



Effect of Hypercapnic Severity on Plasma Ammonia Accumulation and Respiratory Exchange Ratio during Incremental Exercise

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Abstract

We tested the effect of hypercapnic severity on plasma ammonia (Am) accumulation and respiratory exchange ratio (RER) during incremental exercise. Eight male subjects performed incremental cycle exercise to exhaustion under three conditions: breathing (1) Air, (2) 3% CO₂ or (3) 6% CO₂. Am in both hypercapnic conditions was lower than Air at 100% $\dot{V}_{O_{2max}}$ ($P<0.05$) without changing Am threshold (AmT). Am for 6% CO₂ was lower than 3% CO₂ immediately after exercise ($P<0.05$). RER in both hypercapnic conditions was lower than Air throughout the experiment ($P<0.05$). RER for 6% CO₂ was lower than 3% CO₂ at rest and 30% $\dot{V}_{O_{2max}}$ ($P<0.05$). RER for 3% CO₂ at 30% $\dot{V}_{O_{2max}}$ was 0.74 without increasing arterialized blood partial pressure of CO₂, which was near 0.7 (100% fat metabolism). AmT was unchanged by hypercapnic severity and suppression of AM accumulation was observed above AmT. Lowered RER during inhalation of 3% CO₂ and 30% $\dot{V}_{O_{2max}}$ exercise occurred under isocapnic conditions, implying a change in metabolism. 3% CO₂ and 30% $\dot{V}_{O_{2max}}$ was found to be the most effective combination for promoting fat metabolism. We demonstrate that Am accumulation and RER decrease with increasing hypercapnia.

Keywords

Hypercapnia, Ammonia, Respiratory exchange ratio, CO₂ storage, Exercise

Introduction

Blood lactate (La) and ammonia (Am) concentrations are widely accepted markers of metabolism not only in clinical studies but also during exercise. Elevations from normal resting levels

of both are observed in exercise and associated with deleterious effects such as fatigue. A decrease in blood La concentration during exercise under hypercapnic conditions was previously reported [1-4], and La threshold (LT) was unchanged by hypercapnia [4]. Further, we reported that plasma Am concentration was reduced at maximal exercise in a 6% inhaled CO₂ condition [5]. However, the changes in Am dynamics during exercise, including Am threshold (AmT), remain unknown since blood samples were drawn following exercise. Interestingly, a previous study of exercise under hypercapnic conditions of 2, 4, and 6% CO₂ demonstrated that blood La concentration during steady state exercise was the lowest at CO₂ concentrations of 6% in inspired gas [2]. Thus, it is hypothesized that Am accumulation during exercise may be decreased depending on the severity of hypercapnia. Whether this is true has never been previously explored experimentally.

Also, hypercapnic gas inhalation causes a change in respiratory exchange ratio (RER). Previous studies reported a lowered RER during exercise under hypercapnia [2,6]. Substrate utilization during exercise can be estimated from the RER value and a low RER is expected to promote fat metabolism. Thus, a lowering of RER by hypercapnic gas inhalation may promote fat metabolism during exercise. Concerning the role of exercise training intensity on substrate utilization, fat metabolism is best promoted at exercise intensity less than 45% maximal oxygen uptake ($\dot{V}_{O_{2max}}$) [7]. However, the previous reports [2,6] used 45% $\dot{V}_{O_{2max}}$ or greater intensity, so CO₂ inhalation and exercise intensity under 45% $\dot{V}_{O_{2max}}$ for reduction of RER is untried. Moreover, the changes in RER are usually complicated by changes in body carbon dioxide (CO₂) stores tied to changes in

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partial pressure of arterial CO₂ (P_{aCO_2}). A decrease in RER could be due to a transient increase in the amount of CO₂ stored in tissue due to an increased P_{aCO_2} . Arterial CO₂ concentration is approximately linearly proportional to P_{aCO_2} and, when multiplied by cardiac output, leads to a predicted linearly proportional decrease in expired CO₂ flux due to this increase in CO₂ storage. Therefore, to apply a hypercapnic gas inhalation and show a change in fat metabolism, it is required to consider CO₂ storage. An unchanging P_{aCO_2} during RER measurement is the primary requirement.

The aims of the present study were to investigate: (1) the hypothesis that plasma Am concentration would decrease with increasing CO₂ concentration in inhaled gas and (2) the effect of hypercapnic severity and relative exercise intensity on RER and CO₂ storage. To examine these aims, we compared the effects of incremental exhaustive exercise in normocapnic, mild hypercapnic, and severe hypercapnic conditions in human subjects.

Methods

Subjects

Eight healthy, active males with no history of cardiorespiratory diseases volunteered to participate in the present study. Age, height, and weight (mean ± SD) were 23.4 ± 1.8 years, 171.9 ± 6.6cm, and 64.0 ± 6.8kg, respectively. Informed consent was obtained from each subject after a full explanation of the experimental procedure as well as its risks was provided. The experimental protocol was approved by the Human Subjects Committee at the Chukyo University Graduate School of Health Sciences.

Experimental protocol

Each subject performed an incremental exhaustive exercise on three occasions in a climatic chamber with fan at a temperature of 26°C, relative humidity of 50%, and normal barometric pressure, under the following conditions: (1) breathing ambient air (Air), (2) breathing mild hypercapnic gas (21% O₂, 3% CO₂, N₂=balance) (3% CO₂), and (3) breathing severe hypercapnic gas (21% O₂, 6% CO₂, N₂=balance) (6% CO₂). Exercise tests were performed in random order. The interval between each exercise test was at least 1 week. On the day before the exercise test, subjects were advised to avoid strenuous exercise, alcohol, caffeine, smoking, and to fast after supper. Subjects breathed

the allocated gas mixture from 10 min before the start of exercise to 5 min after the end of exercise. The subjects were blinded to the inhaled gas composition (Figure 1). Exercise tests were conducted using an electrically-braked cycle ergometer (AEROBIKE75XL; Combi Wellness, Tokyo, Japan); the workload was set at 30 watts (W) at the beginning of the test and increased by 30 W every 2 min until exhaustion. Subjects were encouraged to maintain a pedaling rate of 60 rpm, with exhaustion being defined as the rate falling to less than 40 rpm.

Measurement of cardiorespiratory parameters

During the experiment, expired gas was sampled and oxygen uptake (\dot{V}_{O_2}), CO₂ output (\dot{V}_{CO_2}), RER = $\frac{\dot{V}_{CO_2}}{\dot{V}_{O_2}}$, expired ventilation (\dot{V}_E), and partial pressure of end tidal CO₂ (P_{ETCO_2}) were analyzed every 30 s using an automatic gas analyzer (RM300, MG360; Minato Medical Science, Osaka, Japan). Heart rate (f_H) was also analyzed every 30 s using a heart rate monitor (Life Scope B; Nihon Kohden, Tokyo, Japan). The automatic gas analyzer was calibrated before each test.

Arterialized venous blood analysis

In order to arterialize venous blood, each subject immersed one hand in warm water (40-43°C) for 30 min before gas inhalation, according to Forster et al.'s method [8]. A catheter (22G × 1 1/4") was inserted in a superficial forearm vein and arterialized blood samples were withdrawn through 3-way valves flushed with saline and heparin. Blood samples were collected before exercise, during the last minute of each intensity level, and immediately after exercise. To determine plasma Am levels, 1 ml blood samples were treated with a kit (Ammonia-Test Wako; Wako Company, Osaka, Japan), and the amount of indophenols produced was measured using a spectrophotometer (UV-2400PC; Shimadzu, Tokyo, Japan) at a wavelength of 630 nm. To measure plasma La concentrations, 1 ml blood samples were placed in chilled tubes containing EDTA-2Na and kept in ice water. Whole blood was separated by cold centrifugation (4°C, 1670 × g) and plasma La concentrations were measured enzymatically (2300 Yellow Springs Instrument Co., Yellow Springs, OH, USA). Each subject's AmT and LT were determined from two linear regression lines on the log [Am or La] against log [% $\dot{V}_{O_{2max}}$] plot based on log-log transformation [9]. Blood samples for determination of pH, HCO₃⁻, and blood partial pressure of CO₂ (P_{CO_2}) levels were drawn into heparinized syringes

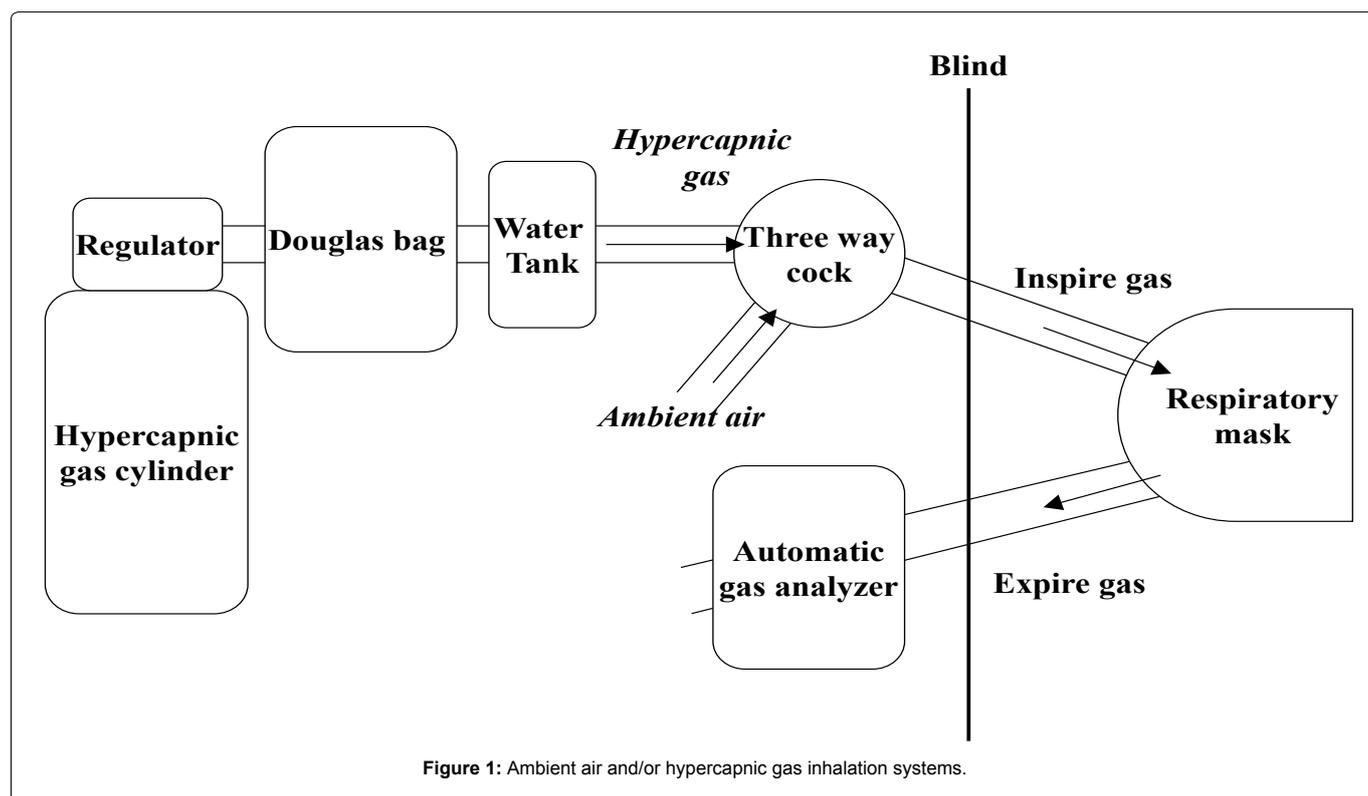


Figure 1: Ambient air and/or hypercapnic gas inhalation systems.

Table 1: Exercise parameters due to incremental exhaustive exercise at the three CO₂ levels.

	Air (n=8)	3% CO ₂ (n=8)	6% CO ₂ (n=8)
Performance time (min)	16'20" ± 1'27"	16'04" ± 1'41"	14'24" ± 1'37"*†
Maximal workload (W)	251.3 ± 15.5	247.5 ± 31.1	228.8 ± 27.5

Values are means ± SD. *P<0.05 vs Air. †P<0.05 vs 3% CO₂.

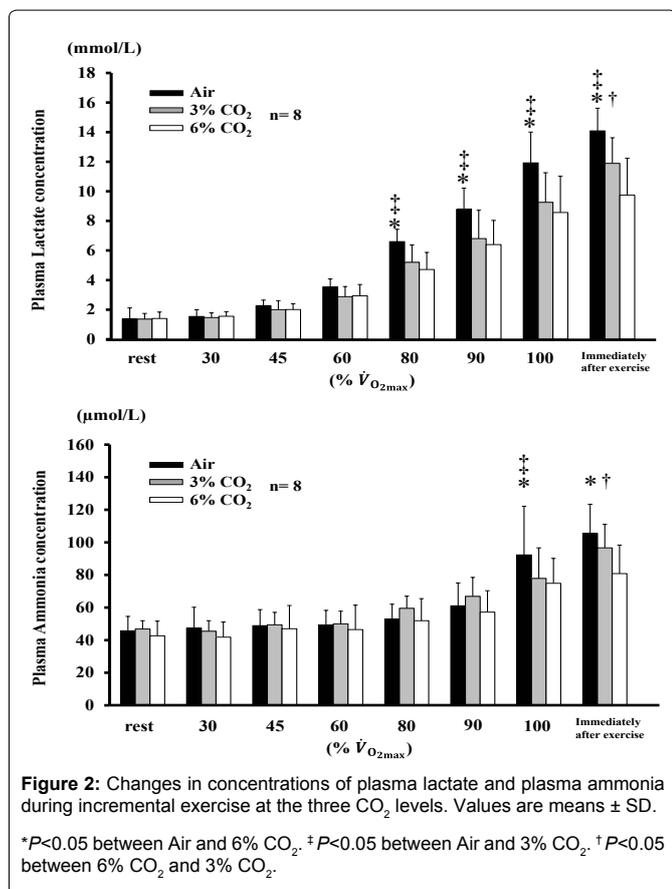


Figure 2: Changes in concentrations of plasma lactate and plasma ammonia during incremental exercise at the three CO₂ levels. Values are means ± SD.

*P<0.05 between Air and 6% CO₂. †P<0.05 between Air and 3% CO₂. ‡P<0.05 between 6% CO₂ and 3% CO₂.

Table 2: Ammonia threshold (AmT) and lactate threshold (LT) at the three CO₂ levels

	Air (n=8)	3% CO ₂ (n=8)	6% CO ₂ (n=8)
AmT (% $\dot{V}_{O_{2max}}$)	63.0 ± 8.6	61.3 ± 9.8	62.3 ± 5.2
LT (% $\dot{V}_{O_{2max}}$)	42.0 ± 8.1	43.8 ± 8.9	44.3 ± 7.1

Values are means ± SD.

and promptly measured using a blood gas analyzer (Rapid lab[™] 348; Bayer, Germany).

Statistics

Mean values with standard deviation (SD) are presented in tabular and graphical format. As maximal workload differed between the subjects, we unified the data by using as a relative intensity of evaluated pre-exercise: 30%, 45%, 60%, 80%, 90%, and 100% $\dot{V}_{O_{2max}}$. For statistical comparisons of changes in cardiorespiratory and blood gas parameters as well as plasma Am and La concentration during exercise, a two-way (gas × time) analysis of variance (ANOVA) with repeated measurements was applied. Differences in exercise performance parameters, AmT, and LT among the three tests were analyzed by one-way ANOVA. When a significant effect was found in ANOVA, Fisher's LSD post-hoc test was used to compare means. The statistical package (PASW statistics 18; SPSS, Tokyo, Japan) was used for statistical analysis. Probability values of P<0.05 were considered significant.

Results

Exercise performance

Parameters for exercise performance are summarized in table

Table 3: Changes in Oxygen uptake (\dot{V}_{O_2}), Carbon dioxide output (\dot{V}_{CO_2}), and Respiratory exchange ratio (RER) at rest and during incremental exercise at the three CO₂ levels

	Air (n=8)	3% CO ₂ (n=8)	6% CO ₂ (n=7)	
$\dot{V}_{O_{2max}}$ (ml/min/kg)	46.6 ± 4.0	45.7 ± 6.1	42.6 ± 4.7	
\dot{V}_{O_2} (ml/min)	Rest	271.8 ± 57.9	284.6 ± 23.0	348.6 ± 148.8
	30% $\dot{V}_{O_{2max}}$	879.8 ± 180.9	873.6 ± 215.6	861.1 ± 155.4
	45% $\dot{V}_{O_{2max}}$	1295.6 ± 301.8	1267.0 ± 317.9	1248.0 ± 182.1
	60% $\dot{V}_{O_{2max}}$	1687.8 ± 259.9	1741.0 ± 300.9	1605.6 ± 220.6
	80% $\dot{V}_{O_{2max}}$	2376.1 ± 405.8	2410.9 ± 434.6	2106.4 ± 373.6
	90% $\dot{V}_{O_{2max}}$	2714.9 ± 459.4	2723.4 ± 521.3	2423.7 ± 389.5
	Max	3002.4 ± 532.8	2940.5 ± 604.9	2719.0 ± 447.4
\dot{V}_{CO_2} (ml/min)	Rest	241.8 ± 44.4	218.9 ± 27.7	162.9 ± 37.6
	30% $\dot{V}_{O_{2max}}$	729.3 ± 169.0	646.8 ± 167.3	563.9 ± 164.4
	45% $\dot{V}_{O_{2max}}$	1233.1 ± 337.7	1052.4 ± 306.1	964.3 ± 175.9
	60% $\dot{V}_{O_{2max}}$	1733.0 ± 298.4	1586.0 ± 299.7	1346.7 ± 201.2
	80% $\dot{V}_{O_{2max}}$	2645.3 ± 491.6	2351.3 ± 404.6	1925.7 ± 408.9*†
	90% $\dot{V}_{O_{2max}}$	3101.8 ± 493.0	2707.9 ± 565.8*	2339.6 ± 436.6*
	Max	3589.6 ± 562.5	2973.3 ± 765.0*	2614.3 ± 629.2*
RER	Rest	0.90 ± 0.11	0.77 ± 0.09*	0.55 ± 0.16*†
	30% $\dot{V}_{O_{2max}}$	0.83 ± 0.07	0.74 ± 0.08*	0.65 ± 0.08*†
	45% $\dot{V}_{O_{2max}}$	0.94 ± 0.07	0.83 ± 0.07*	0.77 ± 0.05*
	60% $\dot{V}_{O_{2max}}$	1.03 ± 0.05	0.91 ± 0.05*	0.84 ± 0.05*
	80% $\dot{V}_{O_{2max}}$	1.11 ± 0.04	0.98 ± 0.07*	0.92 ± 0.10*
	90% $\dot{V}_{O_{2max}}$	1.15 ± 0.05	0.99 ± 0.08*	0.97 ± 0.08*
	Max	1.20 ± 0.05	1.01 ± 0.14*	0.95 ± 0.13*

Values are means ± SD. *P<0.05 vs Air. †P<0.05 vs 3% CO₂.

1. Performance time was lower for 6% CO₂ than Air and 3% CO₂ (P<0.05), while maximal workload was not significantly different among the three conditions.

Am and La

Changes in plasma Am and La concentrations are shown in figure 2. Am for 6% CO₂ was lower than Air at 100% $\dot{V}_{O_{2max}}$ and immediately after exercise (P<0.05), and was also lower for 6% CO₂ than 3% CO₂ immediately after exercise (P<0.05). Am for 3% CO₂ was lower than Air at 100% $\dot{V}_{O_{2max}}$ (P<0.05). La for 6% CO₂ and 3% CO₂ were lower than for Air from 80% $\dot{V}_{O_{2max}}$ (P<0.05), and was lower for 6% CO₂ than 3% CO₂ immediately after exercise (P<0.05). AmT and LT are shown in table 2. AmT and LT were not significantly different among the three conditions.

Cardiorespiratory parameters

Gas exchange data are summarized in table 3. For 6% CO₂, one subject had a higher \dot{V}_{O_2} and a lower \dot{V}_{CO_2} . Consequently, he showed unusually low estimates of RER (0.32-0.4) at 6% CO₂ only, which was considered outliers compared to mean estimates for the other subjects (0.52-0.89). Therefore, gas exchange responses for 6% CO₂ were reported for the remaining 7 subjects. \dot{V}_{O_2} levels were not significantly different among the three conditions. \dot{V}_{CO_2} was lower for 6% and 3% CO₂ than Air at strenuous exercise intensity (P<0.05). Moreover,

Table 4: Changes in Expired ventilation (\dot{V}_E) and Heart rate (f_H) at rest and during incremental exercise at the three CO₂ levels.

		Air (n=8)	3% CO ₂ (n=8)	6% CO ₂ (n=8)
\dot{V}_E (l/min)	Rest	8.8 ± 1.1	14.9 ± 0.8*	26.5 ± 4.3*†
	30% $\dot{V}_{O_{2max}}$	19.1 ± 3.0	29.9 ± 6.0*	45.8 ± 11.5*†
	45% $\dot{V}_{O_{2max}}$	30.6 ± 7.4	41.1 ± 10.0*	62.7 ± 13.2*†
	60% $\dot{V}_{O_{2max}}$	41.7 ± 6.7	58.0 ± 10.0*	75.9 ± 15.6*†
	80% $\dot{V}_{O_{2max}}$	68.6 ± 14.7	87.8 ± 13.1*	94.1 ± 21.1*
	90% $\dot{V}_{O_{2max}}$	85.7 ± 14.7	104.3 ± 17.4*	108.3 ± 22.8*
	Max	109.9 ± 22.3	125.5 ± 22.3*	118.8 ± 23.7
f_H (beats/min)	Rest	71.9 ± 9.2	75.6 ± 7.7	82.0 ± 10.9*
	30% $\dot{V}_{O_{2max}}$	98.0 ± 9.4	97.1 ± 7.5	103.4 ± 6.2
	45% $\dot{V}_{O_{2max}}$	113.9 ± 6.7	112.5 ± 6.8	123.1 ± 6.9*†
	60% $\dot{V}_{O_{2max}}$	133.6 ± 13.6	134.6 ± 12.2	142.0 ± 12.1
	80% $\dot{V}_{O_{2max}}$	166.0 ± 9.6	165.1 ± 8.9	163.1 ± 11.1
	90% $\dot{V}_{O_{2max}}$	176.9 ± 7.5	175.4 ± 8.2	172.1 ± 9.4
	Max	185.3 ± 8.5	182.8 ± 7.8	177.1 ± 7.0

Values are means ± SD. **P*<0.05 vs Air. †*P*<0.05 vs 3% CO₂.

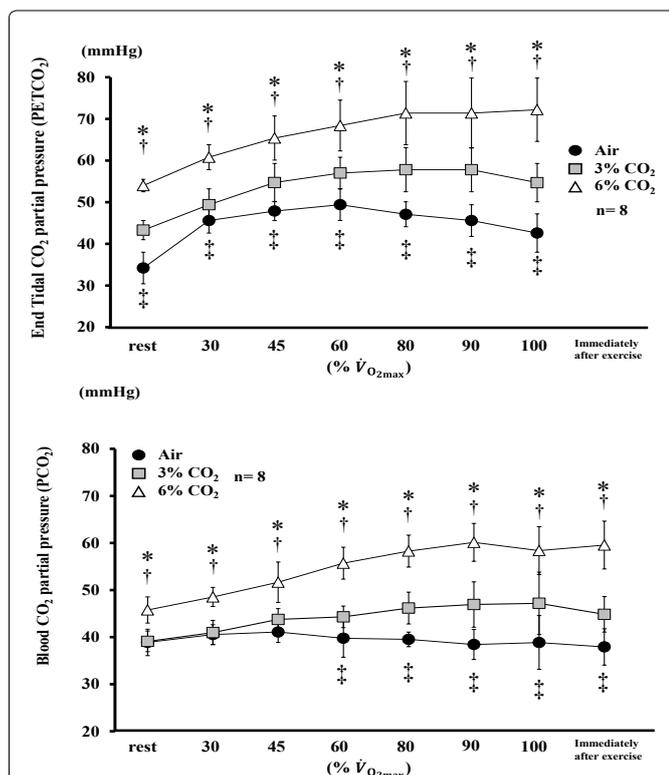


Figure 3: Changes in end tidal CO₂ partial pressure and blood CO₂ partial pressure during incremental exercise at the three CO₂ levels. Values are means ± SD.

**P*<0.05 between Air and 6% CO₂. †*P*<0.05 between Air and 3% CO₂. †*P*<0.05 between 6% CO₂ and 3% CO₂.

\dot{V}_{CO_2} for 6% CO₂ was lower than 3% CO₂ at 80% $\dot{V}_{O_{2max}}$ (*P*<0.05). RER for 3% and 6% CO₂ were lower than Air throughout the experiment (*P*<0.05). Further, RER for 6% CO₂ was lower than for 3% CO₂ at rest and at 30% $\dot{V}_{O_{2max}}$ (*P*<0.05).

\dot{V}_E and f_H data are summarized in table 4. \dot{V}_E for 6% CO₂ and 3% CO₂ was higher than Air during exercise (*P*<0.05). Moreover, \dot{V}_E for 6% CO₂ was higher than for 3% CO₂ up to and including 60% $\dot{V}_{O_{2max}}$ (*P*<0.05). f_H for 6% CO₂ was higher than Air at rest and 45% $\dot{V}_{O_{2max}}$

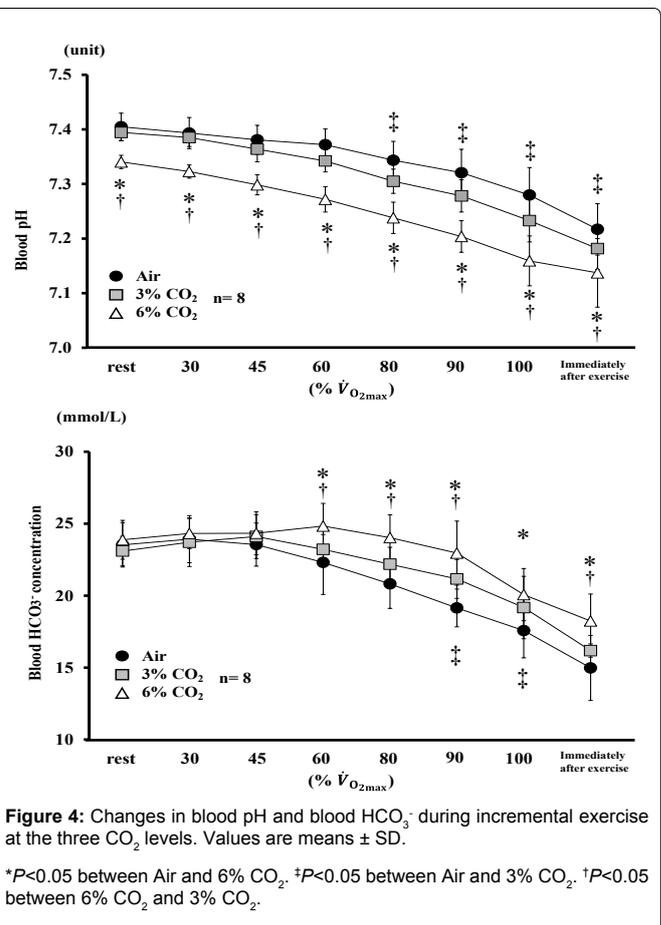


Figure 4: Changes in blood pH and blood HCO₃⁻ during incremental exercise at the three CO₂ levels. Values are means ± SD.

**P*<0.05 between Air and 6% CO₂. †*P*<0.05 between Air and 3% CO₂. †*P*<0.05 between 6% CO₂ and 3% CO₂.

(*P*< 0.05), it was also higher than 3% CO₂ at 45% $\dot{V}_{O_{2max}}$ (*P*<0.05).

P_{ETCO_2} and P_{CO_2}

Changes in P_{ETCO_2} and blood P_{CO_2} are shown in figure 3. P_{ETCO_2} and blood P_{CO_2} for 6% CO₂ were higher than Air and 3% CO₂ throughout the experiment (*P*<0.05). P_{ETCO_2} for 3% CO₂ was higher than Air throughout the experiment (*P*<0.05), and blood P_{CO_2} for 3% CO₂ was higher than Air from 60% $\dot{V}_{O_{2max}}$ (*P*<0.05).

Blood pH and HCO₃⁻

Changes in blood pH and HCO₃⁻ are shown in figure 4. Blood pH for 6% CO₂ was lower than Air and 3% CO₂ throughout the experiment (*P*<0.05). Blood pH for 3% CO₂ was lower than Air from 80% $\dot{V}_{O_{2max}}$ (*P*<0.05). Blood HCO₃⁻ for 6% CO₂ was higher than Air and 3% CO₂ from 60% $\dot{V}_{O_{2max}}$ (*P*<0.05). Blood HCO₃⁻ for 3% CO₂ was higher than Air at 90 and 100% $\dot{V}_{O_{2max}}$ (*P*<0.05).

Discussion

The primary findings of this study were that: (1) the accumulation of Am due to incremental exercise was inhibited with increasing inhaled CO₂ concentration without changing AmT and (2) RER in both hypercapnic conditions were lower compared with Air, but CO₂ storage as indicated by a rise in blood P_{CO_2} for 6% CO₂ occurred at rest, while CO₂ storage for 3% CO₂ was observed from 60% $\dot{V}_{O_{2max}}$. RER at 30% $\dot{V}_{O_{2max}}$ for 3% CO₂ was 0.74 without CO₂ storage, which was close to 0.7 (100% fat metabolism). Without CO₂ storage steady states can be assumed and an effect on metabolism itself inferred. This is not the case when a constant P_{aCO_2} is not maintained.

Incremental exercise performance under hypercapnia

We previously reported that maximal exercise performance for a 6% inhaled CO₂ condition was significantly reduced to approximately 85% of that observed in normocapnia [5]. By contrast, 3% CO₂ gas inhalation had a minor effect on maximal exercise workload [10]. Therefore, we adopted 6% CO₂ as severe hypercapnia, and 3% CO₂ as

a mild hypercapnia in this experiment. Since the present results were in accordance with previous results, this made it possible to compare the Am accumulation and the changes in RER with limited exercise performance status and equivalent exercise performance status.

Effect of hypercapnia on ammonia accumulation

AmT was estimated during graded exercise along with LT [11-13]. LT did not change significantly by hypercapnia [4], but the influence of hypercapnia on AmT was unknown. In the present study, we found a reduced Am at 100% $\dot{V}_{O_{2max}}$, and a reduced La from 80% $\dot{V}_{O_{2max}}$ under both hypercapnic conditions without changing AmT and LT (Figure 2, Table 2). Therefore, these results suggest that AmT may not be influenced by hypercapnic severity and the suppression of Am accumulation under hypercapnia occurs at exercise intensity above AmT.

The suppression of Am accumulation under hypercapnic conditions may relate to the observed reduced exercise performance. However, compared with Air, there was no difference in exercise performance for 3% CO₂ (Table 1), indicating that the lower Am and La were not solely caused by the reduction in incremental exercise performance. A previous study showed that accumulation of La was inhibited as the CO₂ concentration of the inhaled gas increased [2]. In the present study, immediately after exhaustive exercise, plasma Am and La concentrations for 6% CO₂ were lower compared with those for 3% CO₂ and for Air (Figure 2). Moreover, blood pH above 90% $\dot{V}_{O_{2max}}$ decreased with increasing inhaled CO₂ concentration (Figure 4). Thus, these results suggest that the lowered Am and La during exercise were caused by the severity of hypercapnia. Previous studies have suggested that lowered blood La might result from the inhibition of phosphofructokinase (PFK) activity or decreased La transport from muscle cells into the blood by hypercapnia-induced respiratory acidosis [14,15]. In a study of skeletal muscle, metabolic acidosis by NH₄Cl ingestion in skeletal muscle was reported to decrease La production due to inhibition of glycogenolysis [16]. Thus, systemic acidosis with hypercapnia might inhibit La production. Adenosine monophosphate (AMP) deamination as a source of Am is promoted by lactic acidosis [17]. Furthermore, the activity of PFK is promoted by Am [18,19] and there is a strong relationship between the activity of AMP deaminase and PFK activation [20]. Blood HCO₃⁻ is an index of the buffer system [21], and our previous study showed that blood HCO₃⁻ was significantly higher for hypercapnia than Air [5]. In support, we found that the reduction in HCO₃⁻ during incremental exercise decreased with increasing inhaled CO₂ concentration (Figure 4), and this result suggests that lactic acidosis decreased with the severity of hypercapnia. Therefore, it appears that the decrease in accumulation of La during exercise with increasing inhaled CO₂ was caused by increasing hypercapnia-induced respiratory acidosis. As a result, a decreased metabolic acidosis by lowered La production might inhibit the accumulation of Am during exercise under hypercapnia.

Effect of hypercapnia on RER and CO₂ storage

Concerning decreased RER during hypercapnia, it could be caused by a reduction of \dot{V}_{CO_2} due to CO₂ storage. RER reflects the pulmonary exchange of \dot{V}_{CO_2} and \dot{V}_{O_2} [22], and the present results showed that \dot{V}_{O_2} was not significantly changed by hypercapnia (Table 3). Also, some studies reasoned that the lowered RER during steady state exercise under hypercapnia reflected an actual change in substrate utilization [2,6]. Thus, the application of hypercapnic gas inhalation may be effective to promote fat metabolism during exercise. Generally, respiratory quotient (RQ) for various combinations of fat and carbohydrate (CHO) metabolism is constrained to the values between 0.70 and 1.00 [22]. RER reflects RQ only during rest and steady state exercise, because RER rises above 1.00 in exhaustive exercise or decreases below 0.70 during CO₂ storage. In the present study, RER in both hypercapnic conditions showed a lower value than Air and the value of RER for 6% CO₂ at rest and 30% $\dot{V}_{O_{2max}}$ was significantly lower than 3% CO₂ (Table 3), but it was outside of this range. Moreover, as indicated by blood P_{CO_2} and P_{ETCO_2} for 6% CO₂, CO₂ storage occurred before exercise (Figure 3). Thus, the application of the

6% CO₂ to health science is inappropriate. On the other hand, the mean value of RER at 30% $\dot{V}_{O_{2max}}$ for 3% CO₂ was 0.74 (Table 3), which was close to 0.7 (100% fat metabolism). The best exercise training intensity to promote fat metabolism is below 45% $\dot{V}_{O_{2max}}$ intensity as viewed from the "crossover" concept which means that increments in relative exercise intensity result in increasingly greater dependence on CHO and less dependence on fat [7]. Previous studies [2,6], evaluated decreased RER using exercise intensity above 45% $\dot{V}_{O_{2max}}$.

When P_{ETCO_2} increases over 54mmHg, diaphragm fatigue occurs [23]. Also, for endurance cycling load at 80-85% $V_{O_{2peak}}$, P_{ETCO_2} with 3% CO₂ was 51.0 mmHg at end exercise [24]. In comparing the effects of three levels of P_{ETCO_2} (low=29mmHg, medium=47mmHg, high=57mmHg), high CO₂ tensions significantly impaired cognitive and psychomotor performance at atmospheric pressure [25]. From these previous results, a physiologically and psychologically unusual response may occur when P_{ETCO_2} is over 50mmHg. In the present study, P_{ETCO_2} value at 30% $\dot{V}_{O_{2max}}$ for 3% CO₂ was less than 50mmHg and blood P_{CO_2} did not significantly change from Air for this condition (Figure 3). Arterialized blood P_{CO_2} was considered as the best measure of P_{aCO_2} . Concerning the cardiorespiratory responses during the experiment for 3% CO₂, \dot{V}_E was significantly higher, but f_H not significantly different compared to Air (Table 4). Therefore, it seems possible that 3% CO₂ gas inhalation in combination with low intensity exercise might be advantageous for fat metabolism without CO₂ storage and cardiac burden.

Summary

We demonstrated that the accumulation of Am and the mean value of RER due to incremental exercise were reduced with increasing hypercapnia. Above the AmT, the suppression of Am accumulation was observed without changing AmT, while the reduction of steady state RER was indicated at low intensity exercise. When hypercapnic gas inhalation is applied to increase fat metabolism, 3% CO₂ inhalation and 30% $\dot{V}_{O_{2max}}$ intensity exercise was the best combination from the viewpoint of RER value and avoiding CO₂ storage.

Perspectives

We determined the effect of hypercapnic severity on Am accumulation and RER during incremental exercise. Am accumulation during exercise differs from La accumulation depending on exercise intensity and exercise duration [26,27]. During steady state exercise, plasma glycerol and free fatty acids (FFA) were higher under hypercapnia, but this change did not reach statistical significance [1]. Therefore, future studies are required to determine the accumulation of Am and La during steady state and prolonged submaximal exercise along with indices of fat metabolism. The enhancement of lipid metabolism by a combination of moderate hypercapnic gas inhalation and moderate intensity exercise may be applied to the area of rehabilitation and health sciences. In patients with chronic obstructive pulmonary disease (COPD), exercise performance level was lower in comparison with normal lung subjects [28]. In COPD patients, there were the patients whom Am during exercise rose and the patients whom Am did not rise [29]. Thus, it will be beneficial to adjust exercise intensity and hypercapnic severity to minimize Am response during exercise for therapy.

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