Impeding O₂ unloading in muscle delays oxygen uptake response to exercise onset in humans

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Hayashi, Naoyuki, Mutsuhisa Ishihara, Ayumu Tanaka, and Takayoshi Yoshida. Impeding O₂ unloading in muscle delays oxygen uptake response to exercise onset in humans. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1274–R1281, 1999.—We tested whether the leftward shift of the oxygen dissociation curve of hemoglobin with hyperpnea delays the oxygen uptake (V̇O₂) response to the onset of exercise. Six male subjects performed cycle ergometer exercise at a work rate corresponding to 80% of the ventilatory threshold (VT) V̇O₂ of each individual after 3 min of 20-W cycling under eupnea [control (Con) trial]. A hyperpnea procedure (minute ventilation = 60 l/min) was undertaken for 2 min before and during 80% VT exercise in hypocapnia (Hypo) and normocapnia (Normo) trials. In the Normo trial, the inspired CO₂ fraction was 3% to prevent hypocapnia. The subjects completed two repetitions of each trial. To determine the kinetic variables of V̇O₂ and heart rate (HR) at the onset of exercise, a nonlinear least-squares fitting was applied to the data averaged from two repetitions by a monoexponential model. The end-tidal CO₂ partial pressure before the onset of exercise was significantly lower in the Hypo trial than in the Con and Normo trials (22 ± 1 vs. 38 ± 3 and 36 ± 1 mmHg, respectively, P < 0.05). The time constant of V̇O₂ and HR was significantly longer in the Normo trial (28 ± 7 and 34 ± 16 s, respectively) than in the Con trial (21 ± 7, 34 ± 16 s, respectively, P < 0.05). The V̇O₂ time constant of the Hypo trial (37 ± 12 s) was significantly longer than that of the Normo trial, although no significant difference in the HR time constant was seen (Hypo, 41 ± 28 s). These findings suggested that respiratory alkalosis delayed the kinetics of oxygen diffusion in active muscle as a result of the leftward shift of the oxygen dissociation curve of hemoglobin. This supports an important role for hemoglobin-O₂ diffusing in setting the V̇O₂ kinetics at exercise onset.


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METHODS

Six healthy males (23.5 ± 2.4 yr, 173.3 ± 5.2 cm, 66.7 ± 5.4 kg, means ± SD) participated in this study. All subjects received an explanation of the study and gave informed consent before participation.

Each subject performed a 20 W/min incremental ramp-exercise test on an electromagnetically braked cycle ergometer (model 232-C, Combi, Japan), to determine the ventilatory threshold (VT) with gas-exchange criteria and maximal

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IT HAS BEEN PROPOSED THAT the oxygen uptake (V̇O₂) response to the onset of exercise reflects the regulation of oxygen transport to the tissue (10, 15) and oxygen utilization in muscle tissue (2, 8, 24). Grassi et al. (6) recently reported that peripheral O₂ diffusion does not limit the muscle V̇O₂ response to exercise onset in isolated canine muscle. They used hyperoxia and intraarterial administration of RSA-13, which induces a rightward shift of the oxygen dissociation curve of Hb, to increase the O₂ diffusion in the muscle tissue. The result of their study clearly indicated that O₂ diffusion is not a limiting factor in situ dog muscle preparation, but their study did not examine whether O₂ diffusion regulates the V̇O₂ response. Even if we apply their data to humans, it is unclear whether impaired O₂ diffusion impairs the V̇O₂ response. To resolve these issues, it is necessary to determine whether impeding the O₂ diffusion delays the V̇O₂ response at the onset of exercise.

Hyperpnea increases the CO₂ output from the lungs. This excess CO₂ output allows the end-tidal CO₂ partial pressure (PETCO₂) to fall and decreases the arterial CO₂ tension (PA CO₂), which leads to an increase in the arterial pH. Therefore, hyperpnea brings about hypocapnia and consequent respiratory alkalosis (21, 23). This should induce a leftward shift of the oxygen dissociation curve of hemoglobin (Hb), impeding the unloading of O₂ in a working muscle.

We hypothesized that voluntary hyperpnea slows down the V̇O₂ kinetics at the onset of square-wave exercise by a leftward shift of the oxygen dissociation curve of Hb. To test the role of O₂ unloading in muscle capillaries in the V̇O₂ response, we investigated the effect of respiratory alkalosis induced by voluntary hyperpnea on the V̇O₂ response at the onset of exercise. Our preliminary study revealed that hyperpnea slowed the V̇O₂ response to the exercise onset (n = 8, P < 0.05; unpublished data). However, although the V̇O₂ kinetics are decelerated by the hyperpnea, it is impossible to discriminate between the effects of intrathoracic pressure swing and additional work of respiratory muscle produced by ventilation and that of a leftward shift of the oxygen dissociation curve of Hb. To discriminate between the effects of additional ventilation and changes in PETCO₂, we established two comparisons: 1) normocapnia and hypocapnia induced with hyperpnea to test the effect of O₂ unloading in muscle tissue, and 2) normocapnia with and without hyperpnea to test the effect of additional ventilation. Additionally, the effects of hypocapnia, which may induce the Bohr effect due to increased PA CO₂, were investigated.

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oxygen uptake (V\textsubscript{O\textsubscript{2max}}). The VT was determined as the V\textsubscript{O\textsubscript{2}} at which the nonlinear increase of carbon dioxide output (V\textsubscript{CO\textsubscript{2}}) and minute expiration (V\textsubscript{E}) plotted against V\textsubscript{O\textsubscript{2}} was observed. The V\textsubscript{O\textsubscript{2max}} and VT of the subjects were 3.5 ± 0.2 l/min and 2.0 ± 0.2 l/min (means ± SD), respectively.

The subjects performed square-wave exercise protocols on the cycle ergometer. Each protocol consisted of an abrupt work increase for a 6-min period at a work rate corresponding to 80% of the VT of each individual (129 ± 16 W) after an initial 3-min period of 20-W cycling. The subjects kept a constant pedaling frequency of 60 rpm during the cycling. The respiratory rate (FR) was maintained at 30 breaths/min throughout each trial. Four types of trials were conducted. In the control (Con) trial, the tidal volume was not controlled, but the FR was controlled with the inhalation of normal room air (F\textsubscript{ICO\textsubscript{2}}, 0.03%). In the hypocapnia (Hypo) trial, the subjects controlled their V\textsubscript{E} at 60–70 l/min, i.e., hyperpnea, for 2 min before and during 80% VT exercise with room air (F\textsubscript{ICO\textsubscript{2}}, 0.03%). In the normocapnia (Normo) trial, the subjects controlled their V\textsubscript{E} at 60–70 l/min, i.e., hyperpnea, for 2 min before and during 80% VT exercise with a high-fraction CO\textsubscript{2} gas (F\textsubscript{ICO\textsubscript{2}}, 3.00%) to prevent the fall of PET\textsubscript{CO\textsubscript{2}}. In the Hyper trial, PET\textsubscript{CO\textsubscript{2}} was significantly higher than in the other trials due to the CO\textsubscript{2} gas in the Normo trial. In the Hypo trial, PET\textsubscript{CO\textsubscript{2}} fell markedly at the start of hyperpnea and reached a steady state before the work increase and then slightly increased after the work increase.

The mean of steadystate V\textsubscript{O\textsubscript{2}}, V\textsubscript{CO\textsubscript{2}}, V\textsubscript{E}, HR, and PET\textsubscript{CO\textsubscript{2}} rose to new steady-state levels after the onset of hyperpnea. The PET\textsubscript{CO\textsubscript{2}} fell markedly at the start of hyperpnea and reached a steady state before the work increase and then slightly increased after the work increase.

RESULTS

Representative responses of gas exchange variables and HR in the Con trial are shown in Fig. 1A. V\textsubscript{O\textsubscript{2}}, V\textsubscript{CO\textsubscript{2}}, V\textsubscript{E}, HR, and PET\textsubscript{CO\textsubscript{2}} rose to new steady-state levels within 3 min after the work increase. The FR was maintained at 30 breaths/min during the trial. The mean of the steady-state (last 1 min of exercise) V\textsubscript{E} during exercise was 52.2 ± 4 l/min.

Figure 1B shows the time course of the individual cardiorespiratory variables in the Hypo trial in the subject shown in Fig. 1A. The V\textsubscript{E} was maintained at 30 breaths/min during the trial, and V\textsubscript{E} was controlled at ~70 l/min throughout the exercise. V\textsubscript{O\textsubscript{2}} approached the previous baseline levels after the abrupt transient increase at the onset of hyperpnea and thereafter slowly increased to the steady-state level after the exercise increase. V\textsubscript{CO\textsubscript{2}} and HR rose to new steady-state levels after the onset of hyperventilation and then increased after the work increase. The PET\textsubscript{CO\textsubscript{2}} fell markedly at the start of hyperpnea and reached a steady state before the work increase and then slightly increased after the work increase.

The averaged responses of all subjects for V\textsubscript{E}, PET\textsubscript{CO\textsubscript{2}}, HR, and V\textsubscript{O\textsubscript{2}} in these trials are shown in Fig. 3. The time courses were generally similar to the individual examples presented in Figs. 1 and 2. The V\textsubscript{E} during exercise was 65.6 ± 7.4 l/min. The effect of the CO\textsubscript{2} addition to the inspiratory gas was enough to keep the PET\textsubscript{CO\textsubscript{2}} higher (i.e., hypercapnia).

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other trials due to the CO2 added to the inspiratory gas. The VO2 kinetics were clearly delayed in the Hypo trial. Table 1 summarizes the data for the kinetics variables of VO2 and HR. The hyperpnea and/or CO2 addition to the inspiratory gas did not affect the baseline and gain of VO2. The hyperpnea significantly increased the baseline of HR and significantly decreased the gain of HR, and, consequently, there was no significant difference in the steady-state values of HR.

The ANOVA revealed a significant effect of trial on the MRTs of VO2. The MRT of VO2 was significantly longer in the Hypo trial than in the Con, Normo, and Hyper trials (P < 0.05) according to the post hoc comparison. In addition, the time constant was significantly longer in the Hypo trial (37.1 ± 11.5 s) than in the Con (21.0 ± 6.5 s), Normo (28.1 ± 7.2 s), and Hyper (23.8 ± 6.3 s) trials. The MRT and time constant were significantly longer in the Normo trial than in the Con trial. The MRT and time constant in the Hyper trial were not significantly different from those in the Con and Normo trials.

The MRT of HR was significantly longer in the Normo and Hyper trials than in the Con trial. There was no significant difference in this variable among the Normo, Hypo, and Hyper trials.

Figure 4 shows the individual values of the MRT of VO2 and HR. All subjects but one had a longer MRT of VO2 and HR. The main findings in this study were that 1) the hyperpnea procedure significantly slowed the HR and
V\text{\textsuperscript{o}}2 kinetics at the onset of the work increase, 2) the drop in PETCO\text{\textsubscript{2}} induced an additional slowing of the V\text{\textsuperscript{o}}2 response but not of the HR response to the onset of the work increase, and 3) the increase in PETCO\text{\textsubscript{2}} did not significantly change the V\text{\textsuperscript{o}}2 kinetics at the onset of the work increase. These findings suggest that hypocapnia decelerates V\text{\textsuperscript{o}}2 kinetics at the onset of exercise by a leftward shift of the dissociation curve of Hb.

Effect of Hyperpnea Manipulation on Cardiorespiratory Responses to Onset of Exercise (Normo vs. Con)

A significant difference in the MRT of the V\text{\textsuperscript{o}}2 response between the Con and Hypo trials was revealed. This difference is attributed to the effects of intrathoracic pressure swing and respiratory muscle work by an additional lung movement and the effect of the drop in PETCO\text{\textsubscript{2}} in the Hypo trial. By comparing the Normo and Con trials, we can examine the effect of an additional lung movement on V\text{\textsuperscript{o}}2 response using the hyperpnea without hypocapnia. The Normo procedure delayed the HR and V\text{\textsuperscript{o}}2 responses compared with the Con trial. This shows that hyperpnea itself delays the V\text{\textsuperscript{o}}2 response.

It is possible that the delay is due to delay of the central and/or peripheral circulatory response. There is evidence that circulation regulates the V\text{\textsuperscript{o}}2 response (10, 15). However, evidence against a role for the central circulatory response in the regulation of the V\text{\textsuperscript{o}}2 response has been obtained in healthy and heart transplant subjects (7, 9). Grassi et al. (7) reported that changes in the cardiac output response induced by repeated exercise did not affect the V\text{\textsuperscript{o}}2 response to the work increase in heart transplant patients. Haya-ishi et al. (9) suggested that V\text{\textsuperscript{o}}2 responses are regulated by local blood distribution rather than by the central circulatory response during the disorder of vagal withdrawal response to the onset of exercise with facial cooling in healthy subjects. Furthermore, Shoemaker et al. (20) and Hughson et al. (11) suggested that an inadequate blood flow delayed the V\text{\textsuperscript{o}}2 kinetics. The vigorous movement of respiratory muscle needs V\text{\textsuperscript{o}}2,
and normopnea trials for baseline and steady state in the present results did not clearly confirm this.

distribution rather than central circulation, although the Con trial were due to an inadequate local blood flow

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Trial</th>
<th>Baseline</th>
<th>Amplitude</th>
<th>Steady State</th>
<th>MRT, s</th>
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<tr>
<td></td>
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<tr>
<td>VO₂ Con</td>
<td>696 ± 33</td>
<td>1.076 ± 177</td>
<td>1.772 ± 204</td>
<td>32.0 ± 6.0a</td>
<td></td>
</tr>
<tr>
<td>Hypo</td>
<td>668 ± 45</td>
<td>1.097 ± 221</td>
<td>1.765 ± 256</td>
<td>43.7 ± 8.0b</td>
<td></td>
</tr>
<tr>
<td>Normo</td>
<td>698 ± 26</td>
<td>1.087 ± 185</td>
<td>1.785 ± 210</td>
<td>37.2 ± 5.8c</td>
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</tr>
<tr>
<td>Hyper</td>
<td>678 ± 45</td>
<td>1.092 ± 181</td>
<td>1.770 ± 199</td>
<td>34.1 ± 5.6d</td>
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<tr>
<td>HR Con</td>
<td>82.0 ± 7.6a</td>
<td>39.3 ± 8.0d</td>
<td>121.3 ± 13.0</td>
<td>34.1 ± 16.1b</td>
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<tr>
<td>Hypo</td>
<td>92.6 ± 11.9c</td>
<td>26.3 ± 8.8e</td>
<td>118.9 ± 13.8</td>
<td>41.4 ± 27.9c</td>
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<tr>
<td>Normo</td>
<td>86.8 ± 8.5b</td>
<td>35.7 ± 7.9d</td>
<td>122.4 ± 10.8</td>
<td>39.1 ± 18.0c</td>
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<tr>
<td>Hyper</td>
<td>80.5 ± 7.2c</td>
<td>42.5 ± 8.7c</td>
<td>123.0 ± 12.6</td>
<td>39.6 ± 19.3c</td>
<td></td>
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Values are means ± SD for 6 subjects (VO₂ in ml/min; HR, beats/min). MRT, mean response time; VO₂, oxygen uptake; HR, heart rate; Con, control; Hypo, hypocapnia; Normo, normocapnia; Hyper, hypercapnia. Amplitude is steady-state increase from 20-W pedaling. Means with the same letter are not significantly different.

Effect of Hypocapnia on Cardiorespiratory Responses to Onset of Exercise (Hypo vs. Normo)

It is important to understand that the comparison of the Normo and Hypo trials made the effect of hyperpnea itself the same in both trials. This makes it possible to rule out the effect of the hyperpnea. The drop in PETCO₂ induced an additional slowing of the VO₂ response, whereas the hyperpnea procedure itself slows the VO₂ response (Normo vs. Con).

The hyperpnea brought about an increase of VCO₂ and a drop in PETCO₂ in the Hypo trial compared with the Normo trial (Figs. 1B and 3). This drop in PETCO₂ confirms a decrease of the PaCO₂, which leads to an increase in arterial pH. Therefore, the hyperpnea induced hypocapnia and respiratory alkalosis (21, 23). PaCO₂ has been estimated with good accuracy by using the Jones equation (12). The averaged values of PaCO₂ calculated from PETCO₂ for 30 s before the work increase by the Jones equation were 39.8 ± 3.3 (Con trial), 25.1 ± 1.2 (Hypocapnia), and 38.1 ± 1.1 mmHg (Normo trial). In the Hypo trial, 25 mmHg of PaCO₂ corresponds to 7.57 of pH and in the Normo trial, 38 mmHg corresponds to 7.41 of pH according to the acid-base chart for arterial blood (21). In the Hypo trial, the subjects performed the exercise under the hypocapnia and alkalosis condition.

The capacity to release oxygen from the Hb at working muscle tissue is determined by the oxygen dissociation curve of Hb and the tissue PO₂; that is, the capillary-to-tissue PO₂ difference under the condition wherein PaO₂ is the same. In the present study, the PO₂ in working muscle can be assumed to not be different between the trials, supposing the same amount of O₂ was used at the same workload. The oxygen dissociation curve of Hb was influenced by PaCO₂, arterial pH, and temperature. The hyperpnea could not influence the muscle temperature. If one supposes that the PaO₂ in arterial blood was the same among the trials and

Fig. 3. Averaged responses of VE, PETCO₂, HR, and VO₂ for all subjects (n = 6). Solid vertical line indicates 80% VT exercise onset. Error bars represent SD displayed each 15 s.

and, consequently, blood flows to these muscles (1). These results support the speculation that the slower VO₂ kinetics in the present Normo trial compared with the Con trial were due to an inadequate local blood flow distribution rather than central circulation, although the present results did not clearly confirm this.

There is no significant difference between hyperpnea and normopnea trials for baseline and steady state in the VO₂. Previous studies (1, 17) reported higher VO₂ during hyperpnea manipulation than eupnea. The rate of increase in VO₂ on a 1-liter increase of ventilation ranged from 0.5 to 3 ml/l in terms of increasing ventilation (17). In the present study, hyperpnea manipulation increased VE by 30 l/min during 20-W cycling and 10 l/min during 80% VT cycling. The increase of VO₂ was estimated to be 90 ml/min during 20-W cycling and 30 ml/min during 80% VT cycling, when the highest value was applied to the estimation. With strict control of breathing, Aaron et al. (1) measured the oxygen cost of hyperpnea. With the use of their regression, the work of ventilation is calculated to be 47 J/min at 60 l/min of VE. This 47 J/min is 3 W, which costs ~45 ml/min of VO₂ if the efficiency of respiratory muscle is 20%. From these previous studies, oxygen cost for 60 l/min of VE is estimated to be much less than 50 ml/min. So it is not strange that such a small difference was not detectable.

Effect of Hypocapnia on Cardiorespiratory Responses to Onset of Exercise (Hypo vs. Normo)

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Fig. 3. Averaged responses of VE, PETCO₂, HR, and VO₂ for all subjects (n = 6). Solid vertical line indicates 80% VT exercise onset. Error bars represent SD displayed each 15 s.
that the mean capillary Po2 was 30 mmHg (16), the difference in the saturation between arterial and capillary blood clearly decreases in the Hypo trial. The effects of hypocapnia and respiratory alkalosis shifted the oxygen dissociation curve of Hb leftward and consequently impaired the diffusion gradient for O2 between the capillary blood and the exercising muscles. It is known that the O2 half-saturation pressure of Hb (P50) under standard conditions (37°C, pH 7.4) is 26.6 mmHg (18) and, in the relationship D log P50/D pH, 0.48 for a ΔpH of 0.1 units is the standard value. According to these values, the P50 is estimated to be 27.7 mmHg in the Normo and 20.4 mmHg in the Hypo trial. We propose that the oxygen diffusion in muscle tissue thus became impaired, which resulted in the decelerated kinetics of V˙O2 at the onset of exercise. We, therefore, suggest that the oxygen diffusion in muscle tissue is an important component of the V˙O2 response to the exercise onset.

Koike et al. (13) reported slowing of V˙O2 kinetics on inhalation of low concentrations of carbon monoxide. Their study included the effect of a leftward shift of the oxygen dissociation curve and decreased blood O2 content. It was impossible to discriminate between the effect of Hb content and the dissociation curve of Hb in the previous study. Hypo manipulation in the present study did not include the effect of the content of Hb that is able to bind O2.

In a modeling study, Cochrane and Hughson (3) reported that the balance between O2 transport and utilization is very delicate in V˙O2 kinetics. Their model included the Bohr effect on the oxygen dissociation curve of Hb. However, they did not investigate the effect of changes in PAO2 and pH on the shift in the oxygen dissociation curve of Hb or on V˙O2 kinetics.

Grassi et al. (6) recently concluded that the enhancement of peripheral O2 diffusion did not affect the muscle V˙O2 response at the work onset in isolated canine muscle. They also suggested that a faster O2 delivery does not affect the V˙O2 response in isolated muscle (5). Their series of studies in isolated muscle suggested metabolic control of the V˙O2 response. This does not contradict our results. Their results showed that O2 diffusion is not a rate-limiting factor under control conditions. Whereas the present results showed that the decreased peripheral O2 diffusion from Hb did slow the V˙O2 response to the work increase, this did not imply that the O2 diffusion is a rate-limiting factor. It must be noted that the present results merely showed the role of O2 diffusion as a regulator to maintain the O2 uptake response. This means that the O2 uptake slows when this regulator does not work properly.

Shiojiri et al. (19) reported that the poor extraction of O2 and reduced muscle blood flow in exercising muscle at reduced muscle temperatures contributed to the delayed adjustment of V˙O2. Under the reduced muscle temperature condition, the O2 extraction was reduced by the temperature-dependent leftward shift of the oxygen dissociation curve of Hb. The present hypothesis that oxygen diffusion in the muscle tissue plays a major role in V˙O2 kinetics is partly supported by their findings. However, the muscle blood flow was also altered by cold-induced vasoconstriction. The effect of the blood flow distribution in exercising muscle concomitant with a previous manipulation contributes to the adjustment of V˙O2 at the onset of exercise. Therefore, there is less of a positive basis for a role for oxygen diffusion in the muscle tissue in the V˙O2 response. In the present study, the V˙O2 kinetics at the onset of exercise were slowed under the Hypo condition, although the HR kinetics were similar to those observed under the Normo condition. In addition, there is less possibility that the blood flow distribution induced the difference in V˙O2 kinetics between the Hypo and Normo trials.
Ward et al. (22) reported that $\dot{V}e$ and $\dot{V}CO_2$ dynamics were slowed considerably after volitional hyperpnea and that the HR dynamics were unaffected, whereas the $VO_2$ dynamics were slowed only slightly. This result is inconsistent with our present findings. We observed that HR kinetics were not affected by hyperpnea, similar to their findings. However, the $VO_2$ kinetics were significantly slowed by hyperpnea. This difference is due to differences in experimental design. In the study by Ward et al., hyperpnea was induced only before exercise, and there was a brief interval between the cessation of hyperpnea and exercise onset. In contrast, hyperpnea was induced throughout the exercise in our study. The hyperpnea during exercise kept the $PETCO_2$ low for a long time, as shown in Fig. 3. Hypocapnia occurred throughout the exercise. The long duration of hypocapnia contributed to the slowed $VO_2$ kinetics at the onset of exercise.

Limitations. This hypocapnia procedure clearly revealed the effect of $O_2$ diffusion in muscle tissue on the VO$_2$ response. However, this study has some limitations. First, there might be an effect from some metabolic processes that hypocapnia and changes in pH might alter.

Second, hypocapnia would affect the vascular resistance. Kontos et al. (14) measured arterial and venous pressure during hypocapnia with and without increased ventilation to calculate the vascular resistance. They reported that the decreased PaCO$_2$ could increase vascular resistance. Even if vascular resistance had been increased by respiratory alkalosis, the blood flow distribution might not occur in a specific part of the body, because respiratory alkalosis affects the whole body. However, we cannot completely rule out the possibility that the ability to vasodilate appropriately was impaired by the hypocapnia.

Effect of Hypercapnia on Cardiorespiratory Responses to Onset of Exercise (Hyper vs. Normo)

We observed that the $PETCO_2$ was significantly higher in the Hyper trial than the other trials by adding CO$_2$ to the inspiratory gas (Figs. 2B and 3). This increase in $PETCO_2$ represents an increase in PaCO$_2$, i.e., hypercapnia, which leads to a decline in arterial pH. The averaged value of PaCO$_2$ for 30 s before the work increase estimated by the Jones equation was 43.5 ± 1.8 mmHg in the Hyper trial. According to the acid-base chart (21), the pH would be expected to decrease slightly (pH 7.38). This hypercapnia shifted the oxygen dissociation curve of Hb slightly toward the right (Bohr effect). The $P_50$ can be estimated as 28.2 mmHg. Thus the slight difference in pH from the standard value of 7.40 results in little change in the SaO$_2$. The Bohr effect induced by the Hyper improves the extraction of O$_2$ from capillary blood in the exercising muscle tissue. At the same time, the Ve was significantly increased by the CO$_2$ addition to the inspiratory gas (Fig. 3). This spontaneous increase in ventilation might slow the circulatory response at the onset of exercise, as discussed in Effect of Hyperpnea Manipulation on Cardiorespiratory Responses to Onset of Exercise (Normo vs. Con). It is possible that the slowed circulatory kinetics cancel out the effect of facilitated oxygen utilization caused by the Bohr effect.

Grassi et al. (6) reported that increased O$_2$ diffusion by the Bohr effect did not affect the VO$_2$ response in isolated in situ canine muscle. When this increased O$_2$ diffusion is applied to human subjects, though they stated that the application of the study should be limited to muscles with a high aerobic potential, it is plausible that the Bohr effect induced by hypercapnia did not affect the VO$_2$ response.

In addition, the changes of PaCO$_2$ and pH induced by the procedure were smaller in the Hyper trial than the Hypo trial. This would be due to the stronger regulatory system for hypercapnia compared with that for hypocapnia, because the increase in PaCO$_2$ can be regulated downward easily by the increase in Ve. The increase of PaCO$_2$ in the present Hyper trial might not be great enough to change the oxygen dissociation curve for speeding VO$_2$ kinetics. It thus is still unclear whether O$_2$ diffusion increased by the Bohr effect with hypercapnia affects the VO$_2$ response in humans.

In summary, the VO$_2$ kinetics were significantly decelerated by hypocapnia, although the HR kinetics did not change significantly. This finding confirmed that the leftward shift of the oxygen dissociation curve of Hb caused by hypocapnia leads to the slowed kinetics of oxygen diffusion in active muscle, resulting in the decelerated VO$_2$ kinetics at the onset of exercise. These results suggest that the O$_2$ diffusion in muscle tissue is important for regulating the VO$_2$ response to exercise onset.

Perspectives

The present findings confirmed the role of an O$_2$ diffusion mechanism in VO$_2$ kinetics regulation. There has not been a convincing argument for O$_2$ diffusion as a “rate-limiting” step of VO$_2$ kinetics. We did not observe accelerated VO$_2$ kinetics with hypercapnia with a small change in PaCO$_2$. When one examines the possibility of O$_2$ diffusion as a rate-limiting step for the VO$_2$ response, it is essential to show the acceleration of VO$_2$ kinetics with the facilitation of O$_2$ diffusion. It might be that the Bohr effect accelerates the VO$_2$ response to high-intensity exercise in human subjects, as Gerbino et al. suggested (4).


