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Respiratory and Metabolic Responses as Determinants of Exercise Endurance Time in Constant-load Exercise Test in Healthy Adult Volunteers : A Prospective Cross-sectional Study

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Background : Constant-load exercise (CLE) tests have been used to evaluate the effectiveness of treatment with long-acting bronchodilators or pulmonary rehabilitation in patients with chronic obstructive pulmonary disease (COPD). However, exercise limitation in the CLE test is due not only to pulmonary dysfunction but also to leg muscular strength and metabolic efficiency of the muscle, which are affected by daily physical activity and exercise habits. The present study was performed to examine the factors involved in exercise limitation in the CLE test other than pulmonary dysfunction in healthy adult volunteers to establish baselines before applying the study in COPD patients.

Methods : Twenty-seven healthy volunteers performed the CLE test using a treadmill at 80 % of maximum load determined by incremental-load exercise (ILE) test up to a maximum of 20 minutes. Subjects were divided into those who were and were not able to complete 20 minutes of the CLE test. The relative exercise load was calculated as : peak oxygen uptake (V_{O2}) in the CLE test/V_{O2} at anaerobic threshold in the ILE test. The metabolic variables and relative exercise load were compared between the two groups.

Results : In the CLE test, the non-completion group showed significantly higher respiratory exchange ratio (RER, >1) as a variable of the anaerobic threshold. The relative exercise load of the CLE test and the actual exercise load in the non-completion group were higher compared to the completion group.

Conclusion : This study showed that the high relative exercise load in the CLE test causes the subject to exceed anaerobic threshold and limits maintenance of exercise in the CLE test. *Shinshu Med J 69 : 355―362, 2021*

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Key words : cardiopulmonary exercise test, anaerobic threshold, ventilatory inefficiency, exercise tolerance

I Introduction

The cardiopulmonary exercise (CPE) test is performed to determine factors limiting exercise tolerance and to evaluate the efficacy of pulmonary rehabilitation and bronchodilators for exercise tolerance in patients with chronic obstructive pulmonary disease (COPD)[1)-3)]. The CPE test is usually performed as an incremental-load exercise (ILE) test or a constant-load exercise (CLE) test. Generally, the ILE test is used to evaluate maximum oxygen intake as exercise tolerance and respiratory metabolism by symptom-limited exercise and the limiting factors for exercise tolerance. The CLE test is usually performed at approximately 70 %-80 % of peak V_{O2} determined in the ILE test[4)5)], and is also used to evaluate not only the efficacy of treatment with bronchodilators and pulmonary rehabilitation as in the ILE test, but also dynamic lung hyperinflation in patients with

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COPD. In the CLE test, the metabolic kinetics have been reported to reach a plateau at 5-10 minutes after commencement of exercise, and healthy subjects can maintain exercising for at least 10 minutes[1]. However, most patients with COPD are unable to maintain exercising for more than 10 minutes even with the exercise load of the CLE test based on individual exercise capacity determined in the ILE test[6]. Therefore, exercise limitation in the CLE test may be due not only to pulmonary dysfunction but also to leg muscular strength and metabolic efficiency of the muscle, which are affected by daily physical activity and exercise habits. The present study was performed in healthy adult volunteers to determine the factors responsible for exercise limitation in the CLE test while excluding the effects of pulmonary dysfunction prior to applying the study in COPD patients.

The CLE test is characterized by more reflective muscle metabolism and greater promotion of lactic acidosis than the ILE test[7]. We postulated that there may be some differences in muscle metabolism and the threshold of aerobic metabolism in the CLE test between subjects who can maintain CLE for a long time and those who cannot. In the present study, pulmonary metabolic variables were compared between healthy volunteers without pulmonary dysfunction divided into two groups according to whether they could maintain exercise for more than 20 minutes or not in the CLE test.

Ⅱ Materials and Methods

A Subjects

The study population consisted of 27 healthy volunteers (24 males and 3 females) from 18 to 60 years old with normal spirometry results who performed exercise tests at Shinshu University between March 2017 and December 2018. Subjects were included in the study regardless of their exercise habits. Exclusion criteria were current treatment for asthma or heart failure, and musculoskeletal diseases that could affect exercise testing.

B Study design

This study had a prospective cross-sectional design. All subjects underwent spirometry. The symptom-limited ILE test was performed, and then the CLE test was conducted at a load of 80 % of the maximum exercise capacity determined in the ILE test. The endurance time of the CLE test had an upper limit of 20 minutes. The subjects were divided into those who were and were not able to complete 20 minutes of exercise in the CLE test designated as the completion group and the non-completion group, respectively, and metabolic variables in each exercise test were compared between the two groups. A threshold of 20 minutes was used to divide the subjects into the two groups based on a previous study in which the CLE test was performed for at least 20 minutes in healthy subjects[8].

C Evaluation methods

1 Spirometry

Spirometry was performed using a spirometer (Fukuda Denshi Co., Ltd., Tokyo, Japan). All measurements were performed according to the Japanese Respiratory Society guidelines for lung function measurements. For predicted values of FEV_1 and VC, Japanese local reference data developed by the Japanese Respiratory Society were adopted[9].

2 Symptom-limited incremental-load exercise test

Both exercise tests were performed using a treadmill (Auto Runner AT-200 ; Minato Medical Science Co., Ltd., Osaka, Japan), and methods were as described previously[10]. Oxygen uptake (V_{O2}, mL/kg/min), carbon dioxide production (V_{CO2}, mL/kg/min), respiratory exchange ratio ($RER = V_{CO2}/V_{O2}$), end-tidal oxygen (ET_{O2}, %), end-tidal carbon dioxide (ET_{CO2}, %), minute ventilation/oxygen uptake (VE/V_{O2}), minute ventilation/carbon dioxide production (VE/V_{CO2}), minute ventilation (VE, L/min), tidal volume (VT, L), and respiratory rate (RR, times/min) were recorded using the breath-by-breath measurements in each exercise test determined using a breath analyzer system (AE-310S Aeromonitor ; Minato Medical Science). These metabolic variables during exercise measured in a breath-by-breath manner were defined as the average value for 30s before the end of the exercise, which was called the "peak value." We evaluated the anaerobic threshold (AT) point by the V-Slope method, and the respiratory compensation (RC) point was determined by the VE-V_{CO2} slope method according to the ATS/ACCP statement[3]. Pulse rate (PR, beats/min) during exercise was measured continuously with a fingertip monitor (AE-310S Aeromonitor; Minato Medical Science). Percutaneous oxygen saturation (SpO_2, %) was measured with a wrist-worn pulse oximeter (WristOX2™, Model 3150; Philips Electronics Japan Co., Ltd., Tokyo, Japan).

The ILE test protocol was performed on a treadmill adopting the TR-3 protocol, which is designed to increase V_{O2} linearly. In this protocol, the speed and slope changed continuously and this was the same method used for determination of the AT reference value in Japanese subjects[11]. Subjects continued to exercise until limited by symptoms, and maximum exercise capacity and maximum exercise load were evaluated. Subjects walked on a treadmill at a speed of 1 km/h with a slope of 0 % for 3 minutes before and after the ILE test as warm-up and cool-down, respectively.

3 Symptom-limited constant-load exercise test

After sufficient rest, the symptom-limited CLE test was conducted at a load of 80 % of the maximum load in the ILE test as described previously[10]. First, the subjects walked for 3 minutes as a warm-up at a speed of 1 km/h with a slope of 0 % on the treadmill. Next, they began to exercise at up to 80 % of the maximum load determined in the ILE test. The CLE test was performed to the point when the subject could no longer maintain exercise or a time limit of 20 minutes had been reached[10]. After the CLE test, the subjects walked for 3 minutes as a cool-down with the same load as in the warm-up. The subjects who were not able to complete 20 minutes of exercise in the CLE test were evaluated to determine whether they had performed maximum-effort exercise in consideration of 85 % of the maximal age-predicted heart rate[12]. The subjects were divided into those who were and were not able to complete 20 minutes of exercise in the CLE test, defined as the completion group and the non-completion group, respectively. The relative exercise load of the CLE test to the AT point in the ILE test was calculated as: peak V_{O2} in the CLE test/V_{O2} at the AT in the ILE test.

D Statistical analysis

As there have been no similar previous studies, the required sample size could not be calculated. Therefore, we calculated the statistical power by post hoc analysis. Normality was analyzed by the method of Kolmogorov-Smirnov and then Student's t test was used for comparison of metabolic variables between the completion group and the non-completion group. Finally, stepwise multiple regression analysis was performed to determine the factors affecting exercise tolerance for 20 minutes in the CLE test with the completion group or the non-completion group as independent variables and the variables that were significantly different between the two groups in the CLE test and the relative exercise load as explanatory variables. The number of explanatory variables in multiple regression analysis was calculated based on the sample size of the present study. In all analyses, $P<0.05$ was taken to indicate statistical significance. SPSS ver. 25 was used for statistical analyses (SPSS Inc., Chicago, IL).

E Ethics approval and consent to participate

All subjects were given an adequate explanation of the study and provided written informed consent to participation. This study was conducted in accordance with the ethical principles for medical research involving human subjects of the Declaration of Helsinki after obtaining approval from the Shinshu University Medical Ethics Committee (approval number: 3705).

Ⅲ Results

A Characteristics of physical findings, lung function, and complications

Table 1 shows the characteristics of physical findings and spirometry. The study population consisted of a total of 27 subjects. Eleven of the 27 subjects were university students who exercised regularly as sports club members, while the remaining 16 subjects were working persons with no regular exercise habits. Physical findings and lung function were not significantly different between the two groups, and all values of spirometry were within reference ranges in

Table 1 Subject characteristics of basic physical findings, lung function

n	27
Age, years	29.3±2.2
Sex (male/female)	24/3
Height, cm	170.8±1.5
Body weight	65.0±1.8
BMI, kg/m^2	22.3±0.6
%VC, %	91.5±2.4
%FEV_1, %	89.7±2.2
FEV_1/FVC, %	87.1±2.6

Notes: Values represent the means±standard error of the mean.
Abbreviations: BMI, body mass variable; VC, vital capacity; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity.

Table 2 Results of metabolic variables in incremental load exercise test

n	27
V_{O2}, ml/kg/min	44.9±1.7
V_{CO2}, ml/kg/min	56.5±2.2
RER	1.26±0.01
ET_{O2}, %	15.4±0.1
ET_{CO2}, %	6.2±0.1
VE/V_{O2}	38.7±1.4
VE/V_{CO2}	30.3±0.5
VE, L/min	109.7±4.1
VT, L	2.4±0.1
VD/VT	0.27±0.01
RR, breaths/min	44.7±1.2
PR, beats/min	167.2±4.9
SpO_2, %	89.7±5.1
AT point, ml/kg/min	25.9±0.9
RC point, ml/kg/min	38.7±1.4
Minimum VE/V_{CO2}	27.9±0.6

Notes: All values of metabolic variables except the AT point, RC point, and minimum VE/V_{CO2} are shown at the peak of exercise. Values represent the means±standard error of the mean.
Abbreviations: V_{O2}, oxygen uptake; V_{CO2}, carbon dioxide output; RER, respiratory exchange ratio; ET_{O2}, end-tidal oxygen, ET_{CO2}, end-tidal carbon dioxide; VE, ventilation; VT, tidal volume; VD, dead-space gas volume; RR, respiratory rate; PR, pulse rate; SpO_2, percutaneous oxygen saturation; AT, anaerobic threshold; RC, respiratory compensation.

all subjects.

B Comparison of metabolic variables in incremental-load exercise test

Table 2 shows the results of the ILE test. The peak V_{O2} as a general variable of exercise tolerance was 44.9±1.7 ml/kg/min, which was higher in the non-completion group, although the difference was not significant (43.4±8.7 vs. 47.5±3.0 ml/kg/min, respectively). The AT points did not differ between the two groups and were within the reference ranges in Japanese subjects (26.0±4.7 vs. 25.7±4.2 ml/kg/min, respectively) There were also no significant differences in other metabolic variables between the two groups. The speed and angle of the treadmill were higher in the non-completion group, although the differences were not significant. The relationships between these exercise loads, AT points, and peak V_{O2} are shown in **Fig 1**.

C Comparison of metabolic variables in constant-load exercise test

Table 3 shows a comparison of metabolic variables in the CLE tests. Although the actual exercise load was significantly higher in the non-completion group than the completion group, the relative exercise load of the CLE test was also higher in the non-completion group, although the difference was not significant (140.2±28.9 % vs. 158.3±19.2 %, respectively, P=0.052). The non-completion group showed significantly higher values of peak V_{CO2}, RER, and VE than the completion group. The RER in the non-completion group exceeded 1 **(Fig. 2)**. The minimum VE/V_{CO2}, as a variable of ventilatory inefficiency, was significantly higher in the non-completion group than in the completion group.

D Results of stepwise multiple regression analysis

Stepwise multiple regression analysis was performed with the completion or the non-completion group as independent variables and relative exercise load, RER, minimum VE/V_{CO2}, and VE as explanatory variables. The minimum VE/V_{CO2} remained as the final variable ($P<0.05$).

Ⅳ Discussion

The non-completion group showed significantly

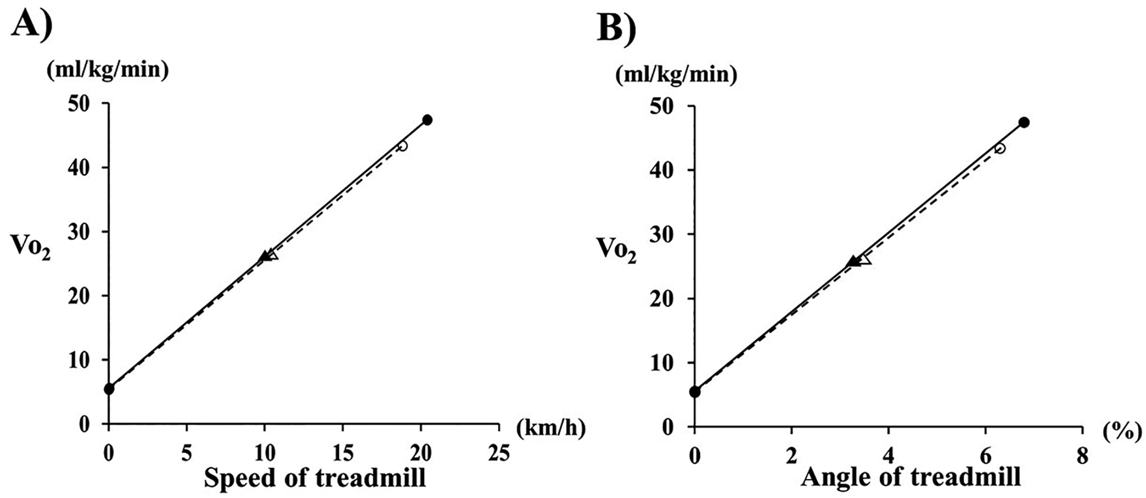

Fig. 1 Relationships between actual exercise load, AT point, and peak V_{O2} in the incremental load exercise test (ILE test).

Note: Panel A (left) shows V_{O2} and treadmill speed in the ILE test. Panel B (right) shows V_{O2} and treadmill angle in the ILE test. Open circles (○), completion group; closed circles (●), non-completion group; open triangles (△), AT point of the completion group; closed triangles (▲), AT point of the non-completion group. The AT point is plotted at the intersection of the V_{O2} lines.

Abbreviations: V_{O2}, oxygen uptake; AT, anaerobic threshold.

Table 3 Comparison of metabolic variables in constant load exercise test

	Completion	Non-Completion
n	17	10
Treadmill speed, km/h	5.0 ± 0.2	5.5 ± 0.3*
Treadmill angle, %	14.2 ± 0.5	16.2 ± 5.5*
Relative exercise load, %	140.2 ± 28.9	158.3 ± 19.2
Endurance time, min	-	9.9 ± 2.0
V_{O2}, ml/kg/min	35.2 ± 1.8	41.5 ± 2.6*
V_{CO2}, ml/kg/min	34.3 ± 1.9	42.7 ± 3.1*
RER	0.97 ± 0.01	1.01 ± 0.02*
ET_{O2}, %	14.1 ± 0.6	15.2 ± 0.8
ET_{CO2}, %	5.7 ± 0.6	5.4 ± 0.6
VE/V_{O2}	33.3 ± 5.0	35.9 ± 4.9
VE/V_{CO2}	34.6 ± 4.1	35.4 ± 3.6
VE, L/min	76.4 ± 4.1	92.2 ± 7.0*
VT, L	1.76 ± 0.08	2.19 ± 0.20
VD/VT	0.27 ± 0.02	0.26 ± 0.03
RR, breaths/min	43.9 ± 1.9	43.7 ± 3.8
PR, beats/min	177.3 ± 4.4	185.2 ± 2.2
SpO_2, %	91.2 ± 1.0	90.7 ± 2.3
Minimum VE/V_{CO2}	27.0 ± 2.9	29.5 ± 2.6*

Notes: All values of metabolic variables except minimum VE/V_{CO2} are shown at the peak of exercise. Values represent the means ± standard error of the mean. *$P < 0.05$ vs. the completion group. The relative exercise load of the CLE test to the AT point in the ILE test was calculated as: peak V_{O2} in the CLE test/V_{O2} at the AT in the ILE test. The completion group consisted of those who continued to exercise for 20 minutes in the constant load exercise test, while the non-completion group consisted of those who could not.

Abbreviations: V_{O2}, oxygen uptake; V_{CO2}, carbon dioxide output; RER, respiratory exchange ratio; ET_{O2}, end-tidal oxygen, ET_{CO2}, end-tidal carbon dioxide; VE, ventilation; VT, tidal volume; VD, dead-space gas volume; RR, respiratory rate; PR, pulse rate; SpO_2, percutaneous oxygen saturation; AT, anaerobic threshold; RC, respiratory compensation.

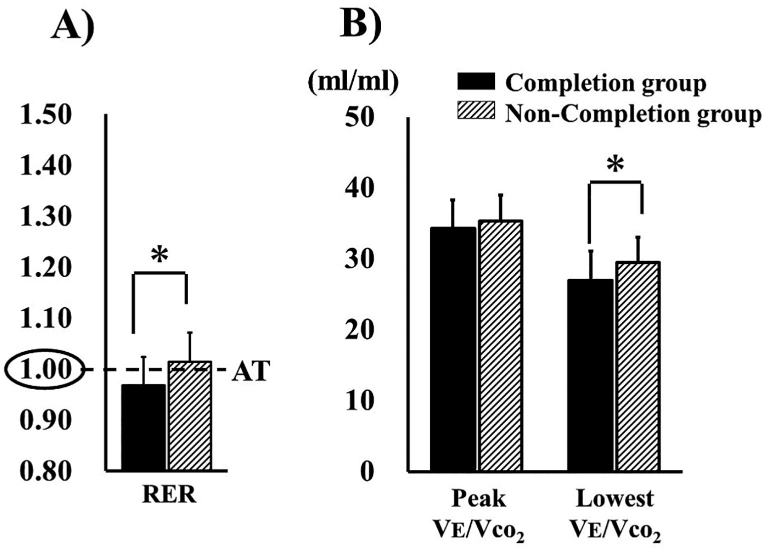

Fig. 2 Comparison of gas exchange ratio and ventilation inefficiency variables in constant load exercise test (CLE test).

Note : Panel A (left) shows a comparison of RER (V_{CO2}/V_{O2}) as a variable of AT in the CLE test. Panel B (right) shows a comparison of minimum VE/V_{CO2} as a variable of ventilatory inefficiency in the CLE test.

Abbreviations : AT, anaerobic threshold ; RER, respiratory exchange ratio ; VE/V_{CO2}, minute ventilation/carbon dioxide production.

higher RER and peak VE than the completion group in the CLE test, and the RER exceeded 1 as the standard of the AT point[13]. Ventilation is more enhanced to compensate for increased CO_2 when exercising beyond the AT point, which promotes lactic acidosis[14]. Therefore, the non-completion group may have exceeded the AT to a greater extent than the completion group in the CLE test, which may have prevented them from completing the CLE test. The non-completion group exceeded the AT point in the CLE test to such a great extent because the relative exercise load of the CLE test was higher in the non-completion group than the completion group. It was reported previously that a higher relative exercise load calculated by the AT point was associated with shorter exercise duration[15]. In the present study, the relative exercise load was higher in the non-completion group, but the difference between groups was not significant. This may have been because the non-completion group exercised to a higher exercise load and showed a higher peak V_{O2} compared to the completion group in the ILE test, even though the AT point was comparable between the two groups **(Fig. 1)**. As peak V_{O2} of the ILE test is affected by motivation and subjective symptoms[3], it may have been more appropriate to determine the exercise load for the CLE test based on the AT point, which is an objective variable that does not depend on motivation[16]. A previous study adopted the AT level as the exercise load for the CLE test in healthy subjects[17]. However, the AT point is 50 %-60 % of maximal capacity[3], and it is recommended to perform the CLE test at 75 %-80 % of maximal exercise capacity in patients with COPD who perform the CLE test frequently[18]. Therefore, it is necessary to conduct the CLE test at 75 %-80 % of the maximal capacity calculated by the AT point, and to examine whether the relative exercise load and exercise duration can be standardized among subjects in such a CLE test in a future study.

The results of stepwise multiple regression analysis showed that the minimum VE/V_{CO2} was the variable with the greatest influence on exercise duration. Minimum VE/V_{CO2}, which is a variable of ventilatory inefficiency in patients with heart failure, was significantly higher in the non-completion group, but was within the reference range and less than the standard value of 36[3]. Keller-Ross et al. reported that minimum VE/V_{CO2} and RER affected by increasing V_{CO2} were related to the type of fibers in skeletal muscle[19]. As daily physical activity influences the proportions of the different types of fibers in skeletal muscle[20], the present study suggested that daily physical activity should be assessed when performing the CLE test to standardize the proportions of types of fibers in skeletal muscle and minimum VE/V_{CO2} among subjects.

This study had several limitations. First, the changes in blood lactate, lower limb muscle strength, and cardiac function, and the differences in relative exercise load between the two groups, which may affect exercise tolerance and ventilation inefficiency, were not measured. Second, we did not evaluate training status or fitness level for modulation of endurance exercise performance. Third, the healthy subjects varied widely in age over the range of 18–60 years old, and the results of the exercise test may have been affected by the lack of adjustment for age. Fourth, we did not evaluate the reasons why the subjects stopped exercising in the CLE test, and it was not determined whether dyspnea or muscle fatigue was responsible for exercise limitation.

Ⅴ Conclusion

This study showed that the high relative exercise load in the CLE test causes the subject to exceed the anaerobic threshold and limits maintenance of exercise in the test. In future, we will conduct a study on the validity of the CLE test in which the exercise load is determined by the AT point to standardize the relative exercise load in the CLE test.

Ⅵ Conflict of interest

The authors declare no conflicts of interest.

Ⅶ Acknowledgments

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