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# Investigating Deep Breathing through Measurement of Ventilatory Parameters and Observation of Breathing Patterns

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June 10, 2019

Dr. Aaron Berard
Science Editor, JoVE

Dear Dr. Berard;

Thank you for giving us the opportunity to submit a revised draft of our manuscript entitled, "***Methodology for investigating deep breathing through measurement of ventilatory parameters and observation of breathing pattern***" to JoVE. The original title was ***Methodology for investigating deep breathing through work of breathing, ventilatory efficiency and breathing patterns***.

We appreciate the time and effort you and each of the reviewers have dedicated to providing insightful feedback on ways to strengthen our paper.

Thus, it is with great pleasure that we resubmit our article for further consideration. We have incorporated changes that reflect the detailed suggestions you have graciously provided.

We also hope that our edits and the responses we have provided satisfactorily address all the issues and concerns you and the reviewers have noted.

To facilitate your review of our revisions, the response to the questions and comments are delivered in attached files.

Again, thank you for giving us the opportunity to strengthen our manuscript with your valuable comments and queries.

Sincerely,

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**TITLE:**

# Investigating Deep Breathing through Measurement of Ventilatory Parameters and Observation of Breathing Patterns

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**KEYWORDS:**

Deep breathing, Natural breathing pattern, Diaphragmatic breathing pattern, Expired gas, Work of breathing, Ventilatory parameters, Video capture

**SUMMARY:**

Here, we present a protocol to assess two deep breathing patterns of natural and diaphragmatic breathing for their effectiveness and ease of execution. Fifteen participants were selected, utilizing an electrocardiograph and expired gas analyzer for measurement of the ventilatory parameters, together with visual assessment by video capture of thoracoabdominal movement.

**ABSTRACT:**

In this protocol, two deep breathing patterns were shown to 15 participants to determine an easy yet effective method of breathing exercise for future application in a clinical setting. The women in their twenties were seated comfortably in a chair with back support. They were fitted with an airtight mask connected to an expired gas analyzer. Three electrodes were placed on the chest connected to a wireless transmitter for relaying to the electrocardiograph. They executed a 5 min rest phase, followed by 5 min of deep breathing with a natural breathing pattern, and terminating with a 5 min rest phase. This was followed by a 10 min intermission before commencing the second instruction phase of substituting the natural breathing pattern with the diaphragmatic breathing pattern. Simultaneously, the following took place: a) continuous collection, measurement and analysis of the expired gas to assess the ventilatory

parameters on a breath-by-breath basis; b) measurement of the heart rate by an electrocardiograph; and c) videotaping of the participant's thoracoabdominal movement from a lateral aspect. From the video capture, the investigators carried out visual observation of the fast-forward motion-images followed by classification of the breathing patterns, confirming that the participants had carried out the method of deep breathing as instructed. The amount of oxygen uptake revealed that, during deep breathing, the work of breathing decreased. The results from the expired minute ventilation, respiration rate and tidal volume confirmed increased ventilatory efficiency for deep breathing with the natural breathing pattern compared to that with the diaphragmatic breathing pattern. This protocol suggests a suitable method of instruction for assessing deep breathing exercises on the basis of oxygen consumption, ventilatory parameters, and chest wall excursion.

**INTRODUCTION:**

The cardiopulmonary physical therapist normally treats the patient according to the individual's needs and requirements. However, in general, the patient is left to carry out preoperative deep breathing exercise by him/herself. Therefore, it is imperative to find a simple and effective instruction method for the patient to carry out deep breathing exercises[1].

Diaphragmatic breathing is such a breathing exercise and one method of breathing control[2,3]. The therapeutic outcome of this method includes a reduction in work of breathing and improvement in efficiency of breathing[2,3], and this brings about an increase in tidal volume, resulting in a reduction in respiratory rate. However, some researchers have pointed out that diaphragmatic breathing exercise may cause asynchronous and paradoxical motion of the rib cage due to abdominal excursions in some patients[4,5]. In such cases, the use of a patient's natural breathing pattern may be efficacious. Regarding the question of deep breathing being effective as a means of a reduction in the mechanical work of breathing and improvement of ventilatory efficiency, it may be useful to quantify ventilatory parameters by an expired gas analyzer.

It is well known that cardiopulmonary exercise testing is carried out using an expired gas analyzer[6,7]. For diaphragmatic breathing, some investigators[8,9] have reported measurement with an expired gas analyzer for patients with chronic obstructive pulmonary disease. Jones et al.[8] compared diaphragmatic breathing, pursed-lip breathing, and a combination of both, with that of spontaneous breathing. Oxygen consumption ($VO_2$) and respiratory rate (f) were measured during these three methods of breathing. They showed that higher resting $VO_2$ may be explained by the increased mechanical work of breathing[8]. Ito et al.[9] examined the immediate effect of diaphragmatic breathing or respiratory muscle stretch on $VO_2$, f and tidal volume ($V_T$). We may expect from the results of the aforementioned studies that similar evidence could be obtained by application of similar breathing exercises to confirm an effective deep breathing method of instruction.

This protocol describes the method for measurement of ventilatory parameters and chest wall excursion in deep breathing with two breathing patterns, together with results and analysis. Continuous and quantitative sampling of ventilatory parameters can measure breathing

precisely compared to alternative techniques. $VO_2$ obtained in this protocol can be regarded as an indicator of work of breathing[8]. Further, f, $V_T$, and minute ventilation are related to ventilatory efficiency, and information on breathing pattern can also be obtained from these ventilator parameters plus inspiratory and expiratory time. This protocol also involves assessment of chest wall excursion through video capture, which corresponds to observation by a physical therapist of the patient's chest wall excursion during breathing exercise. The overall goal of this study was to find a viable and efficient method of deep breathing exercise based on analysis of oxygen consumption, ventilatory parameters, and chest wall excursion.

**PROTOCOL:**

This protocol was in accordance with the ethical principles of the Declaration of Helsinki. The procedure was explained to all participants before commencement of the study.

**1. Participant screening**

1.1. Recruit 15 healthy women in their twenties through convenience sampling. Check medical history verbally. Exclude participants with cardiopulmonary disease.

1.2. Explain the procedure to the participant.

1.3. Ask the participant to refrain from eating and drinking 2 h prior to the commencement of the measurement and to bring a tight-fitting black shirt.

2. **Procedure**

2.1. Preparation for the procedure

2.1.1. Calibrate the expired gas analyzer and flow meters 15 – 30 min before the measurement of gas concentration. Follow manufacturer's protocols.

2.1.2. Attach a video camera to a tripod at a distance of 1.5 m from the chair the participant will sit on. Prepare to record a lateral view of the sitting participant in a range from the top of her cranium to the seat of the chair.

2.1.3. Instruct the waiting participant to put on the tight-fitting black shirt in a cubicle, and when ready, stand by the side of the chair in the laboratory.

2.1.4. Place three electrodes (active, reference and ground) on the skin of the chest in standing, each with a wire to connect to a transmitter that relays to the electrocardiograph.

2.1.5. Seat the participant comfortably for 5 min in a chair with a back support at an angle of 70° and, if necessary, insert a small cushion in the lumbar region.

2.1.6. Explain to the participant deep breathing with a natural breathing (NB) pattern of slow and deep breaths, breathing in through the nose and blowing out through the mouth with no consideration or knowledge given on specific movement of the chest.

2.1.7. Ask the participant to take a normal deep breath without any guidance. Prepare to begin measurement if the investigator is satisfied with breathing performance.

2.2. Measurement of deep breathing with the NB pattern

2.2.1. Fit the participant with a sampling mask over the mouth and nose for measurement of the expired gas. Perform a seal test: close the hole for the sampling tube of the mask with a finger and ask the participant to breathe out gently and confirm whether air leaks from the mask. Connect a sampling tube to the mask for measurement of the ventilatory parameters.

2.2.2. Ask the participant to refrain from talking during the procedure.

2.2.3. Instruct the participant to rest for 5 min, and simultaneously begin recording the expired gas and heart rate, together with video capture. After the 5 min rest phase, instruct the participant to begin deep breathing for 5 min with NB pattern. On termination, instruct the participant to rest for 5 min.

2.2.4. Continue recording and measuring throughout the three phases.

2.2.5. Perform only one three-phase trial for each participant.

2.3. Rest phase

2.3.1. Inform the participant that the experimenter will take off the mask and allow her a 10 min intermission phase.

2.3.2. Instruct the participant that she may sit and talk in the laboratory, but not drink. Begin timing the intermission phase with a stopwatch at the moment of taking off the mask.

**2.4. Measurement of deep breathing with the diaphragmatic breathing (DB) pattern**

2.4.1. Seat the participant as in step 2.1.5.

2.4.2. Explain to the participant deep breathing with a diaphragmatic breathing (DB) pattern. Ask the participant to lace her fingers, place them on her abdomen and take a deep breath in through the nose, expanding the abdomen under the hands and then blowing out through the mouth and gently retracting the abdomen.

2.4.3. Instruct the participant to practice this deep breathing with the DB pattern until the investigator is satisfied.

2.4.4. Fit the participant with a sampling mask over the mouth and nose for measurement of the expired gas. Perform a seal test: close the hole for the sampling tube of the mask with a finger and ask the participant to breathe out gently and confirm whether air leaks from the mask. Connect a sampling tube to the mask for measurement of the ventilatory parameters.

2.4.5. Ask the participant to refrain from talking during the measurement.

2.4.6. Instruct the participant to rest for 5 min, and simultaneously begin recording the expired gas and heart rate, together with video capture. After the 5 min rest phase, instruct the participant to begin deep breathing for 5 min with the DB pattern. On termination, instruct the participant to rest for 5 min. Continue recording and measuring throughout the three phases.

2.4.7. Take the mask off the participant following the 5 min rest phase.

2.4.8. Ask the participant immediately which of the two deep breathing techniques was more comfortable. Record the participant's response on a spreadsheet.

2.4.9. Remove the electrodes, leads and transmitter from the participant and let her leave.

2.4.10. Perform only one three-phase trial for each participant.

## 3. Measurement of the Ventilatory Parameters

3.1. Sample the breath-by-breath expired gas using an expired gas analyzer (see **Table of Materials** and **Figure 2**).

3.1.1. Measure the following ventilatory parameters: oxygen uptake ($VO_2$), carbon dioxide output ($VCO_2$), expired minute ventilation (VE), respiratory rate (f), tidal volume ($V_T$), expiratory time ($T_e$), and inspiratory time ($T_i$).

3.1.2. Measure the heart rate using a medical telemetry sensor for the electrocardiograph (see **Table of Materials** and **Figure 2**) and expired gas analyzer, both of which are connected.

NOTE: The expired gas analyzer is operated using computer software provided by the manufacturer (**Table of Materials**).

3.2. Collect the data for each 5 min phase of rest and deep breathing for NB and DB patterns. Save the data on the ventilatory parameters in CSV format using computer software (see **Table of Materials** and **Figure 3,4**).

3.2.1. Collect mean data for each 5 min phase of this protocol. Average data for the optionally set phase is obtained on a breath-by-breath basis.

3.3. Enter the data for each participant into the spreadsheet program (see **Table of Materials** and **Figure 5**) and determine the mean and standard deviation (SD) for the initial rest and deep breathing phases for NB and DB.

## 4. Assessment of the Breathing Pattern

4.1. Record thoracoabdominal movement from a lateral view of the participant using a video camera (**Table of Materials**).

4.2. Make sure that contrast between the participant and background color is clear.

4.3. Record the video image at 1/30 s per frame, which is the standard speed for the video camera used.

4.4. Upload the motion-images into a personal computer using video editing software 1 (**Table of Materials**).

4.5. Observe 5 min video images of the deep breathing phases at double speed under visual assessment and classify the breathing patterns as upper costal, diaphragmatic or thoracoabdominal. Use video editing software 2 (**Table of Materials**).

NOTE: Video images are analyzed by a cardiopulmonary physical therapist (MY).

## 5. Participants' preferred pattern for deep breathing

5.1. Prepare a spreadsheet for the participant's response.

5.2. Ask the participant which of the two deep breathing techniques is more comfortable following the measurement of the DB pattern.

5.3. Fill in the spreadsheet with the participant's response.

5.4. Be ready to listen to the participant if she wants to talk about the procedure. Do not include the participant's comments in the analysis.

## 6. Statistical Analysis

NOTE: Perform statistical analysis using commercial computer software (**Table of Materials**), and then provide all the button clicks.

6.1. Ventilatory parameters

6.1.1. Do not analyze the 5 min rest phase following the two deep breathing phases in this protocol.

6.1.2. Determine the mean and SD for the initial rest phases and deep breathing phases for each parameter.

6.1.3. Employ two-way repeated-measures analysis of variance (2-way ANOVA) to evaluate the ventilatory parameters and heart rate for the initial rest phases and two deep breathing phases.

NOTE: Factor "instruction" includes two levels NB and DB, and factor "phase" has two levels rest phase and deep breathing phase.

6.1.4. Using the Bonferroni method, evaluate between each factor for parameter measurement, yielding significant interaction following 2-way ANOVA.

6.2. Classification of breathing patterns exhibited by the participants including their preferred pattern for deep breathing.

6.2.1. Categorize the number of participants according to their breathing pattern in upper costal, diaphragmatic or thoracoabdominal breathing.

6.2.2. Compile from the spreadsheet the number of participants according to their preferred pattern of deep breathing.

## REPRESENTATIVE RESULTS[1]:

### The Ventilatory Parameters and Heart Rate

Based on the data (**Figure 5**), the NB and DB patterns were analyzed statistically (**Figure 6** and **Table 1**). The f, $V_T$ and Te were found to have a significant interaction ($p<0.05$, respectively). A significant decrease in the f was found for both the NB and DB patterns during deep breathing compared to the initial rest phases ($p<0.05$, respectively), and, during deep breathing with the NB pattern, the f decreased to a greater extent compared to that for the DB pattern (**Figure 6** and **Table 1**). The $V_T$ and Te revealed a significant increase during deep breathing compared to the initial rest phases for the NB and DB patterns, and, those for both deep breathing with the NB pattern were larger compared to those for the DB pattern (**Figure 6**). All of the parameters except VE and HR revealed main effect for the "phase" factor (**Table 1**).

A reduction in the work of breathing was reflected in a decrease in $VO_2$, and, during deep breathing with the NB and DB patterns, the $VO_2$ decreased with a reduced work of breathing (**Table 1**). The improvement in ventilatory efficiency is reflected in an increase in $V_T$ and a decrease in f or VE. Deep breathing with the NB pattern was superior in ventilatory efficiency compared to that with the DB pattern. Generally, the relationship between VE and alveolar ventilation (VA) is calculated by the formula: $VE = V_T \times f$ and $VA = (V_T$- anatomic dead space$) \times f$. Assuming that VE is a constant, a decreased respiratory rate and an increased $V_T$ indicate an

improvement in VA. As VE yielded no significant interaction and main effect following 2-way ANOVA (**Table 1**), VE for deep breathing with both breathing patterns appeared to be equal. The respiratory rate during deep breathing with the NB pattern was significantly smaller compared to the DB pattern, but, for $V_T$ during deep breathing, it was significantly larger compared to that for the DB pattern (**Figure 6** and **Table 1**). In other words, the alveolar ventilation or gas exchange during deep breathing with the NB pattern appears more efficient than for that during deep breathing with the DB pattern.

**Breathing Patterns and Participants' Preference**

Shown is the result of the visual assessment for thoracoabdominal movement during deep breathing with two methods of instructions (**Table 2**). The majority of the participants presented an upper costal or thoracoabdominal pattern. During deep breathing with the DB pattern, all but one participant showed a thoracoabdominal or diaphragmatic pattern. This result shows that the participants were able to carry out deep breathing as instructed. Thirteen out of the 15 participants expressed that they found the NB pattern easier to carry out than the DB pattern.

**FIGURE AND TABLE LEGENDS:**

**Figure 1. Flow chart for the protocol**

**Figure 2. Ventilatory parameters as were seen displayed on the screen.**

Left, Natural breathing pattern; Right, Diaphragmatic breathing pattern. Display shows a sample of individual data for each of the breath-by-breath samples for the ventilatory parameters and heart rate.

**Figure 3. CSV format for ventilatory parameters during deep breathing with a natural breathing pattern.**

The spreadsheet shows a sample of ventilatory parameters and heart rate following measurement.

**Figure 4. CSV format for ventilatory parameters during deep breathing with a diaphragmatic breathing pattern.**

The spreadsheet shows a sample of ventilatory parameters and heart rate following measurement.

**Figure 4. Raw data for all participants, which was converted from CSV data.**

**Figure 5. Significant interaction between ventilatory parameters.**

Solid blue circle, deep breathing with natural breathing pattern; White circle, deep breathing with diaphragmatic breathing pattern. (**A**) shows the f, respiratory rate, (**B**) shows the $V_T$, tidal volume, and (**C**) shows the Te, expiratory time.

**Table 1. Comparison between the two breathing patterns.**

$VO_2$, oxygen uptake; $VCO_2$, carbon dioxide output; VE, minute ventilation; f, respiratory rate; $V_T$,

tidal volume; Te, expiratory time; Ti, inspiratory time; HR, heart rate; *, $p<0.05$ (Rest vs. Deep breathing during NB); †, $p<0.05$ (Rest vs. Deep breathing during DB); §, $p<0.05$ (NB vs. DB during Deep breathing).
This table has been modified from one published in the Journal of Physical Therapy Science, 2018.

**Table 2. Result of the visual assessment for thoracoabdominal movement during deep breathing with two breathing patterns.**
This table has been modified from one published in the Journal of Physical Therapy Science, 2018.

**DISCUSSION:**

By the use of this protocol, effective instruction for deep breathing can be examined through oxygen consumption, ventilatory parameters, and chest wall excursion. The participants had a mean age of 21.6 years, mean body mass of 51.9 kg, mean height of 159.3 cm and a body mass index of 20.5 kg/m$^2$. No incentives were offered to partake in this protocol. There are three critical steps within the protocol. First is the control of food intake. The ratio of carbon dioxide output to oxygen uptake provides information about the nutrient mixture catabolized for energy[10]. $VO_2$ measured in the expired gas is affected by food intake in lower physical activity than higher one[11]. The result for $VO_2$ (**Table 1**) demonstrates a lower physical activity during deep breathing. Consequently, it is imperative to set conditions for food intake prior to measurement. Second, the participants are required to refrain from talking during measurement so as not to influence expired gas data. Third, to avoid possible learning effects of the DB pattern on the NB pattern, the execution of the breathing pattern sequence for the measurement is important (**Figure 1**).

In general, breathing exercise takes 3-5 min to execute. Therefore, the investigators allocated a 5 min deep breathing phase sandwiched between the two rest phases. The participants carried out one trial for each 5 min deep breathing phase, for this protocol was designed to simulate initial instruction in a clinical setting of a 5 min breathing exercise. However, participants did not have an ideal diaphragmatic breathing pattern when carrying it out for the first time in this protocol (**Table 2**). Modification of this protocol may require further instruction time and practice for learning diaphragmatic breathing to compare the two breathing methods.

For the measurement of the expired gas on a breath-by-breath basis, the number of samples per min for the ventilatory parameters is equal to the respiratory rate per min. The number of samples per min is known to increase during vigorous physical activity, but that for the ventilatory parameters decreases during deep breathing as was shown in this protocol. Considering the above fact, the average time for data collection should be determined.

By the use of a video camera, it is possible for one investigator to carry out this protocol. In addition, a breathing pattern can be easily judged by fast-forwarding the motion-images. During the preliminary testing for this protocol, markers were placed on the sternum and abdomen, followed by videotaping. However, these markers were of no assistance for the

visual judgment. Consequently, it was decided to have the participant wear a tight-fitting black shirt. Furthermore, it may be easier to observe the thoracoabdominal excursion if the color of the shirt is in contrast to that of the wall. In this protocol, the wall's background color is white beige in contrast to the black shirt. The investigators recommend the use of different protocol(s)[12,13] if one intends to study breathing patterns particularly with attention to the kinematics of the thorax.

As for the sample size for this protocol, calculation by post-hoc power analysis[14] yielded 0.75. To satisfy statistical power of 0.8 defined by Cohen[14], a minimum sample size of 17 participants would have been required for this protocol, which meant that it had a shortage of two participants. Further, the distribution of pulmonary ventilation could not be assessed, as would have been possible with electrical impedance tomography[15].

Continuous and quantitative sampling of ventilatory parameters can measure breathing precisely compared to alternative techniques. Ventilatory parameters obtained in this protocol include mechanism of intervention and energy cost in a systematic review of breathing control[2]. Henceforth, the investigators would like to explore how ventilatory parameters in the elderly and individuals in supine and/or side lying would differ from those in this study.

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**DISCLOSURES:**

The authors declare that they have no competing financial interests.

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Figure 1

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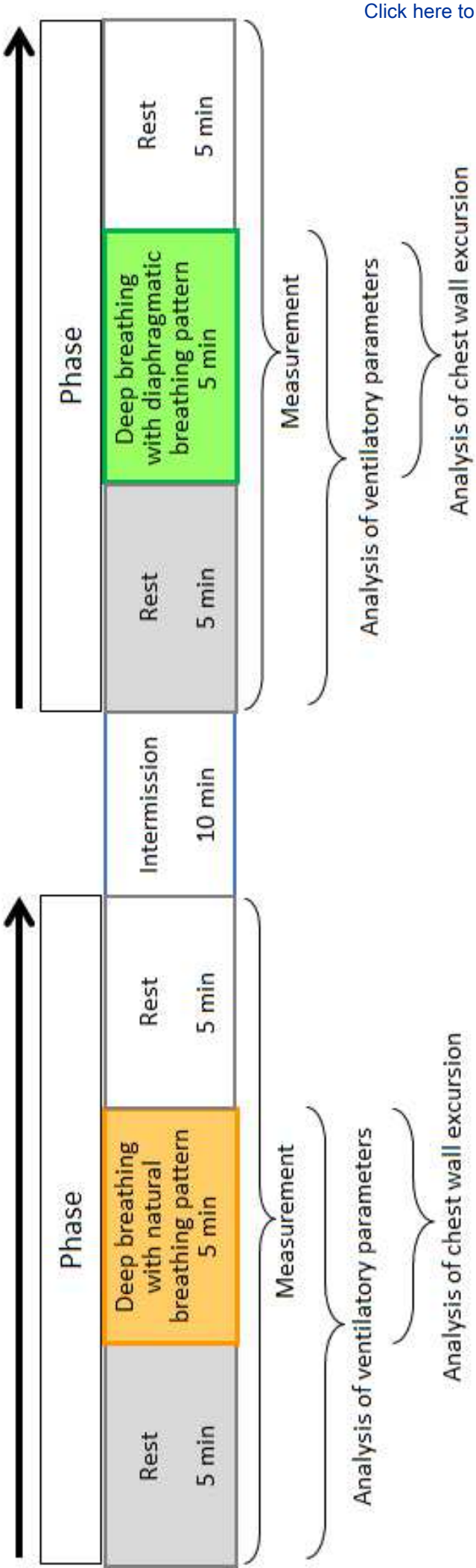

Figure 2

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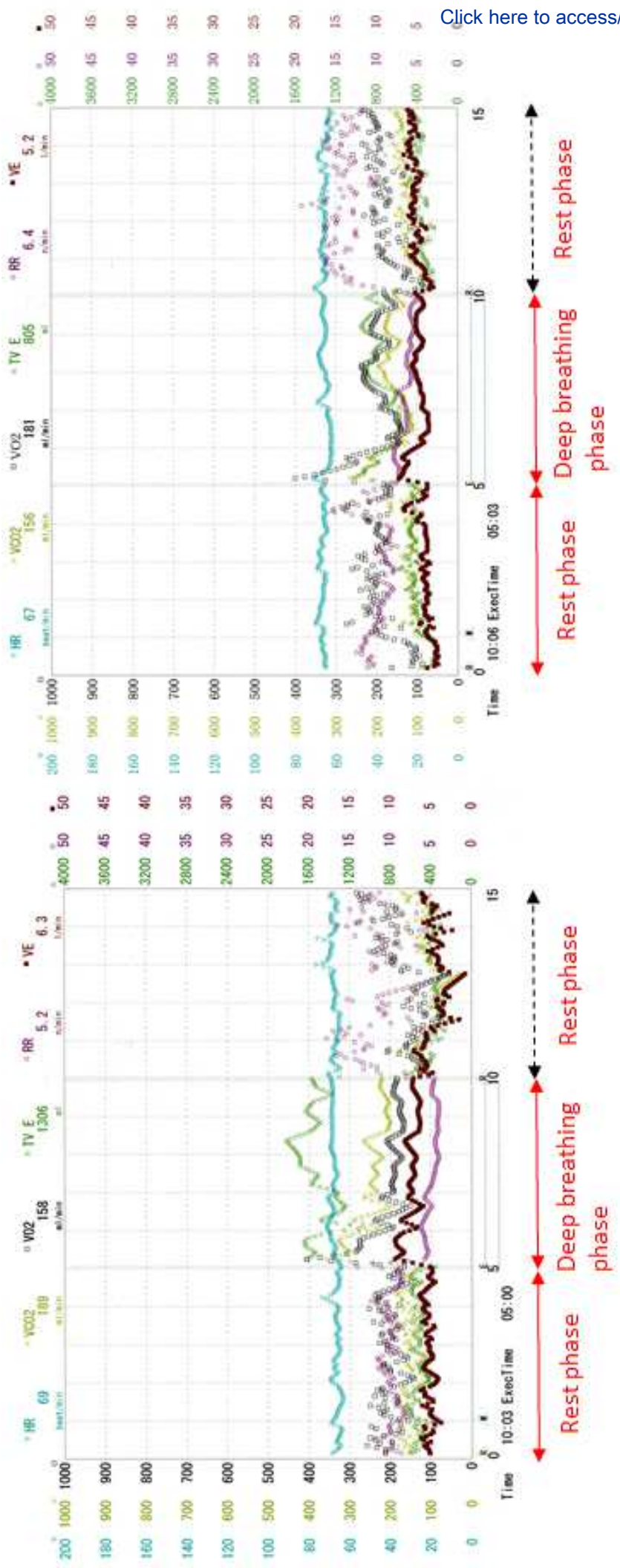
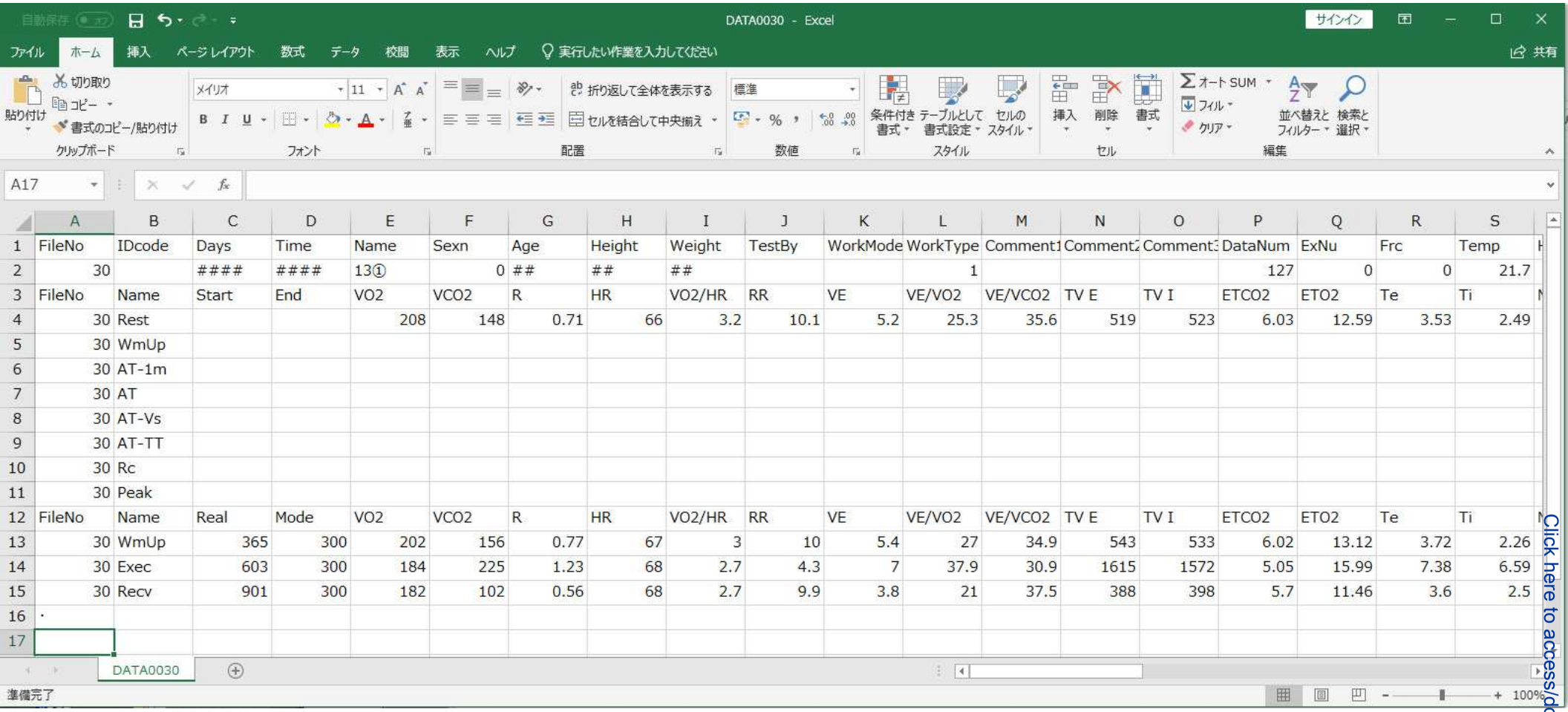
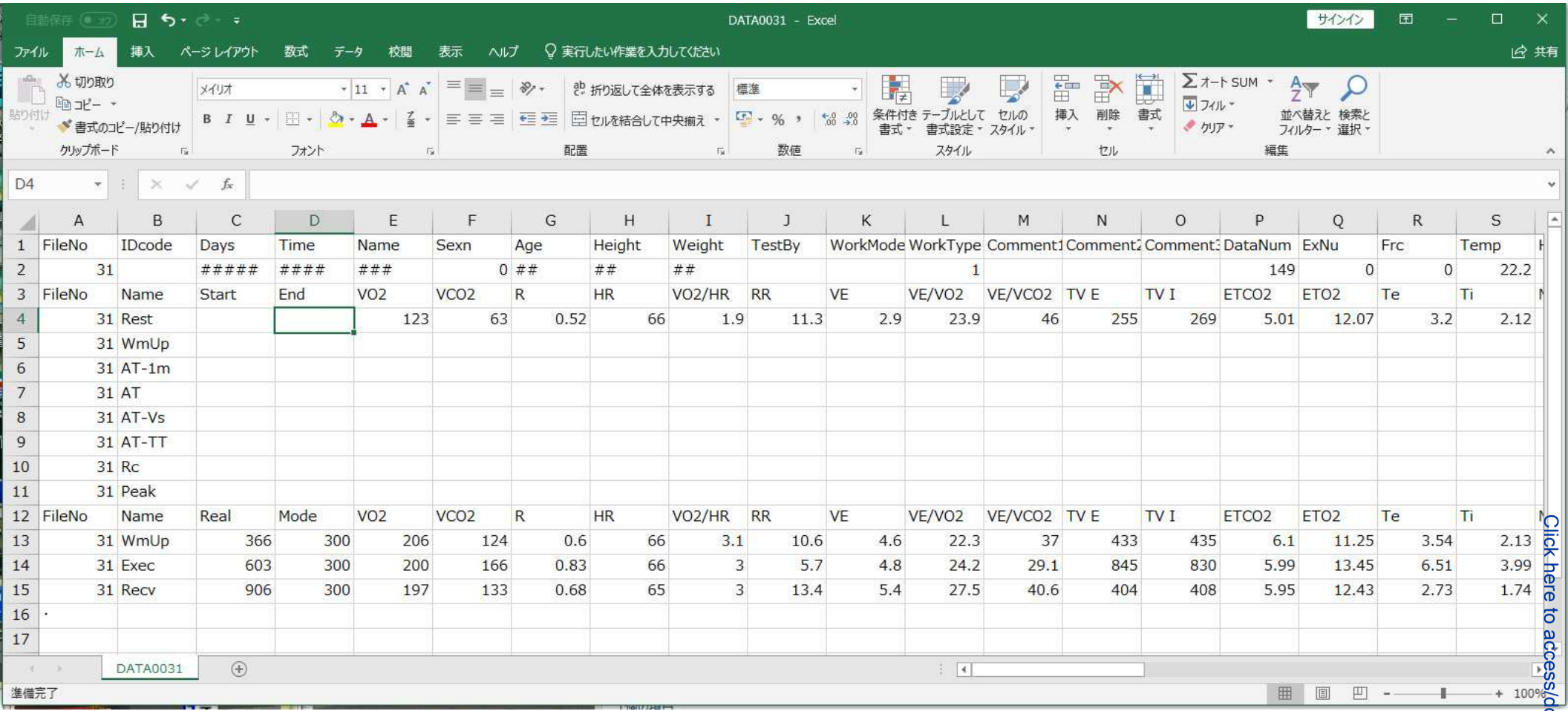


Figure 3




| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | FileNo | IDcode | Days | Time | Name | Sexn | Age | Height | Weight | TestBy | WorkMode | WorkType | Comment1 | Comment2 | Comment3 | DataNum | ExNu | Frc | Temp |
| 2 | 30 | | #### | #### | 13① | 0 | ## | ## | ## | | | 1 | | | | 127 | 0 | 0 | 21.7 |
| 3 | FileNo | Name | Start | End | VO2 | VCO2 | R | HR | VO2/HR | RR | VE | VE/VO2 | VE/VCO2 | TV E | TV I | ETCO2 | ETO2 | Te | Ti |
| 4 | 30 | Rest | | | 208 | 148 | 0.71 | 66 | 3.2 | 10.1 | 5.2 | 25.3 | 35.6 | 519 | 523 | 6.03 | 12.59 | 3.53 | 2.49 |
| 5 | 30 | WmUp | | | | | | | | | | | | | | | | | |
| 6 | 30 | AT-1m | | | | | | | | | | | | | | | | | |
| 7 | 30 | AT | | | | | | | | | | | | | | | | | |
| 8 | 30 | AT-Vs | | | | | | | | | | | | | | | | | |
| 9 | 30 | AT-TT | | | | | | | | | | | | | | | | | |
| 10 | 30 | Rc | | | | | | | | | | | | | | | | | |
| 11 | 30 | Peak | | | | | | | | | | | | | | | | | |
| 12 | FileNo | Name | Real | Mode | VO2 | VCO2 | R | HR | VO2/HR | RR | VE | VE/VO2 | VE/VCO2 | TV E | TV I | ETCO2 | ETO2 | Te | Ti |
| 13 | 30 | WmUp | 365 | 300 | 202 | 156 | 0.77 | 67 | 3 | 10 | 5.4 | 27 | 34.9 | 543 | 533 | 6.02 | 13.12 | 3.72 | 2.26 |
| 14 | 30 | Exec | 603 | 300 | 184 | 225 | 1.23 | 68 | 2.7 | 4.3 | 7 | 37.9 | 30.9 | 1615 | 1572 | 5.05 | 15.99 | 7.38 | 6.59 |
| 15 | 30 | Recv | 901 | 300 | 182 | 102 | 0.56 | 68 | 2.7 | 9.9 | 3.8 | 21 | 37.5 | 388 | 398 | 5.7 | 11.46 | 3.6 | 2.5 |
| 16 | · | | | | | | | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | | | | | | | | |

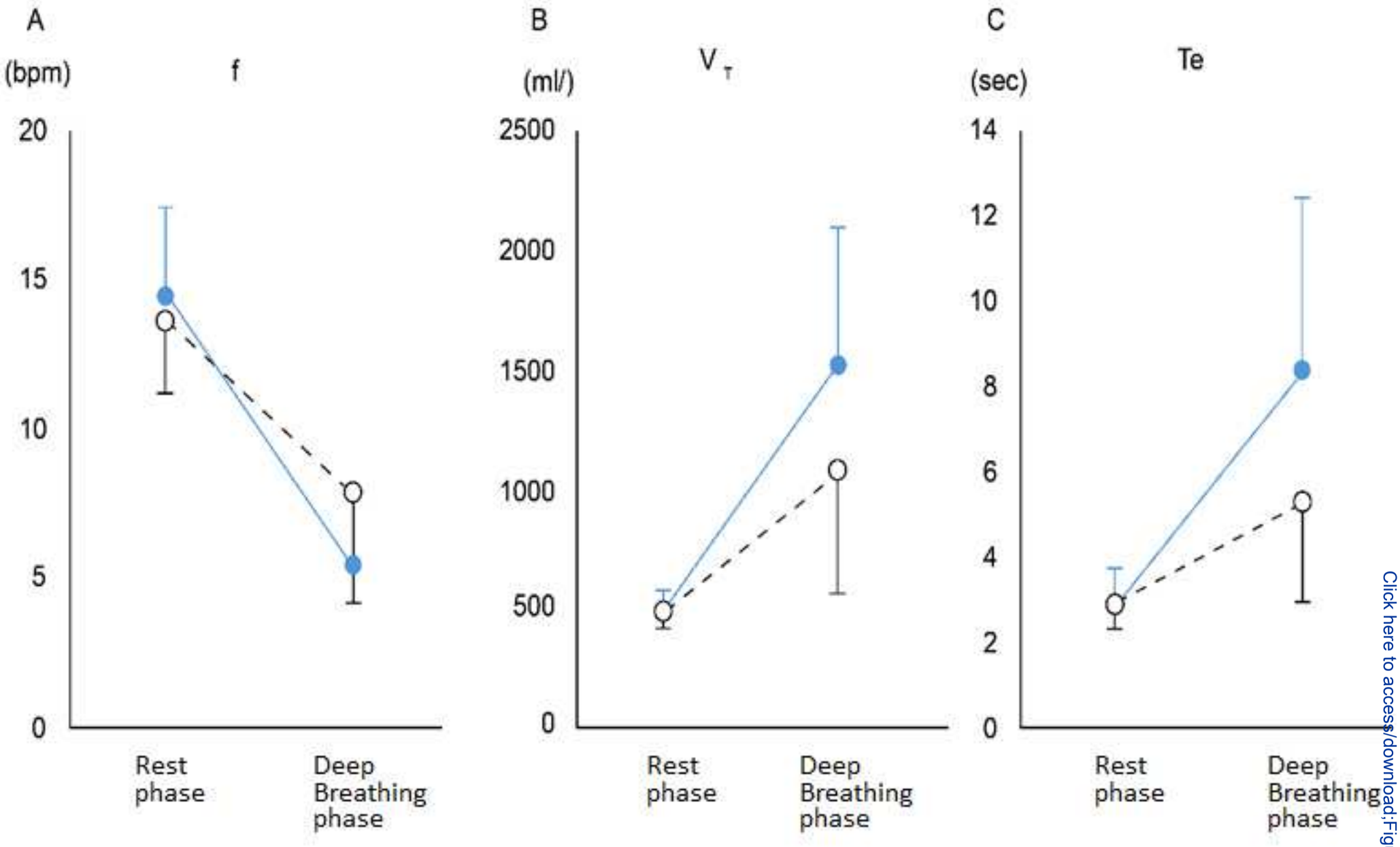
Figure 4




| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R | S |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | FileNo | IDcode | Days | Time | Name | Sexn | Age | Height | Weight | TestBy | WorkMode | WorkType | Comment1 | Comment2 | Comment3 | DataNum | ExNu | Frc | Temp |
| 2 | 31 | | ##### | #### | ### | 0 | ## | ## | ## | | | 1 | | | | 149 | 0 | 0 | 22.2 |
| 3 | FileNo | Name | Start | End | VO2 | VCO2 | R | HR | VO2/HR | RR | VE | VE/VO2 | VE/VCO2 | TV E | TV I | ETCO2 | ETO2 | Te | Ti |
| 4 | 31 | Rest | | | 123 | 63 | 0.52 | 66 | 1.9 | 11.3 | 2.9 | 23.9 | 46 | 255 | 269 | 5.01 | 12.07 | 3.2 | 2.12 |
| 5 | 31 | WmUp | | | | | | | | | | | | | | | | | |
| 6 | 31 | AT-1m | | | | | | | | | | | | | | | | | |
| 7 | 31 | AT | | | | | | | | | | | | | | | | | |
| 8 | 31 | AT-Vs | | | | | | | | | | | | | | | | | |
| 9 | 31 | AT-TT | | | | | | | | | | | | | | | | | |
| 10 | 31 | Rc | | | | | | | | | | | | | | | | | |
| 11 | 31 | Peak | | | | | | | | | | | | | | | | | |
| 12 | FileNo | Name | Real | Mode | VO2 | VCO2 | R | HR | VO2/HR | RR | VE | VE/VO2 | VE/VCO2 | TV E | TV I | ETCO2 | ETO2 | Te | Ti |
| 13 | 31 | WmUp | 366 | 300 | 206 | 124 | 0.6 | 66 | 3.1 | 10.6 | 4.6 | 22.3 | 37 | 433 | 435 | 6.1 | 11.25 | 3.54 | 2.13 |
| 14 | 31 | Exec | 603 | 300 | 200 | 166 | 0.83 | 66 | 3 | 5.7 | 4.8 | 24.2 | 29.1 | 845 | 830 | 5.99 | 13.45 | 6.51 | 3.99 |
| 15 | 31 | Recv | 906 | 300 | 197 | 133 | 0.68 | 65 | 3 | 13.4 | 5.4 | 27.5 | 40.6 | 404 | 408 | 5.95 | 12.43 | 2.73 | 1.74 |
| 16 | · | | | | | | | | | | | | | | | | | | |
| 17 | | | | | | | | | | | | | | | | | | | |

Figure 5

| No. | Condition | W VO2 | W VCO2 | W HR | W RR | W VE | W VE/VCO2 | W TV E | W Te | W Ti | E VO2 | E VCO2 | E HR | E RR | E VE | E VE/VCO2 | E TV E | E Te | E Ti | W Breathing Pattern | E Breathing Pattern | Easier Breathing |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | 1 | 200 | 171 | 49 | 17.1 | 6.8 | 39.8 | 397 | 2.25 | 1.26 | 181 | 153 | 51 | 5.9 | 4.3 | 28 | 730 | 7.05 | 3.17 | 2 | 3 | 2 |
| 5 | 1 | 210 | 189 | 63 | 16.1 | 7.8 | 41.4 | 487 | 2.42 | 1.31 | 208 | 239 | 65 | 5 | 7.2 | 30.1 | 1429 | 8.52 | 3.44 | 2 | 2 | 2 |
| 6 | 1 | 204 | 182 | 73 | 16.6 | 8.1 | 44.5 | 488 | 2.21 | 1.4 | 200 | 212 | 76 | 8.3 | 6.9 | 32.6 | 829 | 4.63 | 2.57 | 3 | 2 | 1 |
| 7 | 1 | 184 | 145 | 69 | 16.6 | 6.7 | 45.9 | 402 | 2.3 | 1.32 | 178 | 172 | 69 | 3.4 | 4.7 | 27.5 | 1371 | 11.81 | 5.63 | 1 | 1 | 1 |
| 8 | 1 | 198 | 182 | 70 | 14.2 | 7.6 | 41.6 | 534 | 2.66 | 1.58 | 200 | 196 | 71 | 2.8 | 5.4 | 27.4 | 1895 | 16.25 | 4.89 | 1 | 1 | 1 |
| 9 | 1 | 177 | 129 | 62 | 15.1 | 5.8 | 44.7 | 382 | 2.52 | 1.46 | 192 | 248 | 75 | 3.7 | 8.5 | 34.3 | 2279 | 10.07 | 5.91 | 3 | 3 | 1 |
| 10 | 1 | 220 | 181 | 78 | 19.1 | 8.1 | 44.7 | 421 | 1.91 | 1.22 | 180 | 162 | 81 | 3.6 | 4.9 | 30.1 | 1364 | 13.06 | 3.76 | 3 | 3 | 1 |
| 11 | 1 | 213 | 173 | 65 | 14.9 | 6.2 | 36 | 415 | 2.54 | 1.48 | 186 | 214 | 66 | 6.4 | 6.6 | 31 | 1033 | 6.09 | 3.28 | 1 | 1 | 1 |
| 12 | 1 | 173 | 140 | 78 | 15.4 | 6.5 | 46.8 | 424 | 2.25 | 1.64 | 185 | 289 | 86 | 10.7 | 14 | 48.5 | 1316 | 2.85 | 2.77 | 3 | 3 | 1 |
| 13 | 1 | 202 | 156 | 67 | 10 | 5.4 | 34.9 | 543 | 3.72 | 2.26 | 184 | 225 | 68 | 4.3 | 7 | 30.9 | 1615 | 7.38 | 6.59 | 2 | 3 | 1 |
| 14 | 1 | 181 | 147 | 72 | 7.1 | 4.5 | 30.7 | 637 | 5.74 | 2.75 | 166 | 150 | 70 | 2.6 | 3.5 | 23.2 | 1335 | 14.26 | 8.75 | 3 | 3 | 1 |
| 15 | 1 | 243 | 235 | 78 | 14.7 | 8.2 | 34.8 | 554 | 2.58 | 1.5 | 187 | 285 | 65 | 6.1 | 10 | 35.1 | 1651 | 6.5 | 3.41 | 3 | 1 | 1 |
| 16 | 1 | 213 | 192 | 68 | 12.4 | 6.7 | 34.8 | 540 | 3.07 | 1.77 | 212 | 212 | 69 | 7.9 | 6.1 | 28.8 | 772 | 3.3 | 4.31 | 3 | 1 | 1 |
| 17 | 1 | 202 | 184 | 75 | 11.9 | 6.6 | 36.1 | 558 | 2.95 | 2.09 | 212 | 350 | 78 | 4.3 | 11.4 | 32.5 | 2622 | 8.43 | 5.46 | 1 | 1 | 1 |
| 18 | 1 | 169 | 143 | 70 | 14.1 | 6.5 | 45.4 | 459 | 2.78 | 1.47 | 188 | 358 | 85 | 6.6 | 15.6 | 43.6 | 2367 | 5.36 | 3.73 | 3 | 1 | 1 |
| 4 | 2 | 199 | 172 | 47 | 18.6 | 6.8 | 39.3 | 365 | 2.04 | 1.19 | 171 | 143 | 49 | 6.1 | 3.9 | 27.5 | 642 | 5.68 | 4.18 | 3 | 3 | 2 |
| 5 | 2 | 197 | 167 | 63 | 15.5 | 7.2 | 42.9 | 463 | 2.49 | 1.38 | 176 | 162 | 67 | 3.7 | 4.3 | 26.4 | 1165 | 11.09 | 5.25 | 3 | 2 | 2 |
| 6 | 2 | 216 | 174 | 74 | 15.4 | 7.5 | 43.2 | 489 | 2.44 | 1.46 | 183 | 170 | 73 | 10.9 | 6.4 | 37.4 | 587 | 3.57 | 1.96 | 3 | 2 | 1 |
| 7 | 2 | 183 | 152 | 70 | 14.7 | 6.5 | 43 | 445 | 2.64 | 1.45 | 165 | 151 | 70 | 5.2 | 4.6 | 30.6 | 898 | 6.31 | 5.33 | 1 | 3 | 1 |
| 8 | 2 | 206 | 195 | 67 | 13.8 | 7.7 | 39.7 | 561 | 2.69 | 1.67 | 183 | 220 | 68 | 6.9 | 7.8 | 35.3 | 1117 | 4.9 | 3.73 | 1 | 3 | 1 |
| 9 | 2 | 171 | 100 | 70 | 12 | 4 | 40.2 | 335 | 3.11 | 1.9 | 177 | 257 | 79 | 6.1 | 10.3 | 40.2 | 1700 | 6.04 | 3.82 | 3 | 3 | 1 |
| 10 | 2 | 214 | 165 | 83 | 12.4 | 6.4 | 38.5 | 515 | 3.44 | 1.41 | 202 | 251 | 80 | 15.2 | 11.8 | 47.3 | 781 | 2.05 | 1.9 | 3 | 2 | 1 |
| 11 | 2 | 226 | 174 | 66 | 14.2 | 5.9 | 34.1 | 416 | 2.68 | 1.53 | 195 | 154 | 64 | 8 | 4.4 | 28.9 | 556 | 4.14 | 3.38 | 1 | 3 | 1 |
| 12 | 2 | 175 | 157 | 74 | 15.4 | 7.6 | 48.3 | 493 | 1.93 | 1.97 | 188 | 215 | 78 | 16 | 11.2 | 52.1 | 700 | 1.55 | 2.2 | 3 | 3 | 1 |
| 13 | 2 | 206 | 124 | 66 | 10.6 | 4.6 | 37 | 433 | 3.54 | 2.13 | 200 | 166 | 66 | 5.7 | 4.8 | 29.1 | 845 | 6.51 | 3.99 | 2 | 2 | 1 |
| 14 | 2 | 228 | 173 | 71 | 11.8 | 5.9 | 34.3 | 504 | 3.41 | 1.67 | 181 | 167 | 61 | 5.2 | 4.6 | 27.6 | 889 | 7.3 | 4.25 | 1 | 1 | 1 |
| 15 | 2 | 201 | 168 | 69 | 11.4 | 5.9 | 35.5 | 519 | 3.24 | 2.01 | 208 | 330 | 72 | 9 | 12.8 | 38.7 | 1428 | 3.96 | 2.74 | 3 | 3 | 1 |
| 16 | 2 | 217 | 176 | 66 | 14.5 | 6.3 | 35.9 | 434 | 2.42 | 1.71 | 197 | 194 | 67 | 7 | 5.8 | 30.1 | 831 | 3.88 | 4.63 | 3 | 2 | 1 |
| 17 | 2 | 168 | 131 | 71 | 10.1 | 4.9 | 37.5 | 484 | 3.5 | 2.42 | 197 | 222 | 75 | 5.8 | 7.1 | 32.1 | 1234 | 6.21 | 4.2 | 3 | 2 | 1 |
| 18 | 2 | 249 | 169 | 70 | 14.3 | 7.2 | 42.8 | 507 | 2.69 | 1.52 | 209 | 375 | 82 | 6.6 | 16.4 | 43.8 | 2481 | 5.53 | 3.55 | 3 | 3 | 1 |



Table



| | Natural Breathing Pattern | | Diaphragmatic Breathing Pattern | | 2-way ANOVA | | |
|---|---|---|---|---|---|---|---|
| | Rest | Deep breathing | Rest | Deep breathing | Instruction | Phase | Interaction |
| $VO_2$ (L/min) | 0.20±0.02 | 0.19±0.01 | 0.20±0.02 | 0.19±0.01 | | <0.01 | |
| $VCO_2$ (L/min) | 0.17±0.03 | 0.23±0.07 | 0.16±0.02 | 0.21±0.07 | | <0.01 | |
| VE (l/min) | 6.8±1.1 | 7.7±3.6 | 6.3±1.1 | 7.7±3.9 | | | |
| f (/min) | 14.4±3.0 | 5.4±2.3$^{*}$ | 13.6±2.3 | 7.8±3.6$^{\dagger,\S}$ | | <0.01 | <0.05 |
| $V_T$ (/ml) | 483±76 | 1507±579$^{*}$ | 464±61 | 1057±509$^{\dagger,\S}$ | <0.05 | <0.01 | <0.05 |
| Te (s) | 2.79±0.92 | 8.37±4.00$^{*}$ | 2.82±0.53 | 5.25±2.31$^{\dagger,\S}$ | <0.05 | <0.01 | <0.05 |
| Ti (s) | 1.63±0.43 | 4.51±1.70 | 1.69±0.33 | 3.67±1.08 | | <0.01 | |
| HR (bpm) | 69.1±7.6 | 71.7±8.9 | 68.5±7.6 | 70.1±8.5 | | | |

Table 2



| Instruction | Upper costal | Thoracoabdominal | Diaphragmatic |
|---|---|---|---|
| Natural breathing pattern | 7 | 6 | 2 |
| Diaphragmatic breathing pattern | 1 | 8 | 6 |

| Name of Material/Equipment | Company | Catalog Number |
|---|---|---|
| Expired gas analyzer | Minato Medical Science, Osaka, Japan | AE-300S |
| Expired gas analyzing software | Minato Medical Science, Osaka, Japan | AT for Windows |
| Medical telemetry sensor for | Nihon Kohden, Tokyo, Japan | BSM-2401 |
| Spreadsheet program | Microsoft, https://www.microsoft.com/ja- | Excel |
| SPSS Statistical Software | IBM, https://www.ibm.com/jp-ja/analytics/spss-statistics-software | Version 23.0 |
| Video camera | Sony, Tokyo, Japan | DCR-SR 100 |
| Video editing software 1 | Sony, Tokyo, Japan | PlayMemories Home |
| Video editing software 2 | Adobe, https://www.adobe.com/jp/ | Premiere Elements 11 |

**Comments/Description**

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**Response:**
We have rephrased Summary as follows in 50 words.
Here, we present a protocol to assess two deep breathing patterns of natural and diaphragmatic breathing for their effectiveness and ease of execution. Fifteen participants were selected, utilizing an electrocardiograph and expired gas analyzer for measurement of the ventilatory parameters, together with visual assessment by video capture of thoracoabdominal movement.

4. Please ensure that the abstract is between 150- 300 words.
**Response:**
Abstract contains 273 words.

5. Please expand all abbreviation during the first-time use.
**Response:**
We have followed this rule accordingly.

6. Please reword lines 52-58 as it matches with previously published literature.

**Response:**
We have reworded lines 52-58 and included them to revise comment #7.

7. Please revise the Introduction to include all of the following:
**Response:**
We have revised Introduction as shown below:

a) A clear statement of the overall goal of this method
**Response:**
We have added a statement on overall goal (lines 95-97).
The overall goal of this study was to find a viable and efficient method of deep breathing exercise based on analysis of oxygen consumption, ventilatory parameters, and chest wall excursion.

b) The rationale behind the development and/or use of this technique
**Response:**
We have revised lines 59-62.
The cardiopulmonary physical therapist normally treats the patient according to the individual's needs and requirements. However, in general, the patient is left to carry out preoperative deep breathing exercise by him/herself. Therefore, it is imperative to find a simple and effective instruction method for the patient to carry out deep breathing exercise[1].

c) The advantages over alternative techniques with applicable references to previous studies
**Response:**
We have added lines 89-93.
Continuous and quantitative sampling of ventilatory parameters can measure breathing precisely compared to alternative techniques. $VO_2$ obtained in this protocol can be regarded as an indicator of work of breathing[8]. Further, f, $V_T$, and minute ventilation are related to ventilatory efficiency, and information on breathing pattern can also be obtained from these ventilator parameters plus inspiratory and expiratory time.

d) A description of the context of the technique in the wider body of literature context
**Response:**
We have added in lines 75-83.
It is well known that cardiopulmonary exercise testing is carried out using an expired gas analyzer[6,7]. For diaphragmatic breathing, some investigators[8,9] have reported measurement with an expired gas analyzer for patients with chronic obstructive pulmonary disease. Jones et al.[8] compared diaphragmatic breathing, pursed-lip breathing, and a combination of both, with that of spontaneous breathing. Oxygen consumption ($VO_2$) and respiratory rate (f) were measured during these three methods of breathing. They described that higher resting $VO_2$ may be explained by the increased mechanical work of breathing[8]. Ito et al.[9] examined the immediate effect of diaphragmatic breathing or respiratory muscle stretch on $VO_2$, f and tidal volume ($V_T$).

e) Information to help readers to determine whether the method is appropriate for their application

**Response:**

We have added in lines 65-67 and lines 70-73.

The therapeutic outcome of this method includes a reduction in work of breathing and improvement in efficiency of breathing[2,3], and this brings about an increase in tidal volume, resulting in a reduction in respiratory rate.

In regard to the question of deep breathing being effective as a means of a reduction in the mechanical work of breathing and improvement of ventilatory efficiency, it may be useful to quantify ventilatory parameters by an expired gas analyzer.

8. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.

**Response:**

We have included an ethics statement as follows (lines 100-101):

This protocol was in accordance with the ethical principles of the Declaration of Helsinki. The procedure was explained to all participants before commencement of the study.

9. Please include a single line space between each step, substep and note in the protocol section. Please use Calibri 12 points throughout.

**Response:**

We have amended the format accordingly.

10. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid the usage of phrases such as "could be," "should be," and "would be" throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a "Note." However, notes should be concise and used sparingly.

**Response:**

The protocol section has been written in the imperative tense.

11. The Protocol should contain only action items that direct the reader to do something. Please move the discussion about the protocol to the Discussion.

**Response:**

We have relocated lines 78-81 to lines 313-315 as follows:

First is the control of food intake. The ratio of carbon dioxide output to oxygen uptake provides information about the nutrient mixture catabolized for energy[10].

We have also relocated lines 130-132 to lines 323-324 as follows:

In general, breathing exercise takes three to five min to execute. Therefore, the investigators allocated a 5-min deep breathing phase sandwiched between the two rest phases.

12. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please ensure that individual steps in the protocol contain only 2-3 actions per step.

**Response:**
We have made sure that individual steps contain no more than 2-3 steps.

13. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.
**Response:**
The protocol steps have been added to accordingly.

14. 4: How is the step performed?
**Response:**
We have revised and expanded the whole protocol (lines 192-203).

15. 6: Do you use any software to perform the statistical analysis? If yes please provide all the button clicks etc.
**Response:**
We have added the following sentence (lines 214-215).
Perform statistical analysis using commercial computer software (see Table of Materials), then provide all the button clicks.

16. There is a 10-page limit for the Protocol, but there is a 2.75-page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.
**Response:**
We have highlighted according to your comment.

17. Please place all the figure legends after the representative results and before the discussion.
**Response:**
We have placed all the figure and table legends after representative results and before the discussion. (lines 271-306)

18. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."
**Response:**
We have uploaded the copyright permission to reuse Tables 1 and 2 from a previous publication.

19. As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:
**Response:**

We have revised the Discussion as follows:

a) Critical steps within the protocol

**Response:**

We have amended the descriptions as follows (lines 312-321):

There are three critical steps within the protocol. First is the control of food intake. The ratio of carbon dioxide output to oxygen uptake provides information about the nutrient mixture catabolized for energy[10]. $VO_2$ measured in the expired gas is affected by food intake in lower physical activity than higher one[11]. The result for $VO_2$ (Table 1) demonstrates a lower physical activity during deep breathing. Consequently, it is imperative to set conditions for food intake prior to measurement. Second, the participants are required to refrain from talking during measurement so as not to influence expired gas data. Third, to avoid possible learning effects of the DB pattern on the NB pattern, the execution of the breathing pattern sequence for the measurement is important (Figure 1).

b) Any modifications and troubleshooting of the technique.

**Response:**

We have amended the descriptions as follows:

(lines 328-330)

Modification of this protocol may require further instruction time and practice for learning diaphragmatic breathing to compare the two breathing methods.

(lines 332-336)

For the measurement of the expired gas on a breath-by-breath basis, the number of samples per min for the ventilatory parameters is equal to the respiratory rate per min. The number of samples per min is known to increase during vigorous physical activity, but that for the ventilatory parameters decreases during deep breathing as was shown in this protocol. Considering the above fact, the average time for data collection should be determined.

(lines 338-347)

By the use of a video camera, it is possible for one investigator to carry out this protocol. In addition, a breathing pattern can be easily judged by fast-forwarding the motion-images. During the preliminary testing for this protocol, markers were placed on the sternum and abdomen, followed by videotaping. However, these markers were of no assistance for the visual judgment. Consequently, it was decided to have the participant wear a tight-fitting black shirt. Furthermore, it may be easier to observe the thoracoabdominal excursion if the color of the shirt is in contrast to that of the wall. In this protocol, the wall's background color is white beige in contrast to the black shirt. The investigators recommend the use of different protocol(s)[12,13] if one intends to study breathing patterns particularly with attention to the kinematics of the thorax.

c) Any limitations of the technique

**Response:**

We have added the following paragraph (lines 349-353).

Limitations: As for the sample size for this protocol, calculation by post-hoc power analysis[14] yielded 0.75. To satisfy statistical power of 0.8 defined by Cohen[14], a minimum sample size of 17 participants would have been required for this protocol, which meant that it had a

shortage of two participants. Further, the distribution of pulmonary ventilation could not be assessed, as would have been possible with electrical impedance tomography[15].

d) The significance with respect to existing methods

**Response:**

We have added the following sentence (lines 355-357):

Continuous and quantitative sampling of ventilatory parameters can measure breathing precisely compared to alternative techniques. Ventilatory parameters obtained in this protocol include mechanism of intervention and energy cost in a systematic review of breathing control[2].

e) Any future applications of the technique

**Response:**

We have added the following sentence (lines 358-359):

Henceforth, the investigators would like to explore how ventilatory parameters in the elderly and individuals in supine and/or side lying would differ from those in this study.

20. Please expand the journal title in the reference section.

**Response:**

We have expanded the journal title accordingly.

21. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.

**Response:**

We have revised and uploaded all tables accordingly.

22. Please remove the figure number and the legends from the figure.

**Response:**

We have removed the figure number and the legends from Figures.

23. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please alphabetically sort the materials table.

**Response:**

We have revised the table of materials accordingly.

# RESPONSE TO REVIEWER #1

To Reviewer #1:

We appreciate the time and effort you have dedicated to providing insightful feedback on ways to strengthen our paper.

Major Concerns:

* As a manuscript written to describe a protocol that anyone reading it should be able to pick up, read, watch and reproduce, more precise detail is needed on several of the protocol steps to clarify:

**Response:**

We have revised our manuscript accordingly.

* Line 74-75, recruit 15 healthy women. Where, and how, are recruits identified, and determined as healthy? Were/are incentives offered? What defines healthy (for example, self-defined, questionnaire about physical health, blood tests, BMI, etc)? How was this sample size determined?

**Response:**

We have added two sentences as follows:

Recruit 15 healthy women in their twenties through convenience sampling. Check medical history verbally. Exclude participants with cardiopulmonary disease. (lines 104-105)

No incentives were offered to partake in this protocol. (line 312)

*Line 74, preparation for the experiment. Are any preparations performed ahead of time for the electrocardiograph? Any standardizations or any control/baseline monitoring? If so, please include as part of preparation for the experiment.

**Response:**

There were no special preparations required for the use of the electrocardiograph. No baseline monitoring was carried out.

*Lines 82-85, calibrate the expired gas analyzer. Does this require any standardized controls to be tested by the machine and the software used to collect patient samples? Do you follow manufacturer's protocols for calibration? If calibration deviates from manufacturer's protocols, this should be noted here. If "calibration" means turning on the machine and no other steps are taken, then "calibration" is not the proper terminology.

**Response:**

We followed the manufacturer's protocol for calibration of the expired gas analyzer before the measurement. (lines 111-112).

*Line 88, the participant receives an explanation and instruction for the procedure. It should be clarified that the explanation of the procedure and instructions given are described later in the protocol, and refer to the specific section/s later in the protocol where these are described.

**Response:**

We have deleted line 88 and amended the entire procedure. (lines 116-172).

*Line 86, conditions and experimental design. The reviewer suggests including a section on how the video capture is set up at this time, since the video data, the electrocardiograph data, and the expired gas data are collected simultaneously. Currently, this section only describes the experimental set up for the electrocardiograph and ventilatory parameters.

**Response:**

We have amended the description of the video capture (lines 113-115, 137, and 164).

*Line 96, fit the participant with a sampling mask. If the proper fit of the sampling mask is important for an accurate collection of data - for example, a specific seal is needed and different mask sizes may be required for accurate collection - this should be mentioned. Is the sampling mask fitted only over the mouth? Or over the mouth and nose? Is a seal test performed on the sampling mask before data collection begins?

**Response:**

As for the sampling mask, please refer to lines 130-133 and 157-160.

*Line 99 - 100, how does quiet breathing differ from rest breathing? Does the protocol require a period of sample collection prior to the quiet breathing, to allow for a baseline reading for comparison during the active protocol? If so, how is this recorded, and how is the comparison made at the end of the experiment?

**Response:**

To avoid confusion, we have changed the terms 'quiet breathing' and 'rest breathing' simply to 'rest'.

*Line 102, has a 10 minute break. Is the participant restricted in activity, or can the participant move around, drink, talk? Do all participants perform the same activity during this ten minute break, to control for variation in activity between patients? Is the break exactly ten minutes, or may testing resume earlier if a participant doesn't use their full ten minutes?

**Response:**

The participant's actions taking place during the 10-min intermission have been described in lines 144-147.

*Line 112, and she gets dressed. In the previous protocol description above this point, it does not state that the participant is undressed. Please clarify.

**Response:**

The description 'the participant is undressed' has been deleted.

*Line 86 - 113, is this procedure only performed once for each participant, or are they asked multiple times to perform the two breathing methods in order to determine which is more comfortable for them?

**Response:**

The participant carried out the two 5-min deep breathing procedures only once.

*Line 143: upload the data using a CSV format…yields the average data for the

randomly set period. The authors need to better clarify what does the randomly set period mean, how is it randomly set, by whom/what? Is there a specific software program used to perform these calculations? If the software needs to be managed by a technician, step-by-step instructions on how to use the software, convert the data to CSV, set any parameters, and any other functions that the technician performs using the software should be listed as part of the protocol.

**Response:**

We have revised the part relating to lines 183-185 including software. We have also listed names of the software in the Table of Materials.

*Line 150, video set up. More information about how the video set up is prepared is needed. What color background is used, are there any stripes on the background or is it a solid wall or drapery (which might have folds that could impair visualizing breathing patterns), are there any other objects observable in the background, how much of an angle does the camera cover (whole body, just the thoracic region, etc), is the video camera on a solid stand or table, or hand-held? Is a particular frame speed used to capture recordings of participants as they breathe through the experiments? How is the camera angled relative to the participant (side, head-on, up towards the ceiling or directly at the thorax/abdomen?) And other useful information that may clarify how breathing patterns are visualized.

**Response:**

We have added the following sentences:

1.2. Attach a video camera to a tripod at a distance of 1.5 m from the chair the participant will sit on. Prepare to record a lateral view of the sitting participant in a range from the top of her cranium to the seat of the chair. (lines 113-115)

(lines 195-203)

2. Make sure that contrast between the participant and background color is clear.
3. Record the video image at 1/30 sec per frame, which is the standard speed for the video camera used.
4. Upload the motion-images into a personal computer using video editing software 1 (see Table of materials).
5. Observe 5-min video images of the deep breathing phases at double speed under visual assessment and classify the breathing patterns as upper costal, diaphragmatic or thoracoabdominal. Use video editing software 2 (see Table of materials).

   Note: Video images are analyzed by a cardiopulmonary physical therapist (MY).

*Line 153, a specific name for the video software used in this experiment should be provided, if available. The same for any software used to capture data from any of the recording devices.

**Response:**

We have listed two video editing softwares in the Table of Materials.

*Line 154, defining the breathing pattern. Does this identification have to be performed by someone who has been trained to identify upper coastal,

diaphragmatic and combined? If so, how are/were they trained? Exactly define how the pattern is scored - is the determination based on every breath taken, an average of those during the experimental time frame, etc.

**Response:**

We have amended 5 under 4. Assessment of the Breathing Pattern as follows: (lines 200-203)

5. Observe 5-min video images of the deep breathing phases at double speed under visual assessment and classify the breathing patterns as upper costal, diaphragmatic or thoracoabdominal. Use video editing software 2 (see Table of materials).

Note: Video images are analyzed by a cardiopulmonary physical therapist (MY).

*Line 158, participant determines which technique is more comfortable and notes the response. Clarify with more detail - are participants asked immediately when they are disconnected from all monitors, before they leave the chair or room, do they answer verbally or in a written survey? Is there a comparison scale given for the different breathing methods? Are multiple questions asked? This is a subjective determination - are the participants asked to give any other "free text" feedback about the experience and if so, how is that data collected and analyzed?

**Response:**

The description has been modified as follows (lines 205-211):

**5. Participants' preferred pattern for deep breathing**

1. Prepare a spreadsheet for the participant's response.
2. Ask the participant which of the two deep breathing techniques is more comfortable following the measurement of the DB pattern.
3. Fill in the spreadsheet with the participant's response.
4. Be ready to listen to the participant if she wants to talk about the procedure. Do not include the participant's comments in the analysis.

* Figure and table legends (listed as in a separate file) were not received, and could not be reviewed. Tables and figures could not accurately be assessed for data presentation.

**Response:**

We have carefully uploaded Tables and Figures in a separate file.

Minor Concerns:

*Line 30, The first sentence of the summary, "to confirm a method of instruction" does not fit with the primary experimental procedure presented in the manuscript, testing of different breathing patterns for efficacy and comfort. The method of instruction was not clearly defined or tested.

**Response:**

We have modified the first sentence as follows: (lines 29-30)

Here, we present a protocol to assess two deep breathing patterns of natural and diaphragmatic breathing for effectiveness and ease of execution.

The method of instruction has been described as follows (lines 123-125 and 151-154):

1.6. Explain to the participant deep breathing with natural breathing (NB) pattern of slow and

deep breaths, breathing in through the nose and blowing out through the mouth with no consideration or knowledge given on specific movement of the chest.

4.2. Explain to the participant deep breathing with diaphragmatic breathing (DB) pattern. Ask the participant to lace the fingers, place them on the abdomen and take a deep breath in through the nose, expanding the abdomen under the hands, then blowing out through the mouth and gently retracting the abdomen.

*Can the authors clarify how an n of 15 was chosen and whether this is considered significant for generating the data presented in this manuscript.

**Response:**

We recruited the participants through convenience sampling (line 104), and have also added a post-hoc power analysis in Discussion (lines 349-352).

*Line 154, reproduce the video images fast-forward - this statement needs clarification.

**Response:**

We have clarified Step 5 under 4. Assessment of the Breathing Pattern (lines 200-202).

*Line 180 - 181, "revealed main effect for the phase factor" needs to be clarified. The intent of this statement is not clear.

**Response:**

We have clarified the definition of factors as follows:

Note: Factor “instruction” includes two levels NB and DB, and factor “phase” has two levels rest phase and deep breathing phase. (lines 224-225)

*Line 199 - 204, and Table 2, the results of the breathing patterns were defined as upper coastal, combined, or diaphragmatic, yet most of the data is referencing either independent breathing method. If the breathing pattern was defined as combined, was this accounted for in the evaluation of results? Under natural pattern, 2 participants were using diaphragmatic breathing, and under DB, this increased to 6; the statement that participants were able to understand and carry out deep breathing as instructed is not fully supported by the data as it is presented.

**Response:**

The overall goal of this method has been modified as follows:

The overall goal of this study was to find a viable and efficient method of deep breathing exercise based on analysis of oxygen consumption, ventilatory parameters, and chest wall excursion. (lines 95-97)

To answer the remaining queries, a paragraph has been added as follows:

In general, breathing exercise takes three to five min to execute. Therefore, the investigators allocated a 5-min deep breathing phase sandwiched between the two rest phases. The participants carried out one trial for each 5-min deep breathing phase, for this protocol was designed to simulate initial instruction in a clinical setting of a 5-min breathing exercise. However, participants did not have an ideal diaphragmatic breathing pattern when carrying it out for the first time in this protocol (Table 2). (lines 323-328)

*Reference 13 is cited in the text but is not included in the reference list.

**Response:**

We have listed it accordingly.

*The major steps of the procedure are outlined, but not specifically highlighted to set them apart.

**Response:**

We have highlighted the major steps of the procedure.

*If specific software programs are used, they should be identified in the table of materials and equipment.

**Response:**

We have done so accordingly.

# RESPONSE TO REVIEWER #2

To Reviewer #2

We appreciate the time and effort you have given to reading our paper.

Methods:
*The author did not clarify the acceptance of the ethical committee or IRB.
**Response:**
We have added an ethics statement as follows:
This protocol was in accordance with the ethical principles of the Declaration of Helsinki. The procedure was explained to all participants before commencement of the study. (lines 100-101)

Table 4 is not clear.
**Response:**
We have revised Table 4.

# RESPONSE TO REVIEWER #3

To Reviewer #3

We appreciate the time and effort for your feedback on improving our paper.

The description of the protocol provided on page 2 is in conflict to what is reported in the results and figure. It's not clear as to when the measures were exactly captured during quiet breathing versus deep breathing for either or the normal breathing pattern or diaphragmatic pattern.

**Response:**

We have revised the manuscript to clarify description of the protocol in more detail.

Additionally the study appears to measure energy expenditure and then Vent efficiency through models and instruments that are arguably not appropriate to measure in this capacity. For example VE/VCO2 slope is measured during exercise. To my knowledge this has not been used to measure that at rest because of the minimal changes in carbon dioxide production even with the minimal change in energy expenditure by changing breathing rate, as done in this study.

**Response:**

We have deleted $VE/VCO_2$ from the results and Table 1.

Further many of the references for this paper are the textbook chapters which are not sufficient evidence to support the claims made in this article. Please provide primary sources or at least peer reviewed manuscripts. Textbook chapters should be reserved generally for technique and not the evidence for a technique.

**Response:**

We have deleted the textbook chapters in the references and provided primary sources as follows:

REFERENCES:


4. Sackner, M.A., Gonzalez, H.F., Jenouri, G., Rodriguez, M. Effects of abdominal and thoracic breathing on breathing pattern components in normal subjects and in patients with chronic obstructive pulmonary disease. *The American review of respiratory disease*. **130**, 584-587 (1984).
5. Gosselink, R.A., Wagenaar, R.C., Rijswijk, H., Sargeant, A.J., Decramer, M.L. Diaphragmatic breathing reduces efficiency of breathing in patients with chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine*. **151**, 1138-1142 (1995).
8. Jones, A.Y., Dean, E., Chow, C.C. Comparison of the oxygen cost of breathing exercises and spontaneous breathing in patients with stable chronic obstructive pulmonary disease. *Physical Therapy*. **83**, 424-431 (2003).
9. Ito, M., Kakizaki, F., Tsuzura, Y., Yamada, M. Immediate effect of respiratory muscle stretch gymnastics and diaphragmatic breathing on respiratory pattern. *Internal medicine*. **38**, 126-132 (1999).
10. Jansson, E. On the significance of the respiratory exchange ratio after different diets during exercise in man. *Acta physiologica Scandinavica*. **114**, 103-110 (1982).

Additionally oxygen uptake should be reported in milliliters per kilogram per minute. Or liters per minute. Please make this change. I would also recommend providing demographics on these patients such as height, age, weight and body mass index.

**Response:**

We have made the change to 'liters per min' and also provided demographics of the participants as follows:

The participants had a mean age of 21.6 years, mean body mass of 51.9 kg, mean height of 159.3 cm and a body mass index of 20.5 $kg/m^2$. (lines 310-312)

日　付: 2019年6月7日

Journal of Physical Therapy Science 編集委員会　御中

**転載許可願**

私は、下記の執筆準備をしております。
書名／雑誌名: Journal of Visualized Experiments　（発行予定年　2019年　）
章名／タイトル: Methodology for investigating deep breathing through work of breathing, ventilatory efficiency and breathing patterns
著者名: Yokogawa M, Kurebayashi T, Soma K, Miaki H, Nakagawa T
出版社／出版元: Boston, Mass. : MYJoVE Corporation

Journal of Physical Therapy Science誌に掲載されました下記について、上記への転載許可をいただきたくよろしくお願いいたします。
尚、本転載許可には、上記の現版およびすべての改訂版における印刷・電子版、およびその翻訳版が含まれます。

Journal of Physical Therapy Science (Vol. 30　No. 4　)
Page: 614-618
Article title: Comparison of two instructions for deep breathing exercise: non-specific and diaphragmatic breathing
Authors: Yokogawa M, Kurebayashi T, Ichimura T, Nishino M, Miaki H, Nakagawa T
Figures/Tables/Lines: Table 1, Table 3,　p617, Lines 29-31

図の転載におきましては、図の説明に下記のように明記します。
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許可いただけます場合は、下記へご署名のうえ、ご返送ください。
また、貴委員会以外に許可が必要な先（著者など）があればご指示ください。　敬具

名　前: 横川　正美

所　属: 金沢大学

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転載を許可いたします。

日　付: 7th June 2019

署　名: Y. Maruyama

~~President, Society of Physical Therapy Science~~
丸山仁司