**ORIGINAL ARTICLE** 



Effect of Forward Trunk Lean Position with Pursed-lip Breathing on Pulmonary Ventilation and Heart Rate during Post-exercise Recovery in Obese Females

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## ABSTRACT

**Background.** Previous studies have reported that obesity is associated with poor respiratory function including reduction of functional residual capacity (FRC) and expiratory residual volume (ERV). **Objectives.** To investigate the effect of forward trunk lean position (FTLP) combined pursed-lip breathing (PLB) on tidal volume ( $V_T$ ), volume of oxygen (VO<sub>2</sub>), the volume of carbon dioxide (VCO<sub>2</sub>), heart rate (HR) during post-exercise recovery. **Methods.** Twenty-two obese females performed two sitting positions, FTLP and upright position (UP) combined with PLB for 2 minutes after 40 minutes of running at moderate intensity. V<sub>T</sub>, VO<sub>2</sub>, VCO<sub>2</sub>, HR were measured in these 2 minutes. The recorded values were averaged in 30s intervals. **Results.** Although the average VT during FTLP+PLB was higher than UP+PLB, there was no statistically significant difference (30s: p=0.718, 60s: p=0.114, 90s: p=0.682, 120s: p=0.927). V<sub>T</sub> in UP+PLB at the 90s decreased while V<sub>T</sub> in FTLP+PLB at the 90s remained close to the highest point in the 60s. However, the difference was not significant. There were no significant differences in VO<sub>2</sub>, VCO<sub>2</sub>, and HR between FTLP+PLB and UP+PLB at all-time points. **Conclusion.** FTLP+PLB and UP+PLB induce comparable changes in V<sub>T</sub>, VO<sub>2</sub>, VCO<sub>2</sub>, and HR during recovery after moderate-intensity continuous exercise in obese females. FTLP+PLB seemed to be better for maintaining V<sub>T</sub> during recovery.

**KEYWORDS:** Forward Trunk Lean Position, Pursed-Lip Breathing, Ventilation, Obesity, Recovery.

# **INTRODUCTION**

Previous studies have reported that obesity (BMI 30–40 kg/m<sup>2</sup>) is associated with poor respiratory function including reduction of functional residual capacity (FRC) and expiratory residual volume (ERV) (1, 2). A reduction of lung volume can induce hypoxemia in obese patients without cardiopulmonary disease (3). Moreover, a recent study observed a progressive reduction of ventilation (low VE/VCO<sub>2</sub>) as BMI progressively

increased in obese males and females  $(30-50 \text{ kg/m}^2)$  and low tidal volume  $(V_T)$  obese females with BMI 30–39.9 kg/m<sup>2</sup> during graded exercise tests (4). The reduction of ventilator response during exercise may be caused by the large fat mass on the thorax, which increases respiratory mechanical impedance and decreases lung volume so that the maximal expiratory flow is limited and ventilation decreases during exercise

(5). Furthermore, two studies have reported a reduction of oxygen saturation during light and moderate-intensity continuous exercise in obese females (BMI >30 kg/m<sup>2</sup>) (6, 7). Females tend to have smaller lung volumes and lower maximal flow rates than males (8) and, therefore, adopt an altered breathing pattern (low V<sub>T</sub> and high breathing frequency) during exercise (9). Moreover, the inspiratory and expiratory muscle strength of females is lower than in males (10).

Forward trunk lean posture (FTLP) is an effective technique that can improve ventilation by adjusting muscle activation and synchronizing thoracic and abdominal movement patterns (11-14). In addition, FTLP also causes an effective length of contraction of the diaphragmatic muscle (11). However, there is another effective technique, pursed-lip breathing (PLB), that can improve ventilation and reduce fatigue. In previous studies, it has been found that PLB increases lung function, and respiratory muscle strength and also reduces work of breathing and fatigue in healthy older adults (15, 16). Therefore, the combination of these two techniques may increase ventilation after exercise in obese persons. Uboluar et al., 2022 investigated the effect of combined FTLP and PLB on regional chest wall volume and ventilatory pattern in older adults (17). The study found that PLB significantly improved ventilation and chest wall volumes compared to quiet breathing (breathing in and out through the nose without controlling the breathing tempo). However, there were no significant differences between combined FTLP with PLB and combined upright position (UP) with PLB (17). However, the study was conducted in resting condition and the participants were healthy older adults with normal body mass index. To the best of our knowledge, Michaelson et al., 2019 is the only study that has investigated the effect of a forward-leaning position as a recovery strategy after exercise (18). The study found that a forward leaning position while standing (hand on the knee) as a recovery posture after high-intensity exercise was more beneficial than upright standing (hand on head) in female soccer players. It provided higher ventilation (an increase of V<sub>T</sub>), carbon dioxide expellant, and heart rate recovery. Therefore, a forward-leaning position seems to be an effective recovery strategy, which may improve ventilation after exercise in obese persons. However, the study did not control the breathing pattern while

performing the forward leaning position. According to physiological differences between genders, obese females seem to have a higher risk of poor ventilation that can induce hypoxemia during exercise than obese males. Even though continuous exercise may increase the risk of hypoxemia, it remains necessary for weight management and preventive health care in obese persons. This study investigated recovery strategies (FTLP technique) that can improve ventilation after exercise. Hence, our study compared FTLP combined with PLB and UP combined with PLB on tidal volume ( $V_T$ ), volume of oxygen (VO<sub>2</sub>) volume of carbon dioxide (VCO<sub>2</sub>), and heart rate (HR) during post-exercise recovery in obese females.

# MATERIALS AND METHODS

**Design.** This study was approved by the Human Research Ethics Committee of Thammasat University (Science) (Protocol number 087/2565). All participants read and signed the consent form before participating in the study.

**Participants.** Twenty-two obese females (BMI 30–40 kg/m<sup>2</sup>) aged 18–30 years participated in this study. They were included if they had no medical condition and were not under medical treatment, had normal lung function (examined by spirometry), and exercised at least three days per week. Participants were excluded from this study if they were not able to run at moderate intensity (60–70% heart rate reserve; HRR) for 40 minutes, could not participate in this study for the entire period, and if they had abnormal symptoms or vital signs that were not in the normal range before, during and after the experiment.

The sample size was calculated by using the G\*power program, version 3.0.10 (Heinrich Heine University Düsseldorf, North Rhine-Westphalia, Germany). According to previous findings, a forward trunk lean position significantly increases ventilation compared to an upright position with a medium effect size, d=0.5 (18). Therefore, the sample size was calculated with an effect size of f=0.25, a significance level of 0.05, and a statistical power of 0.80. These settings resulted in 24 participants needed for this study and considering a 10% attrition rate, the total sample size was 26 participants.

**Intervention.** This research was designed as a crossover study with controlled experiments.

Participants were randomly assigned into two groups (group A and group B). Participants had to attend three visits, on the first visit, the risk factors before exercise had to be evaluated by answering the Physical Activity Readiness Questionnaire Plus (PAR-Q+2019) in Thai version. Furthermore, baseline information was collected. According to ACSM's guideline for exercise prescription of obesity (19), running speed and time were set at 60–70% HRR and 40 minutes running. Moreover, Coli et al., 2020 (6) found a reduction of oxygen saturation during this running speed and time in obese females (BMI>30 kg/m<sup>2</sup>). After that, the participants had to get familiar with the forward trunk lean posture (FTLP) combined with pursed-lip breathing (PLB) and upright position (UP) combined with PLB. On each of the following two visits, participants had to perform one of the two recovery protocols in random order after 40 minutes of running at moderate intensity. After finishing the second visit, they had to rest for one week and then perform another recovery protocol at the third visit (Figure 1).

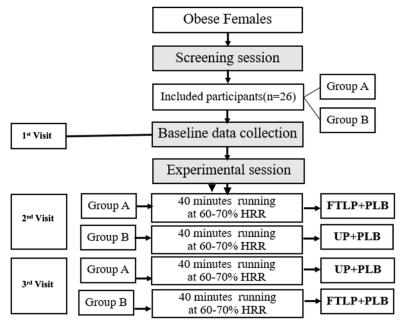


Figure 1. Flow chart of experimental design.

The two recovery protocols were two minutes of FTLP combined with PLB (FTLP+PLB) and UP combined with PLB (UP+PLB). For the FTLP, the participants leaned forward and placed their forearms on their thighs in such a way that their trunk angle were around 45° and knee angle were around 90°. For the UP, the participants had to sit in an upright position and place their hands on their thighs without back support and their trunk angle was around 90° and knee angle was around  $90^{\circ}$  (17) (Figure 2). For the PLB, the participants had to breathe in through their noses and breathe out through their partially closed lips. The duration of breathing-in was 2 seconds and breathing-out was 4 seconds (1:2 breath-in and breath-out ratio) (20, 21). During the two minutes of the recovery period, tidal volume  $(V_T)$ , the volume of oxygen  $(VO_2)$ , the volume of carbon dioxide (VCO<sub>2</sub>), and heart rate (HR) were measured.

## Measurements and data analysis

**Baseline data measurement.** On the first visit, the body compositions of all participants were measured by a body composition analyzer (TANITA MC-780 (MA) S/N 14040018, TANITA Corporation of America, Inc. USA). Lung functions were measured following the ATS/ERS1296 E. guidelines for standardization of spirometry (22) by a standard spirometer (Micro Lab 3.1, Micro Medical Ltd., Kent, UK). Grip strength and leg strength were measured by a handgrip dynamometer and a leg dynamometer (Digital Hand Pressure Meter TKK 5401, Digital Leg Strength Meter TKK 5402, Takei, Japan) following ACSM's Guidelines for Exercise Testing and Prescription 2014 (23). Trunk angles

during FTLB and UP were measured as follows. At first, four markers were placed on the head of the humerus, greater trochanter of the femur, lateral epicondyle of the femur, and lateral malleolus. Then a photo was taken in lateral view and uploaded into Kinovea software (GPL V2 license version 0.9.4) for calculating the trunk angle.



**Figure 2.** Sitting positions during 2 minutes post-exercise recovery; (a) shows the forward trunk lean position (FTLP), (b) shows the upright position (UP).

Measurement on experimental day. On the second and third visits, participants had to perform running at moderate intensity for 40 minutes (60-70% HRR). Target heart rate (THR) was calculated by the formula following ACSM's guidelines (THR=[(HRmax-HRrest) × %intensity desired]+HRrest) (23). Maximal heart rate (HRmax) was calculated by the specific formula for obese persons (HRmax=208-0.7×age) (24). Resting heart rate was measured by a digital blood pressure meter (OMRON Model HEM-7130 L, Omron Co. Japan) after the participants had rested for at least 5 minutes. Running was accomplished on a treadmill with variable speed and inclination (Lexco LT8xA touch screen treadmill, Acme Fitness Pvt. Ltd.. India). Before the experimental day, participants received the preparation guidelines for exercise testing (do not smoke or consume caffeine and alcohol for 12 hours before testing, consume meals 3 hours before testing). On the experimental day, the procedure consisted of 2 minutes of static stretching, 3 minutes of warm-up by running at 40-50% HRR, 40 minutes of running at 60-70% HRR, and 2 minutes of recovery. While running, participants had to wear a gas analyzer (MES VO<sub>2</sub>max tracker Ergo spirometer, Germany) and a heart rate monitor (polar RS300X, UK) for monitoring the cardiorespiratory response. After finishing the running session, participants had to sit on an adjustable chair to perform FTLP+PLB or UP+PLB for 2 minutes. During the 2 minutes of recovery, tidal volume ( $V_T$ ), volume of oxygen (VO<sub>2</sub>), and volume of carbon dioxide (VCO<sub>2</sub>) were measured by a breath-by-breath gas analyzer. All parameters were collected in breath-by-breath for 2 minutes after exercise. After that,  $V_T$ , VO<sub>2</sub> and VCO<sub>2</sub> were averaged in 30s intervals. In addition, the heart rate (HR) was measured by a heart rate monitor and averaged in 30s intervals like  $V_T$ , VO<sub>2</sub> and VCO<sub>2</sub>.

The primary outcome of this study is the tidal volume during the recovery period and secondary outcomes are  $VO_2$  and  $VCO_2$ . and HR response during the recovery period.

**Data analysis.** IBM SPSS Statistics (version 23) was used to analyze all data. Characteristics of participants and experimental data are shown as mean and standard deviation. Shapiro-Wilk test was used to examine the normal distribution of all data. One-way repeated measures analysis of variance (ANOVA) was used to compare the effect of the two sitting positions on the tidal volume, volume of oxygen, volume of carbon dioxide and heart rate at 30s intervals during 2 minutes of post-exercise recovery. Post hoc LSD was used in multiple comparisons. The significance level was set at p=0.05.

#### RESULTS

**Baseline characteristics.** In the beginning, 26 obese females participated in this study, but four of them had to leave because they could not participate in all three visits. Hence, only 22 obese females participated in this study. The baseline characteristics of the participants are shown in Table 1.

**Cardiorespiratory response during postexercise recovery.** The effects of FTLP+PLB and UP+PLB on  $V_T$ ,  $VO_2 VCO_2$ . and HR are shown in Figure 3. Concerning  $V_T$ , the average  $V_T$  was lowest at 30 seconds and highest at 60 seconds in both sitting positions. Test of within-subject effect found a significant difference of average  $V_T$  at each 30s interval during 2 minutes of post5

exercise recovery in both sitting positions (Sum of square=2.530, df=2.483, Mean square=1.019, F=3.124, p=0.042; Greenhouse-Geisser). The pairwise comparison found that there were no significant differences in average V<sub>T</sub> between FTLP+PLB and UP+PLB at 30, 60, 90, and 120 seconds during post-exercise recovery (p=0.718, p=0.972, p=0.682, p=0.927). However, when average V<sub>T</sub> values were compared within each sitting position, several were significantly different as follows and as shown in Figure 3 (a) For FTLP+PLB, average V<sub>T</sub> at 60 and 90 seconds was significantly higher than in 30 seconds (p=0.01, p=0.013); for UP+PLB, average V<sub>T</sub> at 60 and 90 seconds significantly higher than in 30 seconds (p=0.00, p=0.012).

Physical characteristics	Mean ± SD
Age (years)	$22.59 \pm 3.33$
Weight (kg)	$84.45 \pm 8.72$
Height (m)	$160.55 \pm 4.74$
BMI $(kg/m^2)$	$32.89 \pm 3.24$
%Fat (%)	$45.87\pm0.04$
Muscle mass (kg)	$42.43 \pm 2.74$
Absolute/Relative grip strength (kg, kg/BW)	$27.54 \pm 3.66 \ /0.33 \pm 0.05$
Absolute/Relative leg strength (kg, kg/BW)	$81.78 \pm 29.95 \hspace{0.2cm} / 0.98 \pm 0.37$
Lung function	
FEV1 (L)	$2.97\pm0.37$
FVC (L)	$2.95\pm0.36$
FEV1/FVC (%)	$95.00\pm0.06$
Vital signs	
Resting heart rate (bpm)	$75.59 \pm 8.24$
Systolic blood pressure (mm Hg)	$121.33 \pm 12.98$
Diastolic blood pressure (mm Hg)	$82.82\pm8.99$
RPE (scale)	$2.86 \pm 2.23$
Trunk angle (Forward/Upright) (degree)	58.05/90.21

Concerning VO<sub>2</sub>, average VO<sub>2</sub> was highest at 30 seconds and tended to decrease over time in both sitting positions. Test of within-subject effect found a significant difference of average VO<sub>2</sub> at each 30s interval during 2 minutes of post-exercise recovery in both sitting positions (Sum of square=4.860, df=1.893, Mean square=2.568, F=29.968, p=0.000; Greenhouse-Geisser). The pairwise comparison found that there were no significant differences in average VO<sub>2</sub> between FTLP+PLB and UP+PLB at 30, 60, 90, and 120 seconds during post-exercise recovery (p=0.887, p=0.687, p=0.969, p=0.815). However, when average VO<sub>2</sub> values were compared within each sitting position, it was found that the average VO<sub>2</sub> values at all 30s intervals were significantly different from each

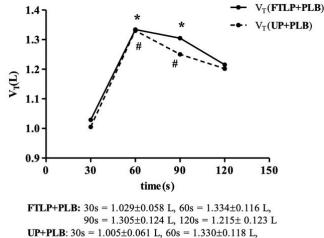
other in both FTLP+PLB and UP+PLB (p=0.000) as shown in Figure 3 (b).

Concerning VCO<sub>2</sub>, average VCO<sub>2</sub> was highest at 30 seconds and tended to decrease over time in both sitting positions. Test of within-subject effect found a significant difference of average VO<sub>2</sub> at each 30s interval during 2 minutes of post-exercise recovery in both sitting positions (Sum of square=2.688, df=2.565, Mean square=1.048, F=33.676, p=0.000; Greenhouse-Geisser). The pairwise comparison found that there were no significant VCO<sub>2</sub> between average differences in FTLP+PLB and UP+PLB at 30, 60, 90, and 120 seconds during post-exercise recovery (p=0.281, p=0.620, p=0.342, p=0.617). However, when average VCO<sub>2</sub> values were compared within

each sitting position, it was found that the average VO<sub>2</sub> values at all 30s intervals were significantly different from each other in both FTLP+PLB and UP+PLB (p<0.01) as shown in Figure 3 (c).

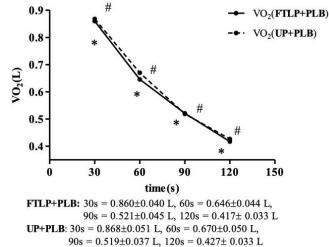
Concerning HR, average HR was highest at 30 seconds and tended to decrease over time in both sitting positions. Test of within-subject effect found a significant difference of average HR at each 30s interval during 2 minutes of post-exercise recovery in both sitting positions (Sum of square=5098.250, df=1.955, Mean square=2607.602, F=40.732, p=0.000; Greenhouse-Geisser). The pairwise comparison

found that there were no significant differences in average HR between FTLP+PLB and UP+PLB at 30, 60, 90, and 120 seconds during post-exercise recovery (p=0.441, p=0.589, p=0.396, p=0.230). However, when the average HR values were compared within each sitting position, it was found that the average HR values at all 30s intervals were significantly different from each other in FTLP+PLB (p<0.001). For UP+PLB, HR at 30, 60, 90, and 120 seconds were significantly different (p<0.01) but HR between 90 and 120 seconds were not significantly different (p=0.261) as shown in Figure 3 (d).



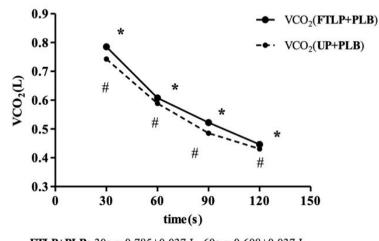
 $\begin{array}{l} 90s = 1.305 \pm 0.124 \text{ L}, \ 120s = 1.215 \pm 0.123 \text{ L} \\ \textbf{UP+PLB:} \ 30s = 1.005 \pm 0.061 \text{ L}, \ 60s = 1.330 \pm 0.118 \text{ L}, \\ 90s = 1.250 \pm 0.117 \text{ L}, \ 120s = 1.202 \pm 0.124 \text{ L} \\ \textbf{*} = \text{Significant difference from } V_{T} \text{ at } 30s \text{ within FTLP+PLB} \\ \# = \text{Significant difference from } V_{T} \text{ at } 30s \text{ within UP+PLB} \end{array}$ 

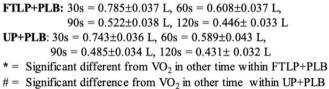
**(a)** 



\* = Significant different from  $VO_2$  in other time within FTLP+PLB

# = Significant difference from VO<sub>2</sub> in other time within UP+PLB





(c)

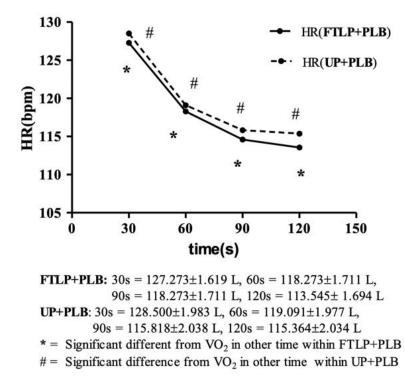


Figure 3 .Pulmonary ventilation and heart rate response during 2 minutes post-exercise recovery; (a) Tidal volume during post-exercise recovery, (b) Volume of oxygen during post-exercise recovery, (c) Volume of carbon dioxide during post-exercise recovery, (d) Heart rate response during post-exercise recovery.

(**d**)

#### DISCUSSION

FTLP+PLB and UP+PLB caused similar changes in  $V_T$ ,  $VO_2$ ,  $VCO_2$ , and HR during the recovery period after moderate exercise intensity in obese females. It seems that the sitting position is less important to increase ventilation when an effective breathing technique like PLB is performed. However, FTLP+PLB seems to be superior for maintaining  $V_T$  during post-exercise recovery. Further investigation is required to confirm this idea.

The main finding of this study was that  $V_T$ ,  $VO_2$ , VCO<sub>2</sub>, and HR were not significantly different between FTLP+PLB and UP+PLB during postexercise recovery. Although the average  $V_T$  of FTLP+PLB was higher than that of UP+PLB at all time points, it was never statistically significantly higher. The change of V<sub>T</sub> during FTLP+PLB and UP+PLB in this study was consistent with the results of Uboluar et al., 2022 (17). The authors reported that FTLP+PLB and UP+PLB similarly increased total and regional absolute chest wall volumes and that FTLP+PLB can induce larger anterior-posterior and medial-lateral chest wall movement than UP+PLB in older adults. In addition, PLB in both sitting positions significantly increased chest wall volumes compared to quiet breathing (QB) with FTLP or UP. A systematic review by Schreuder F., 2009 revealed that PLB can increase tidal volume and decrease respiratory rate in stable chronic obstructive pulmonary disease (COPD) during rest period (25). In COPD patients, PLB improved ventilation by increasing chest wall muscle recruitment during inspiration and expiration, increased abdominal muscle recruitment during expiration, and decreased duty cycle of the inspiratory muscles and respiratory rate (26). In healthy persons, PLB increased lung ventilation by greater chest wall movement and coordination between the ribcage and abdominal regions than OB (17). According to the previous, an effective breathing technique is more important to increase ventilation than the sitting position. However, when the average V<sub>T</sub> at every 30s interval was compared within the same sitting position, FTLP+PLB seemed to be superior for maintaining  $V_T$  during post-exercise recovery. Average V<sub>T</sub> was highest at 60 seconds in both sitting positions, but it decreased at 90 seconds in UP+PLB even though it was not a significant difference. While average  $V_T$  at 90 seconds of FTLP+PLB remained close to V<sub>T</sub> at the highest point (60 seconds). Unfortunately, we cannot clearly explain the effect of FTLP+PLB on maintaining a high level of  $V_T$ . Following previous research (17) that FTLP can induce a larger chest wall movement than UP, it may be a cause for the higher level of  $V_T$ . However, this assumption needs further investigation.

Changes in VO<sub>2</sub> during FTLP+PLB and UP+PLB were similar. Average VO<sub>2</sub> was highest at the first 30 intervals and continuously decreased until the end of the recovery period in both sitting positions. Generally, ventilation and oxygen consumption linearly increase with the intensity of exercise and become steady during moderate exercise intensity. After exercise, the rate of oxygen uptake will slowly decrease and return to a resting level related to ventilation (27). This study hypothesized that FTLP+PLB would cause higher ventilation than UP+PLB and higher ventilation might affect oxygen consumption. However, the results indicated that the sitting position did not affect ventilation and oxygen consumption during post-exercise.  $VCO_2$  changed comparable to  $VO_2$ during FTLP+PLB and UP+PLB. While the average VCO<sub>2</sub> values during FTLP+PLB were higher than those during UP+PLB at all time points, the differences were not significant. In female soccer players, it was found that a forward lean position while standing (hand on the knee) caused higher carbon dioxide release than upright standing (hand on head) by the improved exhalation ability of the abdominal muscles (18). The different result in the present study may have been caused by the lower exercise intensity (moderate; 60-70% HRR) compared to the intensity used for the female soccer players (high: 90–95% HRmax). The lower intensity resulted in lower carbon dioxide production and might not have been enough to make a significant difference. Furthermore, there was no significant difference in HR between FTLP+PLB and UP+PLB. Average HR was highest at the first 30 intervals and continuously decreased until the end of the recovery period in both sitting positions. Higherintensity exercise studies with female soccer players showed different results (18). In the first minute, the heart rate decreased faster in the forward lean position than in the upright position whereas in the present study, the heart response during FTLP and UP was not different. As mentioned above, this might be due to moderate versus high-intensity exercise and their potentially different effect on the cardiovascular response. Other factors might contribute when comparing trained sportswomen to obese women. In any case,

in this comparative study of FTLP+PLB and UP+PLB after moderate exercise intensity, the measured VO<sub>2</sub>, VCO<sub>2</sub>, and HR were not significantly different during the recovery period. There also were several limitations in this study. Firstly, this study did not control the nutrition of the participants. Some participants gained weight during the experimental period and an increase of fat mass around the thorax might affect lung expansion and ventilation. In addition, participants who gained weight could not maintain the target exercise intensity through 40 minutes of running. They requested to lower the running speed a couple of times and went back to the target speed when they were ready. This could be a confounding factor in this study. Secondly, the number of participants was possibly too limited. Originally, the number of participants was as high as calculated in the G\*power program, but four participants left the study after it had already started. Therefore, the number of participants in this study was lower than expected.

#### **CONCLUSION**

Continued exercise is necessary for weight management and health care prevention in obese persons. However, it can cause a risk of poor ventilation that can induce hypoxemia in female obese. Therefore, a recovery strategy that can improve ventilation during the post-exercise is beneficial for female obese. According to our findings, both the forward trunk lean position and upright position are effective in increasing ventilation during post-moderate exercise intensity when combined with effective breathing techniques like pursed-lip breathing.

#### APPLICABLE REMARKS

• This study suggests that female obese should perform pursed-lip breathing during post-exercise to decrease the risk of poor ventilation and hypoxemia.

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## **AUTHORS' CONTRIBUTIONS**

Study concept and design: Supattra Silapabanleng, Piriya Suwondit, Sairag Saadprai. Acquisition of data: Supattra Silapabanleng, Vinitha Puengtanom, Darawadee Panich, Suttirak Artnarong, Thanyaporn Khongthaworn, Chontiya Aumdee, Natiya Thongsen, Pongsiri Onta, Sairag Saadprai. Analysis and interpretation of data: Supattra Silapabanleng, Piriya Suwondit, Sairag Saadprai. Drafting the manuscript: Supattra Silapabanleng, Piriya Suwondit, Sairag Saadprai. Critical revision of the manuscript for important intellectual content: Supattra Silapabanleng, Piriya Suwondit, Sairag Saadprai. Statistical analysis: Supattra Silapabanleng, Sairag Saadprai. Administrative, technical, and material support: Vinitha Puengtanom. Study supervision: Supattra Silapabanleng, Piriya Suwondit, Sairag Saadprai.

# **CONFLICT OF INTEREST**

the authors declared that there is no conflict of interest.

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