be underestimated because the error tended to be larger between $F_ICO_2$ and blood $CO_2$ of the respiratory center than the error between $EtCO_2$ and blood $CO_2$, as in the study by Mannee et al.[10]. In fact, the values of $VRCO_2$(mL/min/mmHg/kg) in normal infants were a median of 34.6 in our study and an average of 48.7 in the study by Wingkun et al.[4] compared to a low average of 10.9 in the study by Ali et al.[5]

This study has some limitations. Normal adults were selected after respiratory function tests and interviews[11, 12]. However, it was difficult to perform spirometry test to infants, so we selected newborns that did not need any medication or monitoring as normal infants. This way of selection was the same as in other studies for normal infants [4, 5]. Next, we measured the $VRCO_2$ once in this study. Some studies of normal adults studied the average measuring the $VRCO_2$ multiple times[7, 11]. We designed to measure the $VRCO_2$ once in this study, because this physiologic test is difficult to perform in infants and the reproducibility of the rebreathing method is high [10]. We plan on evaluating the respiratory center of infants in more detail by performing the $VRCO_2$ to preterm infants, infants with apneic attacks and infants treated with caffeine, using the data of normal infants obtained from this study.

Conclusion:

We measured the $VRCO_2$ with the rebreathing method in normal infants. The value of $VRCO_2$ was 34.6 (29.3 - 42.8) mL/kg/min/mmHg. This study suggested that the rebreathing method can evaluate the ventilatory response in high blood $CO_2$concentrations in a short examination time. We conclude that the rebreathing method is useful even in infants. In the future, we plan on measuring the $VRCO_2$ of preterm infants, infants with apneic attacks, and evaluate the respiratory center of infants more in detail.

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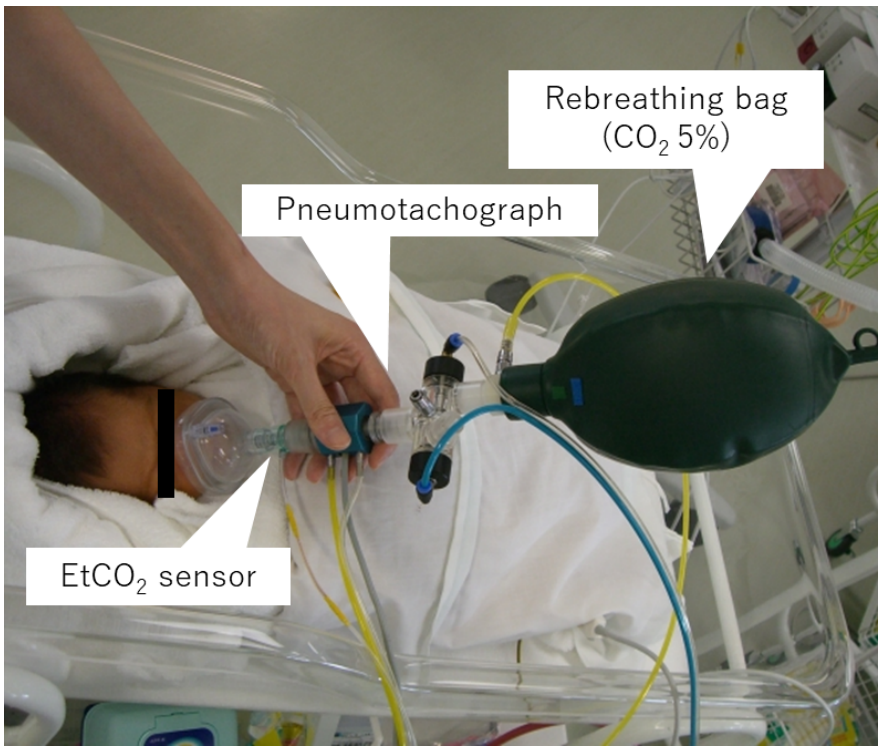
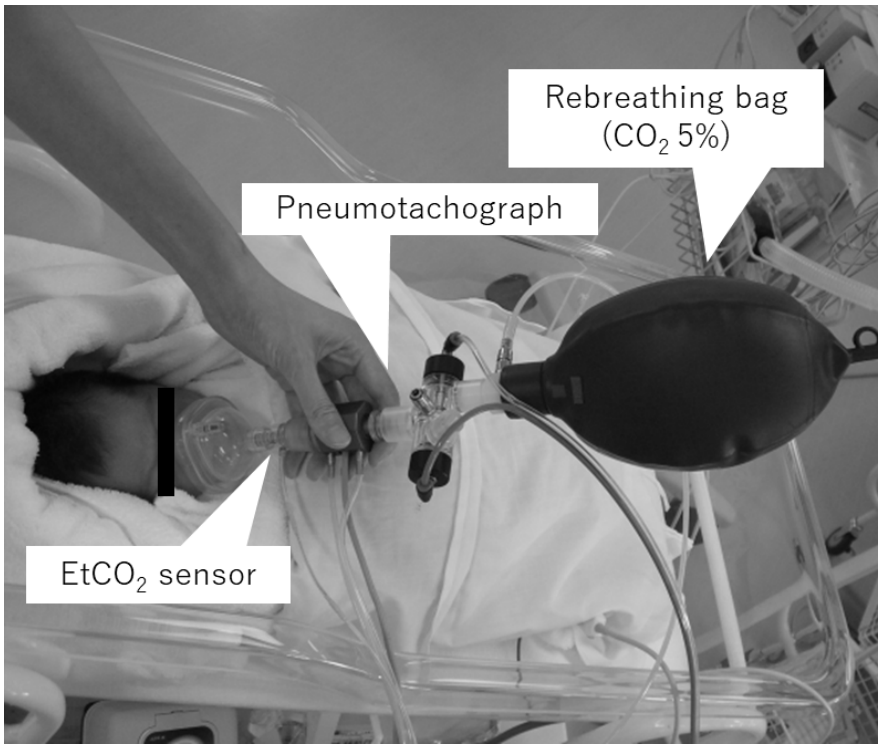
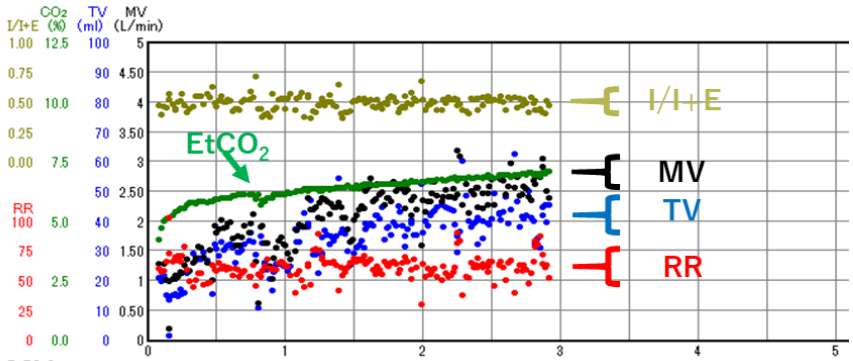
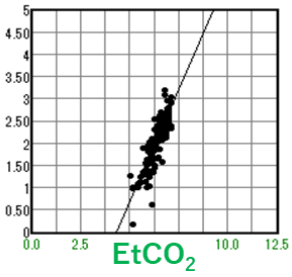
**Hosted file**

`Table 1, 2 VRCO2 with the rebreathing in normal infants.pdf` available at https://authorea.com/users/378145/articles/494693-ventilatory-response-to-co2-with-read-s-rebreathing-method-in-normal-infants

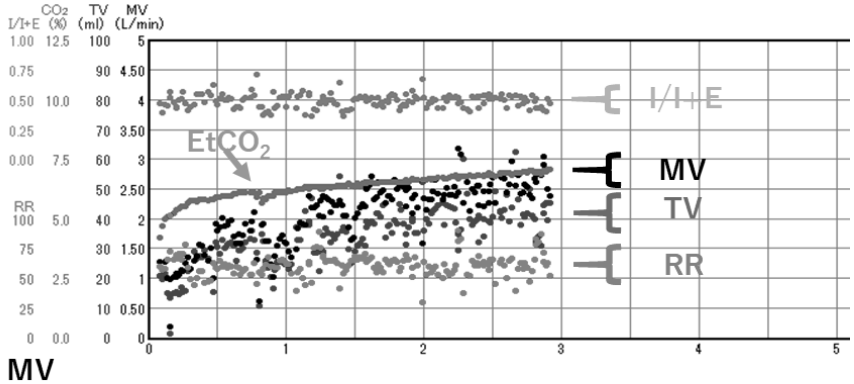


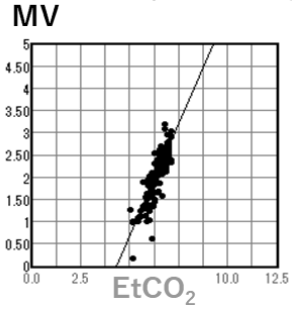



$VRCO_2$ = 36.5 (mL/min/mmHg/kg)

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