Marathoners' breathing pattern protects against lung injury by mechanical ventilation: a pilot study

Yoshiaki Oshima (✉ yoshimatomo@gmail.com)

Yonago Medical Center

Naoto Okazaki

Tottori University Faculty of Medicine

Akihiro Otsuki

Tottori University Faculty of Medicine

Shunsaku Takahashi

Tottori University Faculty of Medicine

Tomomi Harada

Tottori University Faculty of Medicine

Yoshimi Inagaki

Tottori University Faculty of Medicine

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Abstract

Background: Marathoners use the 2:2 breathing pattern. We hypothesized that this ventilation method may protect against ventilator-induced lung injury.

Methods: We studied the 2:2 breathing pattern as a mechanical ventilation mode, by assessing the gas exchange in intact rabbits and the pulmonary protective effects in isolated rabbit lungs. The typical setting of this breathing method was 30 cycles/min of respiratory frequency. The time allocation for one cycle was as follows: 1st inspiratory period, 2nd inspiratory period, 1st expiratory period, and 2nd expiratory period (all 0.3 s long) with intermittent resting periods (all 0.2 s).

Results: The 2:2 breathing pattern caused no problems regarding the efficiency of oxygen uptake and carbon dioxide elimination. The wet-to-dry weight ratio of the lung was lower for the proposed 2:2 breathing pattern than with the inversed ratio ventilation (both inspiratory:expiratory ratio 1:1).

Conclusions: The marathoners' breathing pattern may be a novel method to provide protection against ventilator-induced lung injury in clinical settings.

1. Background

Breathing during a marathon is often done empirically in a so-called "2:2 breathing pattern" [1]. This 2:2 breathing pattern is based on cycles composed of four phases: the 1st inspiratory period, 2nd inspiratory period, 1st expiratory period, and, finally, the 2nd expiratory period. These phases are synchronized with the step rate, e.g., a step with the left foot coincides with the 1st inspiration and 1st expiration phase. Matching rhythmic steps with inspiration and expiration means that the inspiratory:expiratory (I:E) ratio is 1:1 for the 2:2 breathing pattern.

Mechanical ventilation causes lung damage, a pathological condition called ventilator-induced lung injury (VILI) [2,3]. VILI is a factor that increases the mortality rate in acute respiratory distress syndrome (ARDS) [2]. The main factor that causes VILI is alveolar overdistension (volutrauma) and cyclic reopening of collapsed alveoli (atelectrauma) [4]. The ventilation strategy to protect the lungs against deterioration in ARDS is to prevent additional volutrauma and atelectrauma of the remaining normal lung tissue [5]. Recently, low tidal volume ventilation has been established as a lung-protective ventilation strategy; however, it has the disadvantage of PaCO₂ retention [6]. Other candidates for lung-protective ventilation strategies include inversed ratio ventilation (IRV) [7] and high-frequency oscillatory ventilation (HFOV) [8].

During exercise, there is a risk of hypoxemia and pulmonary edema [9-11]. In intense exercise, cardiac output increases as the demand for O₂ increases, and the right ventricular pressure and pulmonary artery pressure rise [12]. The effectiveness of the 2:2 breathing pattern on cardiopulmonary function during a marathon has not been proven yet in a study. However, the 2:2 breathing pattern is empirically favored in marathon runners, presumably because it prevents the progression of additional hypoxemia and pulmonary edema during the marathon. Moreover, since CO₂ is not retained during a marathon, where the

metabolism is increased over an extended time, it has been suggested that the 2:2 breathing pattern provides sufficient capacity for CO₂ release [13].

In the present study, we developed a ventilator prototype that can perform intermittent positive pressure ventilation, mimicking the breathing cycle of the 2:2 breathing pattern (for technical details see Additional file 1). This mode of ventilation was named the marathoners' breathing pattern ventilation (MBV). We assumed that the MBV is a mechanical ventilation method that divides the tidal volume required for conventional mechanical ventilation into the inspiratory volume of 1st and 2nd inspiratory periods. Since the prototyped ventilator was designed to deliver a constant inspiratory flow rate, and the durations of the 1st and 2nd inspiratory periods were set to be the same, the inspiratory volumes of the two periods were the same. Thus, each inspiratory volume can be reduced in the MBV. In addition, the MBV had an I:E ratio of 1:1. Therefore, MBV can be considered as a ventilation mode that combines the features of low tidal volume ventilation and IRV. In this study, the following experiments were conducted for MBV:

Experiment 1) The oxygen uptake and carbon dioxide emission of healthy lungs were compared in vivo between the MBV and IRV (I:E ratio 1:1) in rabbits.

Experiment 2) The effects of the MBV on the pulmonary pre-edema model were compared with those of the IRV (I:E ratio 1:1) in isolated perfused rabbit lungs.

In the MBV method, the 1st inspiratory, 2nd inspiratory, 1st expiratory, 2nd expiratory phases taken together, defined one cycle. In the IRV method, the inspiratory and subsequent expiratory phase together defined one cycle. In both MBV and IRV methods, tidal volume was defined as the total ventilation volume during one cycle. In both MBV and IRV methods, the unit of respiratory rate was cycles/min.

2. Methods

All experimental protocols regarding the use and care of animals in the present study were approved by the Laboratory Animal Care Committee of the Faculty of Medicine at Tottori University. Adult female Japanese white rabbits (2.2–3.1 kg) were purchased from Oriental Yeast Co. (Tokyo, Japan). The rabbits were kept under standard housing conditions with free access to food and water.

2.1. Experiment 1: Assessment of pulmonary oxygenation efficiency

2.1.1. Experimental design

Rabbits (n = 9; body weight, 2.6 ± 0.3 kg) were anesthetized with 30 mg/kg pentobarbital (Abbott Laboratories, North Chicago, IL, USA) intravenously and 25 mg/kg ketamine (Daiichi Sankyo, Tokyo, Japan) intramuscularly. The experiment was conducted with the rabbits in supine position. The animals were tracheotomized, and a catheter was placed into the common carotid artery to measure the arterial blood pressure and heart rate, as well as to allow for blood gas sampling. Another catheter was inserted into the external jugular vein ipsilaterally for continuous infusion of lactated Ringer's solution (Otsuka Pharmaceutical, Tokyo, Japan) (10 ml/kg/h) containing pancuronium bromide (MSD K.K., Tokyo, Japan) (0.5 mg/kg/h) and pentobarbital (5 mg/kg/h). Anesthesia, muscle relaxant dosage, and fluid therapy were according to Maeda et al. (2004) [14] with slight modifications. Animals were ventilated through a 3-ml artificial nose (total volume of the circuit was about 7 ml) using a Harvard Rodent Ventilator 683 (Harvard Apparatus, Holliston, MA, USA) which had a tidal volume of 6 ml/kg, a respiratory frequency of 40 breaths/min, and a positive end-expiration pressure (PEEP) of 2 cmH₂O. To prevent VILI, the tidal volume was set to a low value of 6 ml/kg during the stabilization period [15].

After a stabilization period of 30 min under these conditions, normal blood acid-base balance was confirmed. Then, the MBV and IRV methods were alternately conducted for 20 min. The MBV method was carried out with the five patterns shown in Table 1, fixed individually for each rabbit. For example, a typical setting for MBV involved a respiratory rate of 30 cycles/min; the time allocation for one cycle was as follows: 1st inspiratory period 0.3 s, resting period 0.2 s; 2nd inspiratory period 0.3 s, resting period 0.2 s; 1st expiratory period 0.3 s, resting period 0.2 s; and 2nd expiratory period 0.3 s, resting period 0.2 s. Lungs were hyperinflated at the renewal point of each ventilation mode to prevent microatelectasis. To reduce the number of variables and to simplify the interpretation of the results, this experiment was performed without PEEP.

Table 1. Five patters of the MBV method (Experiment 1)

Time distribution of one respiratory cycle		1 st Insp	Resting	2 nd Insp	Resting	1 st Exp	Resting	2 nd Exp	Resting
Respiratory rate (cycles/min)	25	0.4	0.3	0.4	0.4	0.4	0.2	0.3	0
	30	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2
	30	0.2	0.3	0.2	0.3	0.2	0.3	0.5	0
	30	0.3	0.2	0.2	0.3	0.3	0.2	0.5	0
	35	0.2	0.2	0.2	0.3	0.2	0.2	0.4	0

Values are shown in seconds.

MBV, marathoners’ breathing pattern ventilation; Insp, inspiration period; Exp, expiration period; Resting, resting period

The IRV method was conducted with a tidal volume of 8 ml/kg using the Harvard Ventilator 683, and the respiratory rate was adjusted to one cycle/min increments until the PaCO₂ value was in the normal range. The I:E ratio of the Harvard Ventilator 683 is fixed at 1:1, as the factory default setting. The tidal volume in rabbits under conventional mechanical ventilation is considered to be 8–10 ml/kg (built into the prototype system) [16], the respiratory frequency of ACOMA AR 300 was set to be the same as the respiratory rate, which was allocated to the MBV method, the tidal volume of ACOMA AR 300 was fixed at 8 ml/kg, and the I:E ratio of ACOMA AR 300 was fixed at 1:1.

The tidal volumes in the MBV method were determined from the average gas volume of several exhalation cycles collected by the classic water displacement method [17]. According to findings in a preliminary experiment, the dissolution-dependent decrease in volume after 10 min was less than 0.05 ml per 10 ml of a gas mixture containing 5% CO₂.

2.1.2. Measured variables

Arterial blood pressure and airway pressure were recorded using a PowerLab system (AD Instruments, New South Wales, Australia; software, Chart ver. 5) with transducers (P23 ID, Gould, Oxford, CA, USA) connected to amplifiers (Model 2238; San-ei, Tokyo, Japan). The mean arterial blood pressure (MBP), heart rate, respiratory rate, peak inspiratory pressure (PIP), mean airway pressure (MAP), and minute volume were calculated from the recorded data. Arterial blood gas analysis was performed using iSTAT (iSTAT Corp., East Windsor, NJ, USA) and tidal volume was measured at the end of each ventilatory mode.

Regarding MBV, because different flow volumes and time conditions coexist at the same respiratory rate in the same subject, the highest PaO₂ levels in the normocapnic MBV data were compared to determine the ventilation efficiency, regardless of the abovementioned conditions.

At the end of the experiment, the rabbits were euthanized with bolus injections of an overdose of sodium pentobarbital (100 mg/kg).

2.2. Experiment 2: Assessment of pulmonary protective effects

2.2.1 Experimental design

The isolated perfused rabbit lung model ($n = 14$) was used, and the degree of pulmonary damage during ventilation was compared between MBV and IRV. The isolated perfused rabbit lungs were prepared using the method described in detail by Liu et al. (2001) [18] with minor modifications. Briefly, the rabbits were anesthetized with pentobarbital 30 mg/kg intravenously followed by ketamine 25 mg/kg intramuscularly and anticoagulation with heparin 500 u/kg intravenously. After local anesthesia of the anterior neck and the sternum region with 1% lidocaine, tracheal intubation was performed through a tracheostomy and the rabbits were ventilated mechanically. A median sternotomy was performed, and an incision was made into the right ventricle. The rabbits were euthanized by rapidly exsanguinating whole blood (70ml) from the incision site in the right ventricle. The pulmonary artery and the left atrium were cannulated via the right and left ventriculotomies, respectively. Finally, the lungs were removed en bloc and enclosed in a humidified chamber. The lungs were perfused with bicarbonate-buffered physiological salt solution [PSS (in mM): NaCl, 119; KCl, 4.7; MgSO_4 , 1.17; NaHCO_3 , 22.61; KH_2PO_4 , 1.18; CaCl_2 , 3.2] in a recirculating manner. In every 100 ml of PSS stock solution, we added 100 mg dextrose, 20 mU insulin, 3 g Ficoll® PM70 (GE Healthcare Bio-Sciences, Little Chalfont, UK), and 2 mg indomethacin (Sigma Chemical, St. Louis, MO, USA). The perfusate flow rate was gradually increased to 35 ml/kg/min, while the ventilation gas was changed from air to a mixed gas (O_2 , 21%; CO_2 , 5%; N_2 , balance). Lungs were ventilated with a tidal volume of 6 ml/kg and a respiratory frequency of 40 breaths/min with a PEEP of 2 cmH₂O using a Harvard Ventilator 683. Blood gases were measured using iSTAT. The perfusate pH was adjusted to 7.40 using Meylon® (1 mM NaHCO_3 ; Otsuka Pharmaceutical, Tokyo, Japan), and the temperature of the perfusate was maintained at 38 °C using a regulated heating system.

If an isolated rabbit lung is perfused with Krebs Ringer solution supplemented with 4% (w/v) albumin without the addition of red blood cells to the perfusate, it will develop pulmonary edema within 2 h [19]. Ficoll® PM70, as well as albumin, provide a normal colloidal osmotic pressure at 4% (w/v) [20]. If isolated murine lungs are perfused with Dulbecco's Modified Eagle's Medium containing 4% (w/v) Ficoll® P70 without the addition of red blood cells to the perfusate, an edema will form in the lung interstitium within 1 h [21]. In the current study, a pulmonary pre-edema model was created in the isolated rabbit lung via perfusion with bicarbonate-buffered PSS containing 3% (w/v) Ficoll® PM70 without the addition of red blood cells to the perfusate.

Pulmonary arterial pressure, left atrial pressure, airway pressure, and perfusate flow rate were recorded using a PowerLab system with an electromagnetic flowmeter (MF-1200; Nihon Kohden, Tokyo, Japan). The left atrial pressure was set to 4 mmHg by regulating the height of a reservoir connected to the venous circuit. After a 20-min stabilization period, the baseline values of the pressure-volume (PV) curve and airway pressure were measured as described in section 2.2.2.

The 14 isolated rabbit lung preparations were randomly divided into the IRV and MBV groups. In the IRV group, rabbit lungs ($n = 7$; body weight, 2.5 ± 0.2 kg) were ventilated using a Harvard Ventilator 683 with a tidal volume of 8 ml/kg, a respiratory rate of 30 cycles/min, and a PEEP of 2 cmH₂O. In the MBV group, rabbit lungs ($n = 7$; body weight, 2.4 ± 0.2 kg) were ventilated using the prototype ventilator described in

Experiment 1 with a PEEP of 4 cmH₂O (first step) and 2 cmH₂O (second step). Before the experiment, the tidal volume was adjusted to 6 ml/kg using a test lung and measured by the water displacement method. In Experiment 2, the MBV method had a respiratory rate of 30 cycles/min, and the time allocation for one cycle had exclusively the following pattern: 1st inspiratory period 0.3 s, resting period 0.2 s; 2nd inspiratory period 0.3 s, resting period 0.2 s; 1st expiratory period 0.3 s, resting period 0.2 s; and 2nd expiratory period 0.3 s, resting period 0.2 s.

2.2.2. Measured variables

The airway pressure was recorded using the same device described in Experiment 1, and mean airway pressure (MAP) and peak inspiratory pressure (PIP) were calculated based on the recorded values.

The inflation PV curve was measured using the quasi-static method and a syringe containing mixed gas, while ventilation and perfusion were temporarily removed [4]. This method involves the measurement of airway pressure as the lungs are gradually inflated in 5-ml steps, until a volume of 40 ml is reached. The total inhalation volume was assessed until 40 ml to prevent injuries caused by the measurement method itself. Each inflation interval was set at 15 s to obtain a plateau pressure. The deflation PV curve was not measured.

At the end of all measurements, lungs were clamped in the end-inspiratory state. The left lung was excised, and its wet weight was measured. It was dried at 60 °C in an oven for two weeks, and its dry weight was measured to determine the lung wet-to-dry ratio (W/D) using the formula: $W/D = \text{wet weight} / \text{dry weight}$ [18]. The right lung was washed three times with 5 ml of sterile saline. The lavage fluid was centrifuged at $200 \times g$ for 10 min at 4 °C, and the cell-free supernatant was stored at -70 °C as bronchoalveolar lavage fluid (BALF) for further chemical analyses. The BALF was used to measure total protein concentration and myeloperoxidase (MPO) activity. Total protein concentration was measured using BAC Protein Assay Reagent Kit (Pierce, Rockford, IL, USA). MPO activity was measured using the method of o-dianisidine dihydrochloride oxidation [22]. MPO activity was expressed as the change in optical density (ΔOD) per min and per ml of BALF. W/D, total protein concentration in BALF, and MPO activity in BALF were used to determine histochemical lung injury.

2.3. Statistical analysis

Since no previous published studies were available regarding the study outcome, we could not get an idea regarding the standard deviation. Moreover, as we were interested in finding any level of difference between MVB and IRV, we measured multiple endpoints. Therefore, we used the so-called “resource

equation” method, which depends on the law of diminishing returns for sample size calculation [23]. Experiment 1 was performed using a crossover design to avoid unnecessary waste of resources.

All data are expressed as the mean $\pm$ standard deviation. Prism® ver. 4 (GraphPad Software, San Diego, CA, USA) software was used for statistical calculations and figure preparations. Data were compared using Welch’s t-test; p-values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Experiment 1

Nine rabbits completed Experiment 1. Blood gas analysis and circulatory parameters are shown in Table 2. There were no differences observed in either blood gas composition or circulatory dynamics between the two ventilation methods.

Table 2. Summary of physiological variables (Experiment 1)

	IRV	MBV	P value
pH	7.42 $\pm$ 0.02	7.39 $\pm$ 0.04	0.095
PaCO ₂ (mmHg)	37.7 $\pm$ 2.5	39.5 $\pm$ 2.0	0.112
PaO ₂ (mmHg)	83.6 $\pm$ 5.4	85.2 $\pm$ 3.4	0.465
Base excess (mEq/L)	0.44 $\pm$ 2.51	-1.33 $\pm$ 2.65	0.166
Heart rate (/min)	271 $\pm$ 32	264 $\pm$ 20	0.587
MBP (mmHg)	113 $\pm$ 10	108 $\pm$ 22	0.547

Values are presented as mean $\pm$ standard deviation (n = 9). P values show significant differences between the IRV and MRV methods.

IRV, inversed ratio ventilation; MBP, mean arterial blood pressure; MBV, marathoner's breathing pattern ventilation

Respiratory rate, tidal volume, and minute volume are presented in Fig. 1. No difference was detected in the respiratory rate between groups (IRV: 29.9 $\pm$ 4.1 cycles/min; MBV: 31.3 $\pm$ 2.3 cycles/min; p = 0.39). The tidal volume was significantly different between the two ventilation methods (IRV: 20.8 $\pm$ 2.7 ml; MBV: 16.1 $\pm$ 1.3 ml; p < 0.01). Thus, a significant difference was also observed in the minute volume (IRV: 613 $\pm$ 41 ml; MBV 481 $\pm$ 59 ml; p < 0.01). Regarding the body weight, the mean tidal volume for MBV method was 6.2 ml/kg.

PIP and MAP values are shown in Fig. 2. A significant difference was seen in PIP (IRV: 12.4 ± 1.7 cmH₂O; MBV: 9.3 ± 1.6 cmH₂O; $p < 0.01$) but not in MAP (IRV: 4.23 ± 0.77 cmH₂O; MBV: 3.86 ± 0.78 cmH₂O; $p = 0.33$).

Fig. 3 displays typical waveforms of the airway pressure for both mechanical ventilation methods in the same rabbit. The waveform in the left panel describes the airway pressure in the IRV method, whereas the right panel presents the airway pressure curve in the MBV method. In the MBV method, the airway pressure transiently decreased immediately after the end of the 1st inspiratory period and transiently increased immediately after the end of the 1st expiratory period.

3.2. Experiment 2

Fourteen rabbits completed Experiment 2. Fig. 4 shows PIP and MAP values determined in Experiment 2. PIP in the IRV group was significantly increased after 60 min perfusion ($p < 0.05$). A statistically significant difference in PIP after 60 min perfusion was also observed between the two groups ($p < 0.05$). By contrast, PIP scarcely changed in the MBV group after 60 min perfusion ($p = 0.93$). No significant differences in MAP were observed between the two groups either at baseline ($p = 0.27$) or after 60 min perfusion ($p = 0.40$).

W/D, total protein concentration in the BALF, and MPO activity in the BALF are presented in Fig. 5. A statistically significant difference between the two groups was determined in W/D (IRV: 7.50 ± 0.47 ; MBV: 6.86 ± 0.18 ; $p < 0.05$). However, no significant differences were found in total protein concentration (IRV: 66.5 ± 30.2 µg/ml; MBV: 45.8 ± 32.2 µg/ml; $p = 0.24$) or MPO activity (IRV: 0.56 ± 0.10 ΔOD/ml/min; MBV: 0.42 ± 0.19 ΔOD/ml/min; $p = 0.12$).

The pulmonary PV curves of both groups at baseline and after 60 min perfusion are shown in Fig. 6, in which the pressure is expressed as the average value for the corresponding volume, since no significant differences in pressure values were detected between the two groups.

In comparison to the PV curves of the MBV group, those of the IRV group were slightly shifted towards higher pressures.

4. Discussion

Many marathon runners have adopted the 2:2 breathing pattern. We have named an intermittent positive pressure ventilation that mimics this breathing rhythm of marathon runners as MBV pattern. The ability of the MBV to take in oxygen and release carbon dioxide in healthy lungs was examined in rabbits *in vivo*, and the lung-protective effect of MBV on the pulmonary pre-edema model was examined using an

isolated perfused rabbit lung. In healthy lungs, even if the total ventilation volume for one respiratory cycle was set lower in the MBV than in the IRV, there was no difference in MAP values between the two ventilation patterns. In healthy lungs, there were also no significant differences in hemodynamics and blood gas values between MBV and IRV. In the pulmonary pre-edema model, MBV was, in comparison to IRV, able to reduce the increase in PIP values, the W/D ratio at the end of the experiment was lower, and a protective effect against deterioration of the lung edema was observed.

4.1. Lung-protective ventilation strategy

In recent years, low tidal volume ventilation has secured a leading position among lung-protective ventilation strategies [6]; additionally, HFOV [8] and IRV [7] are also listed as suitable candidates.

Low tidal volume ventilation has a serious disadvantage in that it sometimes causes respiratory acidosis at $\text{pH} < 7.2$ [6]. In a piglet model of VILI, it was found that even with low tidal volume ventilation, high respiratory rates activate transforming growth factor β pathways and exacerbate pulmonary edema [24].

In HFOV, lung volume is secured by the MAP. If the MAP setting is too low, the lung volume decreases, and sufficient oxygenation cannot be obtained; instead, setting the MAP too high can result in decreased cardiac output and increased pulmonary vascular resistance [25]. In the OSCILLATE trial [8], an RCT for ARDS, HFOV did not prove to be superior to low tidal volume ventilation. In the same trial, the high mortality rate found for ARDS patients with HFOV was caused by circulatory suppression due to a high MAP [26]. Moreover, when CO_2 is retained under HFOV, the only possible strategy is to decrease the frequency and increase the tidal volume [27].

In the IRV, PIP values are kept low, but the MAP is maintained at high values [28]. The improved oxygenation capacity by IRV is attributed to the increase in MAP [27] and the occurrence of intrinsic PEEP [29] due to the decrease in expiratory time. However, in mouse experiments with high tidal volume ventilation, lung injury was rather induced by the IRV than by conventional mechanical ventilation [30].

4.2. MBV

In Experiment 1, the total ventilation volume for one respiratory cycle was set lower in the MBV than in the IRV; however, there was no difference in MAP values between the two ventilation patterns. In the MBV, the MAP could be maintained to the same extent as in the IRV. There was no difference in PaO_2 between MBV and IRV, presumably because the MAP was maintained in the MBV. Moreover, PaO_2 was also maintained in the MBV, as division of the expiration into two phases separated by a resting phase generated positive pressure at the end of the expiration, thus possibly reducing the extent of alveolar

collapse. There was no difference in mean blood pressure and HR between the MBV and the IRV, since there was no difference in MAP values between the two methods, as well as no difference in the intrathoracic pressure under muscle relaxant administration. Although the MBV had lower minute volume values compared to the IRV, there was no difference in PaCO_2 between the two methods. This was because in the former, redistribution of the so-called “pendelluft” [31] occurred for the inhaled gas from the short-time constant alveoli to the long-time constant alveoli during the resting phase between the 1st and 2nd inspiration, decreasing the dead space. Similarly, providing an end-inspiratory pause in healthy lungs of piglets [32] and in adult acute respiratory insufficiency [33] decreases the dead space/tidal volume and PaCO_2 .

In Experiment 2, the MBV in the pulmonary pre-edema model reduced the increase in the PIP, and the W/D at the end of the experiment was lower using MBV than using IRV. We suggest that alveolar overdistension was prevented in the MBV because the total ventilation volume for one respiratory cycle was divided into two fractions. In high tidal volume ventilation of isolated perfused rat lung, active sodium transport and Na-K-ATPase activity of the alveolar epithelium are impaired, and lung edema clearance is reduced [34]. According to our results, the MBV reduces pulmonary edema.

4.3. Marathon

During intense exercise, the O_2 demand increases beyond the limit of pulmonary diffusion capacity, thus increasing the alveolar-arterial oxygen difference and inducing hypoxemia [35]. It was shown that the HR increases to 145–180/min during a marathon [36]. In an exercise that corresponds to 80% of the maximum oxygen uptake rate, the mean pulmonary artery pressure increases up to 38 mmHg in young people, while the left atrial pressure increases up to 25 mmHg [37]. Subclinical interstitial pulmonary edema is found in 17% of runners after a marathon [38]. The fact that the 2:2 breathing pattern is favored empirically in situations such as a marathon, which has a long exercise load, suggests that this respiratory technique works effectively to prevent further progression of hypoxia and pulmonary edema. Moreover, the fact that the end-tidal CO_2 is not retained during a marathon, along with the increased metabolism over a long time [13], suggests that the 2:2 breathing pattern provides a sufficient capacity for CO_2 release.

4.4. Experimental models

In Experiment 1, both examined ventilation methods did not utilize PEEP to prevent their influence on the comparison of O_2 uptake and CO_2 elimination in the MBV and IRV. In low tidal volume ventilation, atelectasis progresses [39] even if PEEP is set 2 cmH₂O higher than the lower inflection point. Every time

the ventilation mode was changed in Experiment 1, an alveolar recruitment maneuver was initially performed to overcome the atelectasis, and values were measured after 20 min. In early ARDS, the alveolar recruitment maneuver improves oxygenation after 3 min; however, this effect does not persist, and within 30 min, the oxygenation capacity decreases again [40]. Because arterial blood gas analysis was performed 20 min after changing the ventilation mode in the present study, we believe that the assessment of superiority between IRV and MRV were not affected by recruitment maneuvers.

In Experiment 2, the isolated perfused rabbit lung was used as an experimental model. The PV curves at the end of the experiment were not different between MBV and IRV. The pulmonary pre-edema model we employed can be considered as an early stage of lung injury, since no changes are observed in lung mechanics at early stages of lung injury, and lung mechanics are not a valid marker for early-stage lung injury [41].

In small animals, acute extreme lung stretching rapidly develops into an increased permeability lung edema. The mechanism is thought to involve the destruction of vascular endothelial cells, which induces direct contact between the basement membrane and polymorphonuclear cells, rather than involving the recruitment of inflammatory cells [2]. In Experiment 2, there was no significant difference in MPO activity in the BALF between MBV and IRV, suggesting that polymorphonuclear cells had not yet infiltrated into the alveolus.

4.5. Limitations

The average frequency of steps taken during a marathon is about 180 per minute [42]. In the 2:2 breathing pattern, the average respiratory rate during a marathon is 45 cycles/min, given that one cycle consists of a 1st inspiratory period, 2nd inspiratory period, 1st expiratory period, and 2nd expiratory period. The resting respiratory rate in humans is 12–20 breaths/min, whereas in rabbits, it is 32–60 breaths/min [43]. In the current study, we examined the effects of MBV with respiratory rates of approximately 30 cycles/min in rabbits. Higher respiratory rates should also be examined. O₂ uptake and CO₂ elimination in healthy lungs exposed to MBV, which should also be determined for diseased lungs in future studies. Furthermore, since our experiments do not directly explain how the time allocation of inspiratory, expiratory, and resting phases within a single MBV cycle increases the O₂ uptake and CO₂ elimination efficiency, future studies should address this point.

4.6. Clinical application

The 2:2 breathing pattern in a marathon differs significantly from that in MBV because the former is a spontaneous ventilation, whereas the latter is an intermittent positive pressure ventilation. Thus, before the MBV approach can be applied in the clinic, its effects have to be examined in larger animals which have breathing cycle values that are closer to those in humans during a marathon. In addition, MBV implementation requires the administration of sedatives or muscle relaxants, which needs to be taken into consideration prior to MBV clinical use.

5. Conclusions

The marathoners' breathing pattern ventilation method that we have developed appears to be a novel way of protecting against ventilator-induced lung injury in at-risk patients, thereby tending to decrease mortality rates in such patients.

Abbreviations

I:E: inspiratory:expiratory

VILI: ventilator-induced lung injury

ARDS: acute respiratory distress syndrome

IRV: inversed ratio ventilation

HFOV: high-frequency oscillatory ventilation

MBV: marathoners' breathing pattern ventilation

PEEP: positive end-expiration pressure

PV: pressure-volume

MAP: mean airway pressure

MBP: mean arterial blood pressure

PIP: peak inspiratory pressure

W/D: wet-to-dry ratio

BALF: bronchoalveolar lavage fluid

MPO: myeloperoxidase

Δ OD: change in optical density

Declarations

Ethics approval and consent to participate: All experimental protocols regarding the use and care of animals in the present study were approved by the Laboratory Animal Care Committee of the Faculty of Medicine at Tottori University. Adult female Japanese white rabbits (2.2–3.1 kg) were purchased from Oriental Yeast Co. (Tokyo, Japan). The rabbits were kept under standard housing conditions with free access to food and water.

Consent for publication: Not applicable

Availability of data and materials: All data generated or analyzed during this study are included in this published article and Additional file.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions:

YO – analysis and interpretation of data, revising the manuscript; NO – acquisition of data, drafting the manuscript; AO – conception of the work, revising the manuscript; ST – analysis and interpretation of data, revising the manuscript; TH – analysis and interpretation of data, revising the manuscript; YI – conception of the work, revising the manuscript. All authors have read and approved the manuscript and have ensured that the above-mentioned information is true.

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Figures

Figure 1

Comparison of respiratory rate, tidal volume, and minute volume between the IRV and MBV methods. In both the MBV and IRV methods, the inspiratory and expiratory phases are determined as one cycle, and tidal volume is determined to be the total ventilation volume during one cycle. In both the methods, the unit of respiratory rate is cycles/min. Significant differences are observed in tidal volume and minute

volume between the two methods (*p < 0.01). IRV, inversed ratio ventilation; MBV, marathoners' breathing pattern ventilation

Figure 2

Comparison of peak inspiratory pressure (PIP) and airway pressure between the IRV and MBV methods. A significant difference is observed in the PIP between the two methods (*p < 0.01). IRV, inversed ratio ventilation; MBV, marathoners' breathing pattern ventilation

Figure 3

Typical waveforms of the airway pressure for IRV and MBV methods in the same rabbit. In the MBV method (lower panel), the airway pressure transiently decreases immediately after the end of the 1st inspiratory period and transiently increases immediately after the end of the 1st expiratory period. IRV, inversed ratio ventilation; MBV, marathoners' breathing pattern ventilation

Figure 4

Changes in peak inspiratory pressure (PIP) and airway pressure in the IRV (and MBV groups. The PIP significantly increases after 60 min perfusion in the IRV group († $p < 0.05$). A significant difference is observed in the PIP after 60 min perfusion between the two groups (‡ $p < 0.05$). IRV, inversed ratio ventilation; MBV, marathoners' breathing pattern ventilation

Figure 5

Comparison of myeloperoxidase activity and protein concentration and W/D between the IRV and MBV groups. A significant difference is observed in the W/D between the two groups (§ $p < 0.05$). BALF, bronchoalveolar lavage fluid; IRV, inversed ratio ventilation; MBV, marathoners' breathing pattern ventilation; ΔOD , change in optical density; W/D, wet-to-dry ratio

Pressure Volume Curve

Figure 6

Pulmonary PV curves at baseline and 60 min after perfusion in IRV and MBV groups. Pressure is expressed by the average value against each volume, since no significant differences are observed between groups at each pressure. Compared with the PV curves in the MBV group, the curves in the IRV group have slightly shifted to the high-pressure side. IRV, inversed ratio ventilation; MBV, marathoners' breathing pattern ventilation; PV, pressure-volume.

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