The respiratory and metabolic responses for determinants of exercise endurance time in constant-load exercise test in healthy adult volunteers, prospective cross-sectional design

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Abstract

Although constant-load exercise test is performed at approximately 80% of maximum load determined based on individual exercise capacity of incremental-load exercise test, some subjects cannot maintain exercising in constant-load exercise test. We assumed that the metabolic responses difference between those who are and are not able to maintain exercising in only constant-load exercise. Therefore, present study was performed to elucidate the metabolic responses for determinants of endurance time in constant-load exercise test. Twenty-seven healthy volunteers performed constant-load exercise test at 80% of maximum load determined by incremental-load exercise test. Subjects were separated into those who were and were not able to complete 20 minutes of constant-load exercise test. The metabolic indexes were compared between the two groups. The not-completed group showed significantly higher respiratory exchange ratio (RER) as anaerobic threshold and minimum minute ventilation/carbon dioxide production (VE/V_{CO_2}) as ventilatory inefficiency at peak exercise only in constant-load exercise test ($P < 0.05$). It was suggested that the respiratory and metabolic responses for determinants of endurance time in constant-load exercise test were exercise beyond anaerobic threshold and the increase in CO_2 output due to ventilatory inefficiency related to a reduced skeletal muscle in healthy adults.

Introduction

Cardiopulmonary exercise test (CPET) is performed to evaluate cardiovascular, respiratory, and metabolic function in patients or healthy subjects in clinical practice and in sports science.¹⁻² CPET consists mainly of incremental-load exercise test (ILET) and constant-load exercise test (CLET). Generally, ILET evaluates maximum oxygen intake as exercise tolerance and respiratory metabolism by exercising until limited by symptoms with gradually increasing exercise load and used for evaluation of treatment efficacy.¹ CLET has been used to evaluate kinetic oxygen intake and dynamic lung hyperinflation in chronic obstructive pulmonary disease (COPD) and is performed at approximately 70% – 80% of peak VO_2 exercise capacity determined in ILET.^{1,3-4} As CLET has been reported to reflect the treatment efficacy of pharmacological and non-pharmacological treatments compared with ILET, CLET has been used for evaluation of the effects of bronchodilators, oxygen therapy, and pulmonary rehabilitation.⁵⁻⁷ As the metabolic kinetics reaches a plateau at 5 – 10 minutes after commencement of exercise in CLET, it is recommended that the subject maintain exercising for more than 5 – 10 minutes.⁸ Exercise endurance time of CLET is adopted as exercise tolerance and is often the upper limit up to 20 – 30 minutes.⁹⁻¹⁰ Subjects are separated into those who are and are not able to maintain exercising for a longer duration in CLET even though CLET is performed with a load based on individual exercise capacity determined by ILET.¹⁰ The reason why some healthy subjects cannot maintain exercising in only CLET has not been elucidated. A previous study reported that CLET more reflect metabolism of muscle and had a larger metabolic responses such as progresses of lactic acidosis compared to ILET.¹¹ Therefore, we assumed that the respiratory and metabolic responses which are different between those who are and are not able to maintain exercising in CLET affects exercise endurance of CLET even though CLET is performed with a load based on individual exercise capacity determined by ILET. The present study was performed to

elucidate the respiratory and metabolic responses for determinants of maximum exercise endurance time during CLET in healthy adult volunteers.

Materials And Methods

Subjects

The study population consisted of 27 healthy adult volunteers (24 male and 3 female) from 18 to 70 years old examined between March 2017 and December 2018.

Study design

This study had a prospective cross-sectional design. All subjects underwent spirometry. Symptom-limited ILET was performed, and then CLET was conducted at a load of 80% of the maximum exercise capacity determined by ILET. The endurance time of CLET was upper limit to 20 minutes. The subjects were divided into those who were and were not able to complete 20 minutes of exercise in CLET. We designated these groups as the completed group and the not-completed group, respectively, and compared metabolic indexes in each exercise test between the groups to elucidate the respiratory and metabolic responses for determinants of exercise endurance time in CLET.

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 FEV₁ and VC, Japanese local reference data developed by the Japanese Respiratory Society were adopted.¹²

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 referenced to previous studies.¹⁰ Oxygen uptake (V_{O_2} , ml/kg/min); carbon dioxide production (V_{CO_2} , ml/kg/min); Respiratory exchange ratio ($RER = V_{CO_2}/V_{O_2}$); end-tidal oxygen (ET_{O_2} , %); end-tidal carbon dioxide (ET_{CO_2} , %); minute ventilation/oxygen uptake (VE/V_{O_2}); minute ventilation/carbon dioxide production (VE/V_{CO_2}); 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 indexes during exercise measured in a breath-by-breath manner were assessed by the average value for 10 s before the end of exercise, which was called the “peak value.” We evaluated the anaerobic threshold (AT) point by the V-Slope method and respiratory compensation (RC) point was determined by the $VE-V_{CO_2}$ slope method according to ATS/ACCP statement respectively.¹ Percutaneous oxygen saturation (Sp_{O_2} , %) and pulse rate (PR , beats/min) during exercise were measured continuously with a fingertip monitor (AE-310S Aeromonitor; Minato Medical Science).

The protocol of ILET with a treadmill adopted TR-3 which is designed to increase V_{O_2} linearly. In this protocol, the speed and slope changed continuously and was the same method as used in determination of the AT reference value in Japanese subjects.¹³ 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 ILET as warm-up and cool-down.

Symptom-limited constant-load exercise test

After sufficient rest, symptom-limited CLET was conducted at a load of 80% of the maximum load in the ILET as in the previous study.¹⁰ First, the subjects walked for 3 minutes as warm-up at a speed of 1 km/h with a slope of 0% on the treadmill. Next, the subject began exercise at up to 80% of the maximum load of ILET. The CLET 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 CLET, 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 CLET were evaluated whether they did maximum-effort exercise in consideration of 85% of maximal age-predicted heart rate.¹⁴ The subjects were divided into those who were and were not able to complete 20 minutes of exercise in CLET, defined as the completed group and the non-completed group, respectively.

Statistical analysis

As there have been no similar previous studies, 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 indexes between the completed group and the non-completed group. Finally, single logistic regression analysis was conducted with the completed or non-completed group as the independent variable and metabolic indexes that were significantly different between the two groups as the explanatory variable. In all analyses, $P < 0.05$ was taken to indicate statistical significance. SPSS ver. 25 was used for statistical analyses (SPSS Inc., Chicago, IL).

Results

Characteristics of physical findings, lung function, and complications

Table 1 shows the characteristics of physical findings and spirometry. A total of 27 subjects were enrolled in this study. Physical findings and lung functions were not significantly different between the two groups and all values of spirometry were within reference ranges in all subjects.

Comparison of metabolic indexes in incremental-load exercise test

Table 2 shows the results of comparison in ILET. The peak V_{O_2} as a general index of exercise tolerance were 43.4 ± 2.1 and 47.5 ± 3.0 in the completed group and the non-completed group, respectively, which were within the reference range and were not significantly different. The AT point and RC point were not different between the two groups and were within the reference ranges in Japanese subjects. The other metabolic indexes were also not significantly different between the two groups.

Comparison of metabolic indexes in constant-load exercise test

Table 3 shows the results of CLET. The not-completed group showed significantly higher values of peak V_{CO_2} , RER and VE in comparison to the completed group. The RER in the not-completed group exceeded 1 (Fig. 1). The minimum VE/V_{CO_2} , as an index of ventilatory inefficiency, was significantly higher in the not-completed group than the completed group, whereas VE/V_{CO_2} at peak in CLET were not different between the two groups. The peak V_{O_2} of non-completed group increased until near the RC point of ILET. However, VE/V_{CO_2} which increase after exceeding RC point did not show a significant difference between the two groups.

Results of logistic regression analysis and comparison between the two exercise tests

Table 4 shows the results of single logistic regression analysis with regard to the metabolic indexes. The minimum VE/V_{CO_2} showed the most significant P -value and 95% confidence interval in the regression model to predict the completion of exercise ($P = 0.05$), and the subjects with lower values of minimum VE/V_{CO_2} could complete the 20 minutes of exercise.

Discussion

This study was performed to elucidate the respiratory and metabolic responses for determinants of exercise endurance time in CLET in healthy volunteers. The study population consisted of 27 healthy adult volunteers. CLET was conducted at a load of 80% of the maximum exercise capacity. The subjects were divided into those who were and were not able to complete 20 minutes of exercise (completed group and not-completed group, respectively). We compared metabolic indexes between the two groups. The not-completed group showed significantly higher RER and peak VE than the completed group in CLET, and the RER exceeded 1 as the standard of AT. The minimum VE/V_{CO_2} as an index of ventilatory inefficiency was significantly higher in the not-completed group. Single logistic regression analysis showed that the minimum VE/V_{CO_2} had the greatest predictive capability in the regression model for completing the exercise. It was suggested that the respiratory and metabolic responses for determinants of exercise endurance time in CLET was exercise beyond AT due to ventilatory inefficiency related to a reduced skeletal muscle in healthy adults.

RER (V_{CO_2}/V_{O_2}) is used as an index of AT when RER exceeds 1,¹⁵ and AT is correlated with exercise endurance time.¹⁶ In the present study, the RER was significantly higher and exceeded 1 in the not-completed group. Therefore, the endurance time in the not-completed group could have been restricted by anaerobic metabolism in comparison with the completed group even though the subjects performed CLET based on their individual exercise capacity of ILET. Ventilation is more enhanced to compensate for the increased CO_2 production exceeding AT.¹⁷ Therefore, the not-completed group showed higher V_{CO_2} and VE at peak in CLET. Since the peak V_{O_2} of the not-completed group increased until near the RC point of ILET, the non-completed group may have exercised to near the RC point in CLET. However, as VE/V_{CO_2} which increase after RC point did not show a significant difference between the two groups, the not-completed group may not have exceeded the RC point in CLET.

The not-completed group showed not only a higher RER but also a higher minimum VE/V_{CO_2} as an indicator of ventilatory inefficiency. This could have been caused by enhanced V_{CO_2} production due to reduced skeletal muscle or metabolic inefficiency of the muscles. Although minimum VE/V_{CO_2} as an indicator of ventilatory inefficiency generally reflects V/Q mismatch in patients with heart failure,¹⁸ in healthy subjects, it is related to the mass and strength of skeletal muscle.¹⁹ Gonzales et al. also reported a correlation between VE/V_{CO_2} at AT and the lower limb muscle strength in healthy adults. Furthermore, Keller-Ross et al. reported that minimum VE/V_{CO_2} and RER affected by increasing V_{CO_2} were related to the type of fibers in skeletal muscle.²⁰ Therefore, it was suggested that the increased RER and minimum VE/V_{CO_2} in the not-completed group may have been caused by reduced skeletal muscle, which resulted in decreased exercise endurance time in CLET, and that the minimum VE/V_{CO_2} was the most accurate variable to predict exercise endurance time in logistic regression analysis.

The reason why there were differences in AT and ventilatory inefficiency related to a reduced skeletal muscle in CLET despite the lack of significant differences at all metabolic indexes in ILET is that there were differences in metabolic responses between ILET and CLET. Yamamoto et al. reported that lactic acidosis is more caused by a larger O_2 extraction in CLET compared to ILET.¹¹ O_2 extraction is the release of O_2 from hemoglobin, and this phenomenon is facilitated by the Bohr effect when subjects exceed AT,²¹ namely subjects are more likely to exceed AT in CLET compared to ILET. Therefore, the not-completed group who have a greater V_{CO_2} production affected by reduced skeletal muscle could have exceeded AT and did not maintain exercise only in CLET.

Limitations

This study had several limitations. First, as the required sample size was not calculated, it was unclear whether the sample size had sufficient statistical power. The statistical power of this study for analyzing the difference in minimum VE/V_{CO_2} was calculated as 63.5% by post hoc analysis and it was slightly lower. Second, the changes in blood lactate and muscle strength of lower limbs, which may affect

exercise tolerance and ventilation inefficiency, were not measured. Third, we did not evaluate training status or fitness level for modulating endurance exercise performance between two groups.

Conclusion

It was suggested that the respiratory and metabolic responses for determinants of endurance time in CLET were exercise beyond AT and increased CO₂ output due to ventilatory inefficiency related to a reduced skeletal muscle in healthy adults. Therefore, those respiratory and metabolic responses of endurance time in CLET should be considered when we perform CLET. We will investigate whether the same results as in the present study will be shown in patients with COPD in the future.

Declarations

Ethics approval and consent to participate

All subjects were given an adequate explanation of the study and provided their 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 of Medical Ethics Committee (approval number: 3705).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SK performed the all measurements and analysis in writing the manuscript. Corresponding authors (KF) read and revised the manuscript.

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Tables

Table 1. Subject characteristics of basic physical findings, lung function

	Completed	Not-Completed
<i>n</i>	17	10
Age, years	30.8 ± 3.0	26.9 ± 3.3
Sex (male/female)	15/2	9/1
BMI, kg/m ²	23.1 ± 0.8	21.1 ± 0.9
%VC, %	91.2 ± 2.5	91.9 ± 4.9
%FEV ₁ , %	88.9 ± 2.4	91.0 ± 4.9
<u>FEV₁/FVC, %</u>	<u>86.7 ± 2.2</u>	<u>87.7 ± 2.4</u>

Notes: Values represent the means ± standard error of the mean. The completed group is who continued to exercise for 20 minutes in constant load exercise test, and the not-completion group is who could not that.

Abbreviations: BMI, body mass index; VC, vital capacity; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.

Table 2. The comararison of metabolic indexes in incremental load exercise test

	Completed	Not-Completed
<i>N</i>	17	10
Vo ₂ , ml/kg/min	43.4 ± 2.1	47.5 ± 3.0
Vco ₂ , ml/kg/min	54.2 ± 2.5	60.1 ± 4.0
RER	1.25 ± 0.02	1.28 ± 0.02
ETo ₂ , %	14.1 ± 0.6	15.2 ± 0.8
ETco ₂ , %	5.7 ± 0.6	5.4 ± 0.6
VE/ Vo ₂	37.8 ± 0.9	39.1 ± 1.5
VE/Vco ₂	30.2 ± 0.6	30.6 ± 1.1
VE, L/min	107.6 ± 5.3	113.2 ± 6.7
VT, L	2.3 ± 0.1	2.5 ± 0.2
VD/VT	0.26 ± 0.01	0.25 ± 0.02
RR, breaths/min	47.2 ± 1.4	45.9 ± 2.3
PR, beats/min	148.1 ± 40.2	151.5 ± 37.2

SpO ₂ , %	91.2 ± 4.0	90.7 ± 7.4
AT point, ml/kg/min	26.0 ± 1.1	25.7 ± 1.3
RC point, ml/kg/min	37.2 ± 1.6	41.2 ± 2.6
<u>Minimum VE/Vco₂</u>	<u>27.3 ± 0.5</u>	<u>26.8 ± 0.7</u>

Notes: All values of metabolic indexes except for AT point, RC point and minimum VE/ Vco₂ are at peak of exercise. Values represent the means ± standard error of the mean. The completed group who continued to exercise for 20 minutes in constant load exercise test and the non-completion group who could not that.

Abbreviations: Vo₂, oxygen uptake; Vco₂, carbon dioxide output; RER, respiratory exchange ratio; ETo₂, end-tidal oxygen, ETco₂, end-tidal carbon dioxide; VE, ventilation; VT, tidal volume; VD, dead-space gas volume; RR, respiratory rate; PR, pulse rate; SpO₂, percutaneous oxygen saturation; AT, anaerobic threshold; RC, respiratory compensation.

Table 3. The comararison of metabolic indexes in constant load exercise test

	Completed	Not-Completed
<i>N</i>	17	10
Endurance time, min	19.5 ± 0.1	9.9 ± 2.0
Vo ₂ , ml/kg/min	35.2 ± 1.8	41.5 ± 2.6
Vco ₂ , ml/kg/min	34.3 ± 1.9	42.7 ± 3.1*
RER	0.97 ± 0.01	1.01 ± 0.02*
ETo ₂ , %	14.1 ± 0.6	15.2 ± 0.8
ETco ₂ , %	5.7 ± 0.6	5.4 ± 0.6
VE/Vo ₂	33.3 ± 5.0	35.9 ± 4.9
VE/Vco ₂		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	159.8 ± 6.3	177.4 ± 4.2
SpO ₂ , %	91.2 ± 1.0	90.7 ± 2.3
<u>Minimum VE/Vco₂</u>	<u>27.0 ± 2.9</u>	<u>29.5 ± 2.6 *</u>

Notes: All values of metabolic indexes except minimum VE/Vco₂ were at peak of exercise. Values represent the means ± standard error of the mean. **p* < 0.05 vs. the completed group. The completed group who continued to exercise for 20 minutes in constant load exercise test and the non-

completion group who could not that.

Abbreviations: Vo₂, oxygen uptake; Vco₂, carbon dioxide output; RER, respiratory exchange ratio; ETo₂, end-tidal oxygen, ETco₂, end-tidal carbon dioxide; VE , ventilation; VT, tidal volume;VD, dead-space gas volume; RR, respiratory rate; PR, pulse rate; SpO₂, percutaneous oxygen saturation; AT, anaerobic threshold; RC, respiratory compensation.

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Table 4. Single logistic regression analysis to predict completed exercise in constant load exercise test.

<u>Vriable</u>	<u>Odds ratio</u>	<u>95%CI</u>	<u>P-value</u>
Minimum VE/Vco ₂	0.707	0.50 - 0.10	0.05
RER	0.001<0	0 - 2.52	0.64
<u>VE</u>	<u>0.96</u>	<u>0.92 - 1.00</u>	<u>0.06</u>

Note: Independent variable was completed or not-completed group as categorical variable.

Abbreviations: 95%CI, 95% confidence interval; VE /Vco₂, minute ventilation/carbon dioxide output; VE , minute ventilation.

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Figures

Figure 1

The comparison of gas exchange ratio and ventilation inefficiency indexes in constant load exercise test (CLET). Note: Panel A (left) shows the comparison of RER (V_{CO_2}/V_{O_2}) as an index of AT in CLET. Panel B (right) shows the comparison of minimum VE/V_{CO_2} as an index of ventilatory inefficiency in CLET. Abbreviations: AT, anerobic threshold; RER, respiratory exchange ratio; VE/V_{CO_2} , minute ventilation/carbon dioxide production.