Factors Influencing Excess CO₂ Output During and After Short Duration-Intensive Exercise: Focusing on Skeletal Muscle Characteristics

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The purpose of this study was to investigate the relationship between excess CO₂ output during and after short duration-intensive exercise and skeletal muscle characteristics (i.e., muscle fiber type, muscle capillary density and muscle buffering capacity). Twelve healthy males (age: 22.4±2.9 years, height: 172.3±5.8 cm, weight: 65.0±4.8 kg) performed 30-s maximal cycle ergometer sprinting. Excess CO₂ output during and after exercise was obtained through respiratory gas analysis. Excess CO₂ output per unit of time (Excess VCO₂) was calculated by subtracting VO₂ from VCO₂ during and after exercise. Muscle biopsy samples were taken from the right vastus lateralis muscle, and then muscle fiber type, muscle capillary density and muscle buffering capacity were measured. Excess CO₂ calculated as the sum of Excess VCO₂ from the onset of exercise until 15 min after exercise was not significantly correlated with any muscle fiber types, while it was significantly correlated with capillary-to-fiber ratio (r = 0.791, p < 0.01). A significant negative correlation was also demonstrated between Excess CO₂-to-La peak ratio and muscle buffering capacity (r = -0.645, p < 0.05). These results suggest that Excess CO₂ during and after short duration-intensive exercise is affected by the amount of H⁺ buffered by nonbicarbonate system and the amount of H⁺ diffusion from muscle to blood depending on the development of muscle capillaries.

Keywords: bicarbonate buffering action, nonbicarbonate buffering action, fiber-type distribution, capillary density, lactic acid

1. Introduction

High lactic acid concentration is observed in muscle and blood during short duration-intensive exercise. Lactic acid produced in muscle is dissociated into lactate ions (La⁻) and an equal amount of hydrogen ions (H⁺) within the physiological pH range. The pH decrease associated with accumulation of lactic acid is known to cause muscular fatigue through several mechanisms such as the reduction of glycolytic enzyme activity [Trivedi and Danforth, (1966)], and inhibition of the excitation-contraction coupling [Sutton et al., (1981)].

The body has an ability to restrain the decrease of intracellular pH, which is known as a buffering action. In vivo, buffering action can be classified into two categories, namely the intracellular and extracellular buffering actions. The intracellular buffering action is called the nonbicarbonate buffering system consisting of physicochemical and metabolic buffering actions, while the extracellular buffering action is called the bicarbonate buffering system. The actions of the latter system are mainly dependent on the bicarbonate ion (HCO₃⁻) in the blood.

Recently, several studies have been conducted...
to assess this bicarbonate buffering system through quantifying excess CO\(_2\) output during exercise [Yano et al., (1984); Ito et al., (2001); Yunoki et al., (1999); Maemura et al., (2003)]. Maemura et al. (2003) observed that the process of the ExcessCO\(_2\) accumulation was fitted the process of the [HCO\(_3^{-}\)] decrease during and after intensive exercise (exercise duration: 40-s), suggesting that ExcessCO\(_2\) could reflect the amount of CO\(_2\) produced through the bicarbonate buffering system. In addition, Yunoki et al. (2000) have reported that excess CO\(_2\) output caused by lactic acid buffering (1\(^{st}\)CO\(_2\)excess) during and after intensive exercise (exercise duration: 60-s) is smaller in sprinters than in long distance runners. As the reason, Yunoki et al. (2000) pointed out that skeletal muscle characteristics of each subject might be related. Thus, it is speculated that long-distance runners with smaller muscle buffering capacity [Parkhouse and McKenzie, (1984); Parkhouse et al., (1985)] and greater capillarily density [Andersen, (1975); Brodal et al., (1977)] caused an enhancement of H\(^{+}\) diffusion from muscle to blood compared to sprinters. Based on these results, it seems that the buffering efficiency of bicarbonate buffering system could be influenced by nonbicarbonate buffer value and H\(^{+}\) diffusion from muscle to blood. However, no study directly examining the relationships of these factors has yet been conducted.

Therefore, the purpose of this study was to examine the relationship of ExcessCO\(_2\) and skeletal muscle characteristics, especially muscle capillarization and muscle buffering capacity.

2. Methods

2.1. Subjects

Twelve healthy male subjects (age: 22.4±2.9 years, height: 172.3±5.8cm, weight: 65.0±4.8kg) participated in this study. The purpose, method and risk of this study were explained to all the participants and each participant’s written informed consent was obtained prior to the experiment. This study was approved by the Ethics Committee for Human Experiments of the Research and development center of Nippon Meat Packers Corporation.

2.2. Exercise test

An electronic-braked cycle ergometer (TKK 1254a, Takei Scientific Instruments Co., Ltd., Tokyo), which allows an arbitrary selection of torque and output voltage responding to rotation frequency, was used in the experiments. Each subject performed 30-s maximal cycle ergometer sprinting at a load (kp) corresponding to 7.5% body mass. Power was recorded every 1-s on the personal computer, and thereafter the peak power and mean power/body mass were calculated. Subjects were instructed to adequately practice before the experiments in order to be accustomed to cycle ergometer sprinting. They warmed up by performing 5-min cycle ergometer sprinting at a load of 1 kp, followed by 3-s maximal cycle ergometer sprinting performed twice with 5-min of rest period between exercise. The experiment started about 10-min after the warm-up period ended.

2.3. Measured items and measuring methods

2.3.1. ExcessCO\(_2\) and lactic acid concentration in the blood

CO\(_2\) output (\(\dot{VCO}_2\)) and O\(_2\) uptake (\(\dot{VO}_2\)) were measured using breath-by-breath with an automatic respiratory gas analyzer (OXYCON-ALPHA, Jaeger Mijnhardt) during and after 30-s maximal cycle ergometer sprinting. In this method, gas samples gathered from a facemask were sent to a gas analyzer through the twin tube sample line. The gas analyzer was calibrated by using N\(_2\)-CO\(_2\) standard gas mixture (O\(_2\):0%, CO\(_2\):4.394%). In addition, correction of gas transfer duration through the twin tube and calibration of the flowmeter by using a quantitative syringe were conducted.

Based on obtained data of respiratory gas samples, excess CO\(_2\) output per unit time (Excess \(\dot{VCO}_2\)) during and after exercise and the total amount of excess CO\(_2\) output (ExcessCO\(_2\)) from the start of exercise until 15-min after exercise were calculated using the following mathematical formulas [Maemura et al.,
Factors influencing excess CO$_2$ output


131

(2003)]. (Figure 1).

\[
\text{Excess} \dot{V}_{\text{CO}_2} = \dot{V}_{\text{CO}_2} - \dot{V}_{\text{O}_2}
\]

\[
\text{Excess} \text{CO}_2 = \Sigma \text{Excess} \dot{V}_{\text{CO}_2}
\]

In the present study, ExcessCO$_2$ was calculated from the start of exercise until 15-min after exercise. The sampling duration was determined based on the observation of previous study [Yunoki et al., (1999); Maemura et al., (2003)], in which CO$_2$ is not completely excessively expired during exercise but after the end of exercise, although lactic acid is continuously produced from the beginning of the exercise.

Lactic acid concentration in the blood was measured through an automatic lactate analyzer (1500sport, YSI) immediately after collecting blood samples from fingertips at 1, 3, 5 and 7-min after exercise. In the present study, blood samples were collected until 7-min after exercise, because no greater difference was observed between the value at 7-min after exercise and that of thereafter under the present exercise intensity in the pilot study.

2.3.2. Histochemical analysis

A specimen of the vastus lateralis skeletal muscle of each subject at rest was obtained by using the needle biopsy technique [Bergström, (1962)] 6-7 days after the 30-s maximal cycle ergometer sprinting. The muscle samples were taken from the right vastus lateralis. Parts of these samples were mounted on a specimen holder in O. C. T. compound and frozen in isopentane previously cooled to a viscous fluid with liquid nitrogen. The other parts were immediately frozen in liquid nitrogen. All samples were stored at -80°C until assayed.

The samples frozen in isopentane were used for a histochemical analysis. Transverse sections (10μm) were cut from each muscle using a cryostat, and the sections were mounted on cover glass. Myosin adenosine triphosphatase (ATP ase) staining was performed after preincubation at pH 10.3 following the techniques described by Gollnick et al. (1983).

Muscle fibers were identified as Type I and Type II fibers on the basis of the myosin ATP ase [Brook and Kaiser, (1970); Pereira et al., (1997)]. A composite photomontage of each ATP ase preparation was made using micrographs, and each fiber was then identified and counted with a hand counter. A muscle cross-sectional area was estimated with a 3-CCD video camera (KY-F55B, Victor, Kanagawa). The video camera was attached to a microscope (Nikon, Tokyo). The software used for storing and analyzing the microscopic images were Image Grabber PCI and NIH image 1.57, respectively.

The section was fixed with PBS buffer containing 4% formaldehyde for 4-min at room temperature, and then myosin ATP ase (preincubation at pH10.3) was performed. The stained section were photographed, and the capillary density and capillary-to-fiber ratio were determined [Suwa et al., (2003)].

The measurement of buffering capacity was performed by adding 1 ml of homogenate solution (145 mmol/L KCl, 10 mmol/L NaCl, 5 mmol/L iodoacetic acid) to the 3-10mg dry wt muscle sample which was then allowed to homogenate for about 1 minute on ice. The sample was then placed in a water bath at 37°C for 5-min. After initial pH was measured, the pH was set at 7.1. Then by titrating 0.01 mmol/L HCl every 10μl, H$^+$ concentration necessary to decrease the pH from 7.1 to 6.5 was determined by using interpolation [Weston et al., (1997)].

2.4. Statistical analysis

All data are expressed as means±SD. Peason’s product moment correlation analysis was used to calculate...
correlation between the two variables. The statistically significant level was set at $p < 0.05$.

3. Results

The peak power/BW and mean power/BW during the 30-s maximal cycle ergometer sprinting were 11.8±1.1 W/kg and 8.4±0.8 W/kg, respectively.

During the 30-s maximal cycle sprinting, La peak was 11.7±0.4 mmol/L, ExcessCO$_2$ was 89.6±11.8 ml/kg, and ExcessCO$_2$-to-La peak ratio was 6.4±8.9 ml/kg/mmol/L (Table 1).

Muscle fiber composition (% fiber type) was 49.1±11.3 % for Type I and 50.9±11.3% for Type II. Muscle fiber area ratio (% fiber area) was 48.2±11.6 % for Type I and 51.8±11.6 % for Type II. Capillary density per unit area was 4174±79 capillaries/ mm$^2$, and capillary-to-fiber ratio was 1.57±0.21 capillaries/fibers. In contrast, the buffering capacity ($\beta_{\text{titr}}$) was 131.0±10.2 μmol/g dry muscle (Table 2).

Table 3 shows the correlation coefficients of ExcessCO$_2$ with % fiber type, % fiber area, capillary density, capillary-to-fiber ratio and $\beta_{\text{titr}}$. ExcessCO$_2$ did not show a significant correlation with % fiber type and % fiber area. Although ExcessCO$_2$ did not show a significant correlation with capillary density, a significant positive correlation was observed between ExcessCO$_2$ and capillary-to-fiber ratio ($r=0.762, p < 0.01$; Figure 2).
Factors influencing excess CO$_2$ output

Also, a significant negative correlation was observed between $\beta_{\text{titr}}$ and ExcessCO$_2$ ($r$=-0.585, $p<0.05$). Figure 3 shows the correlation between $\beta_{\text{titr}}$ and ExcessCO$_2$ -to-La peak ratio. A significant negative correlation was observed between these variables ($r$=-0.645, $p<0.05$).

4. Discussion

The main findings of the present study are that excessCO$_2$ output (ExcessCO$_2$) during and after short duration-intensive exercise were significantly correlated with buffering capacity ($\beta_{\text{titr}}$) and capillary-to-fiber ratio. These results suggest that ExcessCO$_2$ is affected by the amount of H$^+$ buffered by nonbicarbonate system and the amount of H$^+$ diffusion from muscle to blood depending on the development of muscle capillaries.

The body has an ability to restrain the decrease of pH and to maintain the homeostasis of pH, which is known as a buffering action. These actions are primarily accomplished by buffer substance such as muscle protein, dipeptide (carnosine, anserine) and phosphate, and HCO$_3^-$ in the blood (bicarbonate buffering system). In general, the buffering capacity ($\beta$) is shown as the amount of strong acid or strong base necessary to change one unit of pH, and two methods for determining buffering capacity have been used. The first method measures pH change by titrating with acid ($\beta_{\text{titr}}$). The second method measures the decreasing rate of muscle lactic acid and muscle pH from resting state to immediately after exercise ($\beta_{\Delta\text{La}/\Delta\text{pH}}$). The physicochemical contribution of muscle protein, dipeptide (carnosine, anserine) and phosphate can be assessed with $\beta_{\text{titr}}$. In addition, $\beta_{\text{titr}}$ would reflect noncarbonate buffering action, since transmembrane transport of H$^+$ and HCO$_3^-$ (i.e.,La$^-$/H$^+$ transport, Na$^+$/H$^+$ exchange) is not reflected [Malin and Harris., (1991)]. In contrast, it is reported that $\beta_{\Delta\text{La}/\Delta\text{pH}}$ shows higher values than the buffer capacity using $\beta_{\text{titr}}$, because it involves H$^+$, La$^-$ and HCO$_3^-$ defluxion [Manion et al., (1993)]. The present study used $\beta_{\text{titr}}$ to determine the buffering capacity. Thus, $\beta_{\text{titr}}$ is considered to be valid for evaluating of the contribution of both bicarbonate and nonbicarbonate buffering systems responded to lactic acid generated during intensive exercise.

Lactic acid generated in muscle during intensive exercise completely dissociates into La$^-$ and H$^+$. H$^+$ dissociated from lactic acid will immediately be buffered by nonbicarbonate system with pK close to intermuscular pH, subsequently buffered by bicarbonate system [Beaver et al., (1986)]. Suzuki et al. (2000) have reported that increase in muscle volume following resistance training, which means enhancement of nonbicarbonate buffering contribution, caused decrease of bicarbonate buffering contribution. This indicates the possibility that the buffering efficiency in bicarbonate buffering system could be influenced by the buffer value in the nonbicarbonate buffering system. Therefore, this study examined the relationship between $\beta_{\text{titr}}$ which is the indicator of nonbicarbonate buffering system and ExcessCO$_2$ which is the indicator of bicarbonate buffering system. The results showed that a significant negative correlation was observed between them (Table 3). This study also examined the ExcessCO$_2$-to-La peak ratio, since ExcessCO$_2$ is directly influenced by blood lactate. As a result, $\beta_{\text{titr}}$ showed a significant negative correlation with ExcessCO$_2$-to-La peak ratio (Figure 3). These results suggest that the greater contribution of the nonbicarbonate buffering system, the more H$^+$

Figure 3  Relationship between $\beta_{\text{titr}}$ and ExcessCO$_2$-to-La peak ratio
buffering will be conducted in muscle cells and less H\(^+\) will diffuse into the blood. Conversely, the less contribution of the nonbicarbonate buffering system, the less H\(^+\) buffering will be conducted in muscle cells and more H\(^+\) will diffuse into the blood. Concerning this issue, Yunoki et al. (2000) have reported that excess CO\(_2\) output caused by lactic acid buffering (1\(^{st}\)CO\(_2\)excess) is lower in sprinters when compared to long-distance runners. As the reason, Yunoki et al. (2000) pointed out the possibility that the contribution of bicarbonate buffering system in blood would decrease as a result of the more H\(^+\) buffering in muscle cells and the less H\(^+\) diffusion into the blood because sprinters are superior to buffering actions caused by buffering substance including muscle protein, dipeptide (carnosine, anserine) and phosphate compared to long-distance runners. The present results seem to confirm these suggestions, and this is the first study to examine the relationship between the bicarbonate and the nonbicarbonate buffering systems.

In addition, Yunoki et al. (2000) suggested that capillarization might be responsible for the differences in 1\(^{st}\)CO\(_2\)excess among subjects. Capillary density is generally high in long-distance runner who perform endurance training everyday [Andersen, (1975); Brodal et al., (1977)] and their H\(^+\) diffusion from working muscles into the blood is rapid [Yano, (1987); Hirakoba et al., (1990); Hirakoba et al., (1992); Inaki et al., (1993); Suzuki et al., (2000)]. Also, since bicarbonate buffering system generates CO\(_2\) in reaction of H\(^+\) + HCO\(_3\)^\(-\) + H\(_2\)O + CO\(_2\), the buffering efficiency in bicarbonate buffering would reduce unless CO\(_2\) is emitted efficiently. In fact, Hultman and Sahlin (1980) have reported that CO\(_2\) removal from the muscle tissue plays crucial roles in the bicarbonate system because the buffering capacity decreases under the condition in which CO\(_2\) removal is limited (closed system). Therefore, Differences in capillarization would influence CO\(_2\) output from muscles, thereby affects the bicarbonate buffering efficiency. This hypothesis is supported by the present results that significant positive correlation was observed between ExcessCO\(_2\) which is a bicarbonate buffering indicator and the capillary-to-fiber ratio which reflects the magnitude of capillarization. However, the relationship between ExcessCO\(_2\) and capillary density per unit cross-section area, which similarly reflects the magnitude of capillarization, tended to show a positive correlation but was not statistically significant. Mathieu Costello (1994) pointed out the possibility that capillary density could not properly assess the number of capillary, since this is influenced by the size of muscle fiber. Therefore, capillary density can not be considered as an appropriate indicator to reflect the magnitude of capillarization which performs various exchanges of substances including CO\(_2\) and metabolites.

In the present study, we also examined the relationship of excessCO\(_2\) with % fiber type and % fiber area. Since blood lactate directly influences ExcessCO\(_2\) [Yunoki et al., (2000)], the characteristics of muscle fiber relative to lactic acid production and oxidation would affect ExcessCO\(_2\). It has been shown that Type II fiber with high in contractile rate and anaerobic capacity relates to lactic acid production, whereas Type I fiber with low in contractile rate and high in aerobic capacity relates to lactic acid oxidation [Gleeson, (1996)]. Therefore, we hypothesized that ExcessCO\(_2\) could closely relate to both Type I and Type II fiber. However our hypothesis was not supported because the present study did not show significant correlations between ExcessCO\(_2\) and both Type I and Type II fibers. This suggests that muscle fiber characteristics relative to lactic acid production and oxidation would not clearly affect production of ExcessCO\(_2\).

In conclusion, the present study observed that excessCO\(_2\) output (ExcessCO\(_2\)) during and after short duration-intensive exercise were significantly correlated with buffering capacity (\(\beta_{tite}\)) and capillary-to-fiber ratio. These results suggest that ExcessCO\(_2\) is affected by the amount of H\(^+\) buffered by nonbicarbonate system and the amount of H\(^+\) diffusion from muscle to blood depending on the development of muscle capillaries.

References
Factors influencing excess CO₂ output


