**CLINICAL RESEARCH** 

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Received: 2022.01.11 Accepted: 2022.04.27 able online: 2022.05.04 Published: 2022.05.30		Effects of Wearing Face Capacity and Ventilato Healthy Subjects Durin	e Masks on Exercise ry Anaerobic Threshold in og the COVID-19 Epidemic
Authors' Contribution: Study Design A Data Collection B Statistical Analysis C Data Interpretation D Manuscript Preparation E Literature Search F Funds Collection G	BCDEF 1 BC 1 CD 2 CD 2 CD 2 E 1 F 1 F 3 AG 1	Qiang Lin D Yuxing Cai Changjun Yu Wei Gu Yan Tan Li Wang Anliang Chen Kai Cheng Ting Meng Xueping Li	<ol> <li>Department of Rehabilitation Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu, PR China</li> <li>Department of Respiratory and Critical Care Medicine, Nanjing First Hospital, Nanjing Medical University, Nanjing, Jiangsu, PR China</li> <li>The First Clinical Medical College of Nanjing University of Traditional Chinese Medicine, Nanjing, Jiangsu, PR China</li> </ol>
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Back Material/N	ground: Nethods: Results:	Face masks have become an important part of the C the effect of wearing masks on exercise ability and Thirty-four young, healthy volunteers were include subjects were randomized to perform 2 cardiopulm exchange analysis, one with and another without for all subjects and indicators from the 2 exercise to collected. In cardiopulmonary exercise tests, wearing a mas rate (WR), oxygen consumption per kg body weigh carbon dioxide ventilation equivalent (VE/VCO <sub>2</sub> ) ar quency (BF), dead space ratio (VD/VT), and VE/VCC There was a positive linear correlation between WR VAT and $\delta$ VO <sub>2</sub> @VAT, respectively) (r=0.495, <i>P</i> =0.003 VO <sub>2</sub> /kg, and VE were significantly decreased in the VAT group with mask ( <i>P</i> <0.05). Logistic regression fluences on VAT.	COVID-19 prevention approach. This study aimed to explore I ventilatory anaerobic threshold (VAT). ed in this study, consisting of 18 men and 16 women. The nonary exercise tests (CPET) on a cycle ergometer with gas wearing a face mask (cross-over design). The general data ests performed with and without wearing a face mask were k significantly ( $P$ <0.05) decreased peak indexes (eg, work t (VO <sub>2</sub> /kg), heart rate (HR), ventilation per minute (VE) and nd anaerobic threshold indexes (eg, WR, HR, VE, breath fre- O <sub>2</sub> ). However, the PETCO <sub>2</sub> at peak was significantly higher. difference and VO <sub>2</sub> difference at VAT (abbreviated as $\delta$ WR@ 3). Subgroup analysis of the VAT indexes showed that WR, r advanced VAT group with mask compared with the stable showed that $\delta$ VE, $\delta$ BF, and $\delta$ VE/VCO <sub>2</sub> had independent in-
Cond	lusions:	Wearing masks advances VAT in healthy young sub changes in VE, BF, and VE/VCO <sub>2</sub> while wearing mas	bjects during CPET. The advanced VAT was associated with sks.
Ke	ywords:	Anaerobic Threshold • COVID-19 • Exercise Test	
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## Background

The coronavirus-19 (COVID-19) pandemic has had a long-term and profound effect on people's lives, studies, work, and social activities around the world. Wearing face masks, which prevent the transmission of infectious respiratory pathogens, has become a major strategy to prevent its spread [1,2].

Some clinical trials have investigated the effects of wearing face masks on subjects' performance during exercise. Fikenzer et al conducted repetitive cardiopulmonary exercise tests on 12 healthy male subjects in a randomized order: no mask, surgical mask, and an FFP2/N95 mask; the forced expiratory volume and peak expiratory flow of the subjects decreased during the lung function tests, while peak power and ventilation also decreased during the cardiopulmonary exercise test [3]. Shaw et al showed that wearing a face mask during vigorous exercise had no discernable detrimental effect on blood or muscle oxygenation or exercise performance in young, healthy participants [4]. The conclusions of these studies were based on comparisons of maximum exercise capacity, which does not necessarily represent the actual exercise conditions during a routine exercise.

However, there is a lack of studies on continuous exercise capability (anaerobic threshold), which we believe is the crux of the issue. Any physical activity that exceeds the anaerobic threshold can lead to anaerobic metabolism and unsustainable exercise. Continuous physical activity is only possible if the exercise intensity does not exceed the anaerobic threshold. According to previous studies, the endurance threshold for an 8-h shift of heavy manual occupational labor is 33%-40% of an individual's maximum oxygen uptake [5]. Even in physically highly demanding occupational tasks, the ventilatory threshold of an individual was only exceeded for short periods at 35-69% of maximal oxygen uptake [6]. To study the harm caused by wearing a mask during exercise, it is important to determine an individual's ventilatory threshold. However, there are limited studies on the effect of exercising with a mask on the ventilatory anaerobic threshold.

Two separate incidents of the sudden death of students wearing face masks in China have raised concerns about whether wearing face masks during exercise results in physiological changes that endanger human life. Considering that wearing masks is thought to prevent oxygen inhalation, increase repetitive inhalation of carbon dioxide, and increase work of breathing [7], we hypothesized that wearing face masks might advance both the peak and ventilatory anaerobic threshold (VAT) during cardiopulmonary exercise testing. This study aimed to investigate the effect of wearing face masks on exercise performance and the VAT in healthy young subjects through a cardiopulmonary exercise test (CPET), as well as the mechanisms involved.

## **Material and Methods**

#### Subjects

A total of 34 young, healthy volunteers were recruited from the medical staff of our Rehabilitation Department. The inclusion criteria were: 1) 18-30 years of age, 2) healthy and without cardiopulmonary diseases, 3) not using drugs that affect heart rate, and 4) signed the informed consent form. The exclusion criteria were: 1) any signs of hemodynamic instability during exercise, such as pale complexion and decreased blood pressure, or 2) unable to complete the cycling task for any reason.

#### **Study Design**

This study was registered at <u>http://www.chictr.org.cn/</u> (ChiCTR2000034457) and approved by the Ethics Committee of Nanjing First Hospital (KY20210125-02). The study was conducted as a randomized, counterbalanced, cross-over trial. Study participants were randomized to perform an incremental cycle ergometer exercise test wearing a surgical face mask or not on 2 separate occasions, with at least 48 h between 2 separate occasions. Randomization was performed using a computerized random number generator. Subjects with odd number were first subjected to cardiopulmonary exercise test without mask, and subjects with even number were first subjected to cardiopulmonary exercise test with mask. All participants wore the same brand and model of surgical face masks.

#### Cardiopulmonary Exercise Test (CPET) Protocol

All CPETs were performed by the same operator, who has been a CPET operator at the Rehabilitation Department for >10 years. The fit of the surgical face mask beneath the Schiller mask was standardized; the correct fit was confirmed by exhaling with maximal force before each test to identify air leakage. At the beginning of each exercise test, subjects rested for 3 minutes, then performed warm-up exercise for 3 minutes (unload cycling), followed by a ramp incremental exercise stage until voluntary exhaustion occurred. Each subject continued an additional 5-min recovery period at a work rate of 20 W. The increase in the work rate varied from 10 to 25 W/min according to the estimated exercise capability of each subject and ensured that the subject reached the peak within 10 minutes. The power increasing scheme for each subject's 2 exercise tests was the same so that the detection of exercise peak and the anaerobic threshold were not affected. Gas exchange was measured using a breath-by-breath instrument (Schiller AT-104) with a mask with 80 to 100 mL of dead space. All values were averaged across 10-second epochs. At the end of each CPET test, the physician estimated the location of the subject's VAT. At the end of all tests, the location of the VAT for each exercise test was re-verified by another physician. The VAT point of each

test was determined using the V-slope method [8] and other ventilatory parameters, including the VE/VCO<sub>2</sub> nadir, PETCO<sub>2</sub> response, and RER transition point.

#### **Collected Indicators**

General information about each subject was collected, and all indicators from the 2 exercise tests were recorded, with or without wearing a face mask, including work rate (WR), oxygen uptake (VO<sub>2</sub>), heart rate (HR), ventilation per minute (VE), tidal volume (VT), breath frequency (BF), dead space ratio (VD/VT), carbon dioxide ventilation equivalent (VE/VCO<sub>2</sub>), and end-tidal CO<sub>2</sub> pressure (PETCO<sub>2</sub>). The VAT was denoted by work rates and oxygen uptake, abbreviated as WR@VAT and VO<sub>2</sub>@VAT, respectively.

#### **Statistical Analysis**

R version 4.0.3 (R version 4.0.3 (2020-10-10) - "Bunny-Wunnies Freak Out" Copyright (C) 2020 The R Foundation for Statistical Computing Platform: https://www.r-project.org/; x86 64-w64mingw32/x64 (64-bit)) was used for statistical analysis. Continuous variables were represented as mean ± standard deviation. Categorical variables were expressed as frequencies and percentages. The paired t test was used to compare data from 2 exercise tests with and without masks. For correlation analysis. Pearson correlation analysis was used if both variables had normal distribution; otherwise, the Spearman correlation analysis was used. After grouping the participants, the group *t* test was used to compare the 2 groups. The cor.test() function in R was used for correlation analysis and test. The glm() function was used to fit the logistic regression model. The step() function was used for variable screening, and the model was automatically selected according to the Akaike information criterion (AIC) (stepwise regression method). P<0.05 was considered statistically significant. The graphs were drawn using GraphPad PRISM 5.01 for Windows (GraphPad Software, Inc., San Diego, CA, USA).

# Results

## **Demographic and Baseline Data**

A total of 34 young, healthy volunteers were enrolled, consisting of 18 men and 16 women (**Figure 1**). All 34 participants completed the 2 CPETs. The main characteristics were an age of  $23.4\pm3.6$  years, a height of  $170.3\pm9.7$  cm, a weight of  $63.3\pm13.5$  kg, and a BMI of  $21.7\pm3.7$  kg·m<sup>-2</sup>. The physiological indexes at rest included heart rate of  $87.7\pm12.5$  bpm, systolic blood pressure of  $114.8\pm17.8$  mmHg, and diastolic blood pressure of  $77.0\pm9.6$  mmHg. All pulmonary function test indexes were good (**Table 1**).



Figure 1. Patient flowchart.

 Table 1. Baseline characteristics and physiological indexes of participants.

Parameter	Total (n=34)
Age (year)	23.4±3.6
Male/Female (%)	52.9/47.1
Height (cm)	170.3±9.7
Weight (kg)	63.3±13.5
BMI (kg/m²)	21.7±3.7
Heart rate (bpm)	87.7±12.5
Systolic blood pressure (mmHg)	114.8±17.8
Diastolic blood pressure (mmHg)	77.0±9.6
FVC (%predicted)	86.6±8.6
FEV1 (%predicted)	87.0±9.1
FEV1/FVC (%)	86.6±6.6
PEF (%predicted)	82.2±17.9

Data are presented as mean±SD unless otherwise indicated. BMI – Body Mass Index; bpm – beats per minute; FVC – Forced Vital Capacity; FEV1 – Forced Expiratory Volume in 1 second; FEV1/FVC – the percentage of the FVC expired in one second; PEF – Peak Expiratory Flow.

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Indexes	Without mask	With mask	P value*
Indexes at peak			
WR (Watts)	146.9±42.1	133.6±37.3	<0.001
VO <sub>2</sub> /kg (ml/kg/min)	24.2±5.9	22.3 <u>+</u> 6.1	0.018
HR (bpm)	170.1±12.1	163.7±13.2	0.008
VE (l/min)	41.4±11.1	32.9 <u>±</u> 8.6	<0.001
VE/VCO <sub>2</sub>	23.5±2.9	21.3 ±2.8	<0.001
PETCO <sub>2</sub> (mmHg)	47.4±4.9	48.7 ±5.1	0.004
Indexes at VAT			
WR (Watts)	84.1 ±20.6	76.4 ±23.6	<0.001
VO <sub>2</sub> /kg (ml/kg/min)	14.1 ±2.6	13.3 ±3.3	0.08
HR (bpm)	133.6 ±14.3	128.3 ±14.6	0.005
VE (l/min)	21.2 ±4.8	17.2 ±4.7	<0.001
VT (l/min)	1.05±0.31	1.07±0.41	0.556
BF (bpm)	21.2±5.3	17.3±5.0	<0.001
VD/VT	0.22±0.09	0.15 <u>±</u> 0.11	<0.001
VE/VCO <sub>2</sub>	25.3 ±2.6	23.1 ±3.0	<0.001
PETCO <sub>2</sub> (mmHg)	42.7±3.6	43.2±4.0	0.248

Table 2. Comparison of peak indexes and VAT indexes between 2 exercise tests with and without a mask.

Data are presented as mean $\pm$ SD. \* *P* values for paired *t* test between 2 exercise tests with and without a mask. VAT – ventilatory anaerobic threshold; WR – work rate; VO<sub>2</sub>/kg – oxygen consumption per kg body weight; HR – heart rate; VE – ventilation per minute; VE/VCO<sub>2</sub> – carbon dioxide ventilation equivalent; VT – tidal volume; BF – breath frequency; VD/VT – dead space ratio; PETCO<sub>2</sub> – end-tidal CO<sub>2</sub> pressure.

## Comparison of Peak Indexes and VAT Indexes Between 2 Exercise Tests with and without a Mask

As shown in **Table 2**, the peak indexes of the cardiopulmonary exercise test while wearing face masks, such as WR,  $VO_2/kg$ , HR, VE, and  $VE/VCO_2$ , were significantly lower than those in the cardiopulmonary exercise test without a face mask (all *P*<0.05). However, the PETCO<sub>2</sub> was significantly higher than those in the cardiopulmonary exercise test without a face mask (*P*=0.004). The VAT indexes of the cardiopulmonary exercise test while wearing face masks, such as WR, HR, VE, BF, VD/VT, and VE/VCO<sub>2</sub>, were significantly lower than those in the cardiopulmonary exercise test without a face mask (all *P*<0.05).

# Correlation Analysis of VAT Power Change Value with VAT VO, Change Value ( $\delta$ WR@VAT vs $\delta$ VO,@VAT)

There was a positive linear correlation between  $\delta WR@VAT$  and  $\delta VO_{2}WAT$  (r=0.495, *P*=0.003, **Figure 2**).

## Subgroup Analysis

According to the aggregation characteristics of scattered points,  $\delta$ WR@VAT=-6 watts at the X-axis position was selected as the

vertical dividing line, and the 34 subjects were divided into 2 groups: the stable VAT group (VS group) and the advanced VAT group (VA group). The subgroup analysis of the VAT indicators showed that all subjects in the VS group and VA group had similar VAT indicators in the exercise test without a mask. Compared with the VS group, WR, VO<sub>2</sub>/kg, and VE were significantly decreased in the VA group after wearing masks (all *P*<0.05) (**Table 3**).

As shown in **Table 4**, subjects in the VA group demonstrated a significant difference in  $\delta$ HR,  $\delta$ VE,  $\delta$ VT,  $\delta$ VE/VCO<sub>2</sub>, and  $\delta$ PETCO<sub>2</sub> compared with subjects in the VS group (all *P*<0.05). All demographics and physiological characteristics were not significantly different between the 2 groups. Multivariable logistic regression analysis revealed that the factors independently associated with decreased VAT were  $\delta$ VE,  $\delta$ BF, and  $\delta$ VE/VCO<sub>2</sub> (**Table 5**).

## Discussion

This study aimed to investigate the effect of wearing a mask on the aerobic capacity of healthy young subjects, especially on the VAT. Wearing a mask significantly impaired the participants' performance during CPET. Significant differences in



**Figure 2.** Scatter plots of the  $\delta$ WR and  $\delta$ VO<sub>2</sub> at the VAT point. The scatter plot shows a positive linear correlation between  $\delta$ WR@VAT and  $\delta$ VO<sub>2</sub>@VAT (r=0.495, *P*=0.003). VAT – ventilatory anaerobic threshold;  $\delta$ WR – work rate difference between 2 exercise tests with and without a mask;  $\delta$ VO<sub>2</sub> – oxygen consumption difference between 2 exercise tests with and without a mask.

	Without mask			With mask		
Indexes at VAT	VS Group (n=20)	VA Group (n=14)	P value*	VS Group (n=20)	VA Group (n=14)	<i>P</i> value**
WR (Watts)	85.7±19.9	81.9±22.2	0.608	85.9±19.4	62.8 <u>±</u> 22.8	0.003
VO <sub>2</sub> /kg (ml/kg/min)	14.0 ±2.4	14.3±2.9	0.718	14.5±2.9	11.5±3.0	0.006
HR (bpm)	132.8±14.1	134.7±15.1	0.700	131.3±14.3	124.1±14.6	0.167
VE (l/min)	21.6±4.2	20.6±5.6	0.530	19.0±4.0	14.6±4.5	0.005
VT (l/min)	1.05±0.3	1.05±0.33	0.973	1.15±0.37	0.96±0.46	0.187
BF (bpm)	21.5±4.6	20.7±6.2	0.675	17.5±4.2	17.1±6.1	0.831
VD/VT	0.22±0.11	0.21±0.07	0.660	0.14±0.13	0.16±0.07	0.619
VE/VCO <sub>2</sub>	25.7±2.6	24.8±2.7	0.329	22.8±3.3	23.6±2.3	0.394
PETCO <sub>2</sub> (mmHg)	42.5±4.1	43.0±3.0	0.708	44.17±4.36	41.9±3.0	0.106

Data are presented as mean $\pm$ SD. \* *P* values for group *t* test between the VS and the VA groups without mask; \*\* *P* values for group *t* test between the VS and the VA groups with mask. VAT – ventilatory anaerobic threshold; VS Group – stable VAT group; VA Group – advanced VAT group; WR – work rate; VO<sub>2</sub>/kg – oxygen consumption per kg body weight; HR – heart rate; VE – ventilation per minute; VT – tidal volume; BF – breath frequency; VD/VT – dead space ratio; VE/VCO<sub>2</sub> – carbon dioxide ventilation equivalent; PETCO<sub>2</sub> – end-tidal CO<sub>2</sub> pressure.

	VS Group (n=20)	VA Group (n=14)	P value*
Demographics and physiological characteristics			
Age (year)	24.3±3.9	22.2 <u>+</u> 2.7	0.095
Male/Female (%)	60/40	42.9/57.1	0.324
BMI (kg/m²)	21.8±3.6	21.6±4.0	0.915
FVC (%predicted)	85.1±9.8	88.9±6.1	0.207
FEV1 (%predicted)	86.4±9.6	87.8±8.6	0.669
FEV1/FVC (%)	87.4±7.0	85.6±6.1	0.436
PEF (%predicted)	84.9±18.9	78.4±16.3	0.306
Delta indexes at VAT between 2 exercise tests			
δHR (bpm)	-1.5±7.8	-10.6±10.6	0.007
δVE (l/min)	-2.6±2.5	-6.0±3.7	0.004
δVT (l/min)	0.1±0.18	-0.09±0.27	0.021
δBF (bpm)	-4.1±2.2	-3.6±3.4	0.674
δVD/VT	-0.08±0.07	-0.05±0.05	0.133
δVE/VCO <sub>2</sub>	-2.95±2.63	-1.14±1.79	0.033
δPETCO <sub>2</sub> (mmHg)	1.7±2.7	-1.0±2.4	0.004

Table 4. Comparison of demographic and physiological characteristics, and delta indicators at VAT after subgrouping.

Data are presented as mean±SD unless otherwise indicated; Delta index represents the index of exercise test with a mask minus the corresponding index of exercise test without a mask. \* *P* values for group *t* test between the VS group and the VA group. VAT – ventilatory anaerobic threshold; VS Group – stable VAT group; VA Group – advanced VAT group; BMI – Body Mass Index; FVC – Forced Vital Capacity; FEV1 – Forced Expiratory Volume in 1 second; FEV1/FVC – the percentage of the FVC expired in one second; PEF – Peak Expiratory Flow; HR – heart rate; VE – ventilation per minute; VT – tidal volume; BF – breath frequency; VD/VT – dead space ratio; VE/VCO<sub>2</sub> – carbon dioxide ventilation equivalent; PETCO<sub>2</sub> – end-tidal CO<sub>2</sub> pressure.

 Table 5. Factors associated with being in the advanced VAT group rather than the stable VAT group via multivariable logistic regression analysis.

	β	Odds ratio	95% CI	<i>P</i> value
δνε	-1.628	0.196	0.054-0.712	0.013
δBF	1.154	3.171	1.152-8.73	0.026
δVE/VCO <sub>2</sub>	0.48	1.161	1.014-2.575	0.043

AIC: 29.391. Delta index represents the index of exercise test with a mask minus the corresponding index of exercise test without a mask. VAT – ventilatory anaerobic threshold; CI – confidence interval; VE – ventilation per minute; BF – breath frequency; VE/VCO<sub>2</sub> – carbon dioxide ventilation equivalent.

key variables, including the peak indexes (eg, WR, VO<sub>2</sub>, HR, VE, VE/VCO<sub>2</sub>, and PETCO<sub>2</sub>) and the VAT indexes (eg, WR, HR, and VE), suggested that exercising while wearing a face mask negatively impacted the maximum exercise capacity and aerobic capacity.

We have to admit that some subjects did not achieve the true exhaustion required in the exercise test while wearing masks. Therefore, the CPET peak we measured when wearing masks is not reliable. Other researchers have produced inconsistent results on the peak value of CPET in mask-wearing. The study by Driver et al showed that wearing a cloth face mask led to a significant reduction in exercise time (P<0.001), maximal oxygen consumption ( $VO_2max$ ) (P<0.001), ventilation (P<0.001), and maximal heart rate (P<0.01) [8]. However, Shaw et al and Mapelli et al showed that wearing face masks did not affect performance (time to exhaustion and peak power) [4,9]. Similarly, Epstein et al found that heart rate, respiratory rate, blood pressure, oxygen saturation, and time to exhaustion did not differ significantly [10]. A major difference among these studies is that most studies indicating reduced exercise performance while wearing a face mask also used a mask (ie, rubber or silicone) for collecting gases, which was placed over the face mask; whereas the studies by Shaw et al and Epstein et al did not use a breath-collection mask. Therefore, the breath-collection mask is likely to interfere with gas exchange through the surgical face mask as it would seal the surgical face mask tightly to the face. Usually, a surgical face mask would not be worn this tight to the face during normal exercise. When the breath-collection mask covered the surgical masks, the reduced exercise performance may become less meaningful for the usual exercise of wearing face masks.

Still, we think this study is of some value because we found that wearing a mask reduces the WR@VAT in some participants. Since the 2 exercise testing schemes of increasing power were the same, and the power was linearly increasing with time, it is indicated that wearing a mask makes part of the subjects' ventilatory anaerobic threshold occur earlier. Therefore, this study suggests that wearing masks might affect the process or mechanism of VAT. We believe that the peak is the ceiling of anaerobic threshold, and the ventilatory anaerobic threshold may occur early or late due to some factors, which are independent of the peak. This is why we used absolute intensity rather than peak percentage to represent anaerobic threshold in this study. We hope to objectively report the phenomenon that wearing masks causes individuals' VAT to occur earlier and further analyze the related factors involved in this phenomenon. The anaerobic threshold is usually defined as "slightly lower than the power or oxygen consumption at which metabolic acidosis and related gas exchange changes occur" [11]. When the intensity of exercise exceeds a certain power (said to be the same point at which the production of lactic acid is significantly increased), "additional" carbon dioxide is produced, which is assumed to be due to bicarbonate (HCO<sub>3</sub>) buffering lactic acid [12]. This point of view has been called into guestion in recent years. More recently, several authors found that sodium bicarbonate is not the principal buffer in skeletal muscle and that carbonate-buffered lactic acid does not produce "excess" carbon dioxide; hyperventilation causes an increase in carbon dioxide discharge, not vice versa [13-15]. Therefore, the mechanism of VAT remains unclear. Recently, Sales et al proposed that peripheral stimuli and autonomic adjustments during exercise are physiological factors affecting anaerobic threshold [16]. It may also be related to the role of the prefrontal cortex (PFC), as advocated by Vasconcelos et al [17]. It is widely accepted that less "healthy" patients enter anaerobic metabolism earlier during exercise testing. In this study, although WR, VE, and HR at VAT were significantly reduced while wearing a mask, the decrease in VO,@VAT was not significant, indicating that VO,@VAT decreased to some extent but was interfered with by other variables (Table 2). This was potentially due to increased oxygen consumption by respiratory muscles in response to the increased respiratory resistance caused by wearing a mask.

To explore the possible mechanism of VAT advanced occurrence, we designed a subgroup analysis based on whether the anaerobic threshold occurred in advance. Since the value of δWR@VAT represents the time of early emergence of anaerobic threshold, scatter plots of  $\delta WR@VAT$  and  $\delta VO2@VAT$  were plotted. There was a positive linear correlation between  $\delta WR@$ VAT and  $\delta VO_0$  VAT. According to the characteristics of scattered point aggregation, we finally selected the X-axis position of  $\delta WR@VAT=-6 W$  as the dividing line after trying multiple segmentation points and divided the participants into the VS group and the VA group. Subgroup analysis showed that there were no significant differences between the 2 groups in all indicators of the exercise test without wearing masks. In the exercise test with a mask, except for WR and VO<sub>2</sub>, which were significantly different due to the grouping method, only VE showed significant differences compared with the exercise test without a mask, which suggests that VE might be the most important factor in whether VAT occurs early or not. Logistic regression analysis showed that  $\delta VE$ ,  $\delta BF$ , and  $\delta VE/VCO_2$  were independent factors affecting whether VAT occurred earlier, suggesting that higher  $\delta VE$  decreases the risk of VAT occurring in advance, but higher  $\delta BF$  and  $\delta VE/VCO_2$ , increase the risk of VAT occurring earlier. Since  $\delta$  is equal to the value of a masked index minus the value of the unmasked index, it indicates that higher VE, lower BF, and lower VE/VCO, reduce the risk of VAT occurring earlier in masked exercise tests. Thus, deep and slow breathing patterns might better cope with movement while wearing a mask. When this deep and slow breathing pattern with a mask succeeded in increasing the ventilation rate by 1 L/min (which was evident in some subjects), the risk of VAT earlier occurring was decreased by about 80%. In addition, slower respiration rates also contribute to adequate blood gas exchange and reduce respiratory work and oxygen consumption by respiratory muscles. If the adjustment of the breathing pattern does not significantly increase VE, whether VAT occurs earlier also depends on changes in ventilatory effectiveness indicators (VE/VCO<sub>2</sub>). During incremental exercise trials, VE/VCO, usually continues to decline until the point of respiratory compensation, which implies a decrease in the physiological dead space fraction. However, in exercise trials using masks, repeated inhalation of the remaining exhaled air in the mask increases dead cavities, and these 2 forces battle each other to influence whether VAT occurs in advance and how much time in advance it occurs.

#### Limitations

Although the tightness test of the mask was assessed in a calm state, we could not guarantee the absence of gas leakage during the exercise test. Due to many missing values, systolic blood pressure data could not be provided, which is important because it may be closely related to the risk of wearing masks. However, we did detect a decrease in systolic blood pressure in

many subjects, whether at peak or VAT. When portable finger pulse oximetry was added,  $SpO_2$  did not significantly decrease during the exercise test while wearing a mask, and in some cases, it even increased. Since  $SpO_2$  values cannot be recorded synchronously in real-time, we could not provide  $SpO_2$  values at each observation time point. Blinding was not possible for the participant nor any study personnel in the same room as the participant. Finally, no body composition tests were performed, and follow-up studies will be conducted.

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#### Conclusions

Our data show that wearing surgical masks causes ventilatory anaerobic threshold to occur earlier in some healthy young subjects during cardiopulmonary exercise test. Whether VAT occurs in advance is related to the changes in VE, BF, and VE/VCO<sub>2</sub>.

#### **Declaration of Figures' Authenticity**

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or part.

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