

## Evaluation of relationship between aerobic fitness level and range of isocapnic buffering periods during incremental exercise test

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**Abstract:** The purpose of this study is to examine the relationship between the amount of O<sub>2</sub> uptake (VO<sub>2</sub>) in the range of isocapnic buffering (ICB) periods and aerobic fitness levels of subjects with different exercise tolerance levels. A total of 50 young male subjects (20.8±0.4 years) performed an incremental exercise test using a cycle ergometer to determine their anaerobic threshold (AT), respiratory compensation point (RCP) and maximal exercise capacity (Wmax). The ICB period is defined as the region between AT and RCP. Pulmonary gas exchange parameters were measured breath-by-breath using a respiratory gas analyser. The subjects' fitness levels, as indicated by peak O<sub>2</sub> uptake to body weight ratios (VO<sub>2peak</sub>/BW), ranged from 28 ml/min/kg to 58 ml/min/kg, and Wmax capacity to body weight ratio (Wmax/BW) ranged from 1.94 W/min/kg to 3.96 W/min/kg. The VO<sub>2</sub> in the range of ICB periods ranged from 101 ml to 793 ml (with an average of 295±157 ml). There was a positive linear correlation between VO<sub>2peak</sub>/BW, Wmax/BW and range of ICB: R=0.76542 (p<0.0001), and R=0.92135 (p<0.0001), respectively. The results of this study suggest that the range of ICB periods may be related to aerobic fitness. Importantly, aerobic fitness levels should be evaluated and considered important data, in addition to AT, VO<sub>2peak</sub>/BW and Wmax/BW.

**Key words:** Exercise; Isocapnic buffering period; Anaerobic threshold; Aerobic fitness; Respiratory compensation point.

### Introduction

Cardiopulmonary exercise testing has been increasingly used for evaluating the causes of exercise intolerance in subjects with different aerobic fitness levels (1, 2). The concept of optimal exercise intensity is important in the fields of exercise and clinical science (3). Incremental exercise testing is a widely used method for challenging the mechanisms of pulmonary gas exchange and detecting physiological abnormalities (4). During incremental exercise tests, arterial blood lactate concentration is sensitive to changes in exercise intensity and increases only when a specific work rate is reached; this point is called the anaerobic threshold (AT) (3).

It is generally accepted that during an incremental exercise test, minute ventilation (V<sub>E</sub>), CO<sub>2</sub> output (VCO<sub>2</sub>) and O<sub>2</sub> uptake (VO<sub>2</sub>) increase linearly with increasing work rate until the onset of metabolic acidosis, i.e. the AT (3). Above the AT, the increase of blood lactate concentration is accompanied by an almost equal decrease in bicarbonate concentration (5), releasing additional non-metabolic CO<sub>2</sub>. Thus V<sub>E</sub> increases out of proportion to VO<sub>2</sub>, leading to an increase in the ventilatory equivalent for VO<sub>2</sub> (V<sub>E</sub>/VO<sub>2</sub>), and also in end-tidal O<sub>2</sub> partial pressure (P<sub>ET</sub>O<sub>2</sub>). However, for a short period, V<sub>E</sub>/VCO<sub>2</sub> and P<sub>ET</sub>CO<sub>2</sub> become relatively constant due to the close relationship between V<sub>E</sub> and VCO<sub>2</sub>. With further increases in work rate above a specific level, a steeper increase in V<sub>E</sub> against VCO<sub>2</sub> occurs, causing an increase in V<sub>E</sub>/VCO<sub>2</sub> and a decrease in P<sub>ET</sub>CO<sub>2</sub> (3, 6). The onset of hy-

perventilation during incremental exercise is called the respiratory compensation point (RCP), which marks the point at which the loss of linearity between V<sub>E</sub> and VCO<sub>2</sub> begins. During incremental exercise, the region from AT to RCP, where P<sub>ET</sub>CO<sub>2</sub> becomes constant, has been termed an isocapnic buffering (ICB) period (3, 7).

The concept of an anaerobic threshold has been used for the last half century for sedentary, trained and patient groups, to assess cardiopulmonary and metabolic fitness status in order to determine appropriate exercise intensity (3, 8). The amount of VO<sub>2</sub> present during the ICB period may indicate the substantiality of metabolic systems. The range of ICB periods may be related to the magnitude of the stimulation of a carotid body (7). The short period of ICB may vary among subjects, and reflects the status of the body's general buffering capacity toward exercise-induced metabolic acidosis, especially in increased lactate accumulation (3, 9, 10). The onset of hyperventilation might reflect the furthest point at which the body's metabolic systems attempt to compensate for metabolic acidosis. There is no elucidative investigation that evaluates the correlation between VO<sub>2</sub> in the range of ICB periods, and the fitness status of the subjects.

Despite the many studies concerning AT, peak O<sub>2</sub> uptake (VO<sub>2peak</sub>) and aerobic fitness (1-3, 8), few studies have been performed on the ICB period and its relation to aerobic fitness (11,12). The purpose of this study is to examine the possible relationship between the range of ICB periods and the level of aerobic fitness in male

subjects with different exercise tolerance levels.

## Materials and Methods

### Subjects

A total of 50 males were subjects of this study; the mean values ( $\pm$ SD) of their age, height and weight were  $21.2 \pm 2.7$  years,  $177.8 \pm 7.9$  cm and  $73.2 \pm 9.8$  kg, respectively.

The study protocol was approved by the Ethic Committee of Firat University. Before the subjects participated in the study, the experimental procedures, benefits and risks of the study were fully explained to them. Written informed consent was obtained from all subjects.

The inclusion criteria for participants in this study were that the subjects were young healthy males (fitness levels ranging from sedentary to high), that their body mass indexes ranged from  $18.5 \text{ kg/m}^2$  to  $25.0 \text{ kg/m}^2$ , that they were free of any acute or chronic disease (cardiac, renal, liver or metabolic), and refrained from drugs, smoking or alcohol. A previous physical examination, including electrocardiographic, echocardiographic, hormonal and biochemical evaluations, conducted within the months prior, ensured that no participant had a health problem.

The subjects underwent a training session in order to familiarise themselves with the equipment before the test. During the test, subjects adopted the upright cycling posture. Tests were performed under similar envi-

ronmental conditions ( $21\text{--}22^\circ\text{C}$ ).

The subjects' fitness levels ranged from sedentary to undergoing regular training. The peak  $\text{O}_2$  uptake to body weight ratio ( $\text{VO}_{2\text{peak}}/\text{BW}$ : from  $28 \text{ ml/min/kg}$  to  $58 \text{ ml/min/kg}$ ) (3) and maximal work production capacity to body weight ratio ( $\text{Wmax}/\text{BW}$ : from  $1.94 \text{ W/min/kg}$  to  $3.96 \text{ W/min/kg}$ ) differed markedly among the subjects, due to varying training conditions and physical fitness statuses (13).

### Incremental exercise test

The subjects performed an incremental exercise test on an electromagnetically braked cycle ergometer (VI-Asprint 150P). Before the test, the subjects were carefully monitored for hyperventilation, which can cause a pseudo-threshold phenomenon (14). The exercise test consisted of three phases. Initially, subjects pedalled for 4 minutes at a power of 20 W (at 60 rpm) as a warm-up. The work rate was subsequently increased by 15 W/minute (5 W/20 seconds) with a work rate controller, until the subjects could no longer maintain the work rate. Finally, subjects cycled for a further 4 minutes at 20 W for recovery (15).

Throughout the test, subjects wore 12-lead heart rate monitors so that electrocardiograms and ST segment deviation could be monitored. During the incremental exercise test, ventilatory and pulmonary gas exchange responses were measured breath-by-breath using a metabolic and respiratory gas analyser system (Master Screen CPX, Germany). Ventilation was measured by using a precise, bidirectional, digital volume sensor (Triple V volume sensor). Before the each test, a volume and gas calibration of the system were performed.

### Estimation of AT and RCP

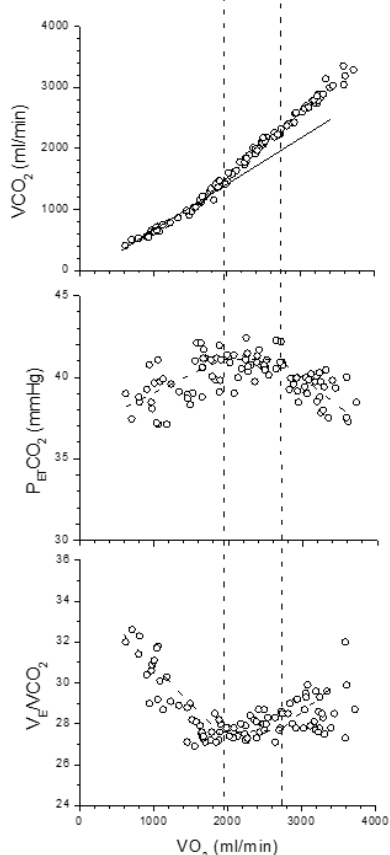
AT was estimated using the criteria of a systematic increase in  $\text{VCO}_2$  as a function of  $\text{VO}_2$  (i.e. the V-slope method) (Figure 1) (16). Other ventilatory and pulmonary gas exchange criteria, including increases of  $\text{P}_{\text{ET}}\text{O}_2$  and  $\text{V}_E/\text{VO}_2$  with no increase in  $\text{V}_E/\text{VCO}_2$  and with no decrease in  $\text{P}_{\text{ET}}\text{CO}_2$ , were also used to confirm the AT estimation (3, 4). The RCP was estimated as the point at which  $\text{V}_E/\text{VCO}_2$  began to increase and  $\text{P}_{\text{ET}}\text{CO}_2$  began to decrease. The range of ICB was defined as the  $\text{VO}_2$  between AT and RCP (Figure 1) (3, 5).

### Statistics

Data were expressed using mean and standard deviation ( $\pm$ SD). Statistical analyses of physiological data were performed by the determination of the Pearson correlation coefficient ( $r$ ) and linear regression analysis. For all tests, findings were considered significant when  $P < 0.05$ .

### Results

The  $\text{VO}_2$  was found to be  $1.79 \pm 0.22 \text{ L/min}$  at the AT ( $1.34 \text{ L/min}$  minimum and  $2.26 \text{ L/min}$  maximum),  $2.08 \pm 0.33 \text{ L/min}$  at the RCP ( $1.57 \text{ L/min}$  minimum and  $2.80 \text{ L/min}$  maximum) and  $2.72 \pm 0.41 \text{ L/min}$  at the end of the test (i.e.  $\text{VO}_{2\text{peak}}$ ) ( $2.10 \text{ L/min}$  minimum and  $3.69 \text{ L/min}$  maximum). The  $\text{VO}_{2\text{peak}}$  for each kilogram of body weight ranged from a minimum  $28.4 \text{ ml/min/kg}$  to maximum of  $57.8 \text{ ml/min/kg}$ , and averaged  $38.2 \pm 7.7$



**Figure 1.** Ventilatory and pulmonary gas exchange responses, as a function of  $\text{O}_2$  uptake, to an incremental exercise test performed to the limit of tolerance in a representative subject. The first dashed line indicates the estimated anaerobic threshold (AT) and the second dashed line indicates the respiratory compensation point (RCP).

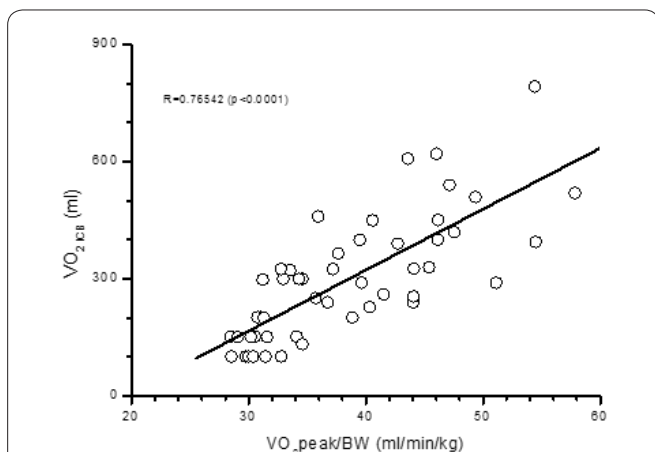
ml/min/kg. The mean ( $\pm$ SD) workload at the end of the exercise and at the AT were found to be  $200\pm 30$  W (150 W minimum and 265 W maximum) and  $130\pm 29$  W (90 W minimum and 210 W maximum), respectively.

The  $P_{ET}CO_2$  was constant between AT and RCP. The  $VO_2$  at the ICB periods varied widely among the subjects, with a minimum value of 101 ml to a maximum value of 793 ml. The mean ( $\pm$ SD)  $VO_2$  during the ICB period was  $295\pm 157$  ml. The  $P_{ET}CO_2$  decreased with an increase in work rate beyond the RCP.

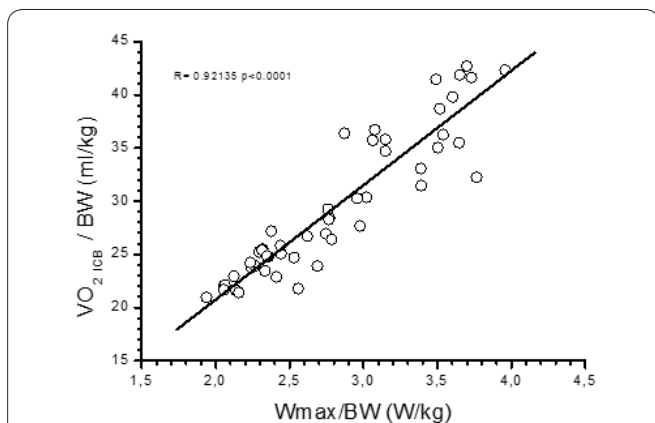
As illustrated in Figure 2, there was a positive linear correlation between increased aerobic fitness levels, as determined by the  $VO_{2peak}$  per kilogram of body weight, and the range of ICB ( $R=0.76542$ ,  $p<0.0001$ ) (Figure 2). In addition, there was a significant linear correlation between the maximal work rate production capacity to body weight ratio ( $W_{max}/BW$ ) and the range of ICB ( $R=0.92135$ ,  $p<0.0001$ ) (Figure 3).

## Discussion

This study shows that the amount of  $VO_2$  during the ICB period is increased and that RCP is delayed in subjects with higher aerobic fitness levels, as determined  $VO_{2peak}/BW$  and  $W_{max}/BW$ . We also found that aerobic fitness capacity was positively correlated with an



**Figure 2.** The relationship between aerobic fitness, as indicated by the peak  $O_2$  uptake to body weight ratio, and  $O_2$  uptake, in the range of the isocapnic buffering (ICB) periods in individual subjects. The solid line indicates the regression equation parameters.



**Figure 3.** The relationship between aerobic fitness, as indicated with maximal exercise capacity to body weight ratio, and  $O_2$  uptake, in the range of isocapnic buffering (ICB) periods in individual subjects. The solid line indicates the regression equation parameters.

increased  $O_2$  uptake across the range of ICB periods.

Incremental exercise testing is becoming more widespread among researchers (3, 15). The fundamental matching mechanisms between  $V_E$ ,  $VO_2$  and  $VCO_2$  during different stages of incremental exercise testing have not yet been well documented. Generally, the rapid increase in  $V_E$  above AT has been attributed to the stimulation of peripheral chemoreceptors by an increase in lactate concentration in exercising muscles (3, 17). However, the major issue is the ICB region, which reflects a constant phase of  $P_{ET}CO_2$  beyond the AT. This is the result of a close relationship between  $V_E$  and  $VCO_2$ , despite increased metabolic acid production. The main reason for delayed hyperventilation in response to developing metabolic acidosis above the AT, has not been clarified. Exercise-induced lactic acidosis (3, 5) and stimulus from the exercising muscle (18) are involved in hyperventilation, which starts at RCP.

The observation of the ICB period during incremental exercise testing may provide useful information on the validity of the non-invasive estimation of AT through ventilatory and pulmonary gas exchange parameters (19, 20). Research shows that exercise training based on workloads associated with AT can help reduce the risk of several diseases and health conditions, and improve overall quality of life (3, 6). It has been shown that the ICB period can be used as an effective training tool (21). It has also been shown that training causes an improvement of the ICB period in older subjects (i.e. enlargement of ICB period) (22). The  $VO_2$  during the ICB period varies widely among subjects. However, an increase in the rate of power output, which may have an effect on the ICB period, was not considered in this study because of the similar power output increase applied by all subjects (23). The sensitivity of carotid bodies to exercise-induced metabolic acidosis may play an important role in the length of an ICB period (24, 25). A longer period of ICB may relate to low sensitivity to acute metabolic acidosis in carotid bodies (26). The result of one study performed at high altitude, where ventilation increased above metabolic demands, showed the disappearance of the isocapnic buffering period (27). In addition, the ICB period did not appear during an incremental exercise test with breathing 50% of  $O_2$  (28).

The suggestion has also been made that the range of ICB is closely related to the aerobic fitness level of the subjects (11, 12). It has been shown that subjects with high aerobic fitness have a longer ICB period than subjects with a low level of aerobic fitness (11). In clinical medicine, the ICB period could be an indicator of patients with impaired cardiopulmonary capacity (29). In the present study, we used subjects with a wide range of aerobic fitness levels and observed significantly positive correlation between fitness and the ICB period (Figure 2, 3). The bicarbonate buffering capacity may have important effects on exercise-induced metabolic acidosis and the ICB period (30). More specifically, high buffering capacity may cause diminishes lactate to increase.

During an incremental exercise test performed under acute hypoxia, many factors are involved, including a change in metabolic system, substrate utilisation, enzyme activity and the body's buffering capacity, which may also have an effect on the balance between  $V_E$  and metabolic requirement (31-33). The observation

of a significant positive correlation between aerobic fitness levels and range of ICB periods in heterogeneous subjects raises the suggestion that ICB range depends on carotid body stimulation. Discounting extreme conditions such as acute hypoxia or acute hyperoxia, the subjects who had high levels of aerobic fitness revealed a longer period of ICB when compared to subjects with relatively low aerobic fitness.

As a conclusion, the range of ICB periods is an important criterion for the determination of aerobic fitness levels.

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