Blood Lactate Disappearance during Breathing Hyperoxic Gas after Exercise in Two Different Physical Fitness Groups —On The WorkLoad Fixed at 130% AT—

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Abstract. This study aimed to investigate the effects of hyperoxic gas breathing on the disappearance of blood lactate after exercise in two groups having different physical fitness and to determine the most effective O₂ concentration in consideration of workload. Our previous study has demonstrated that hyperoxic gas breathing brought out different effects among subjects. In respect of these effects, it was thought necessary to pay attention to exercise intensity. Therefore, the exercise intensity of this study was set by using relative workload of anaerobic threshold (AT) from the aspect of blood lactate. Ten healthy male students participated as subjects and were divided into 2 groups; a group consisting of 5 active students whose mean AT was 60.4% $\dot{V}O_2$ max (Higher AT group) and the other group consisting of 5 inactive students having the mean AT of 48.8% $\dot{V}O_2$ max (Lower AT group). All subjects underwent three cycles of ergometer exercise on a bicycle (workload; 130% AT) for 5 min and recovery session for 6 min. The hyperoxic gas breathing was given only for the recovery session. The conditions of breathing were air, 30, 40, 60 or 80% O₂. Blood for determination of the blood lactate level was taken only in the recovery session. When compared with air-breathing, the blood lactate level was significantly reduced in the condition of more than about 60% O₂ breathing in Higher AT group, but not in Lower AT one. Together with the previous findings, it was found that the blood lactate level was markedly reduced by more than 60% O₂ breathing in Higher AT group without relation to the workload in the range of about 70 to 80% $\dot{V}O_2$ max. In Lower AT group, however, the effects of hyperoxic gas breathing were dependent on the exercise intensity; 30% and 40% O₂ breathings were effective for the subjects with more than about 65% $\dot{V}O_2$ max, but not in a lower intensity than it. These results indicate that the effects of hyperoxic gas breathing on the disappearance of blood lactate are dependent on the exercise intensity and the physical capacity.

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Keywords: blood lactate, hyperoxia, anaerobic threshold (AT), physical fitness, recovery

Introduction

Breathing of hyperoxic gas during exercise has been found effective for prolongation of exercise and reduction of blood lactate level (Bannister and Cunningham, 1954; Wilson and Welch, 1975; Hogan et al., 1983). However, there are some confusion (Murphy, 1986; Shell et al., 1986; Maeda et al., 1993) on the effects of hyperoxic gas breathing after exercise on the disappearance of blood lactate. Thus, it was suggested that some individual differences, especially in physical capacity might be involved in such effects (Maeda and Yasukouchi, 1997). One of the physical fitness activities, anaerobic threshold (AT) is closely correlated to oxidative activity of the muscle which mediate the initiation of the lactate accumulation (Rusko et al., 1980; Tanada et al., 1993; Karamizrak et al., 1994). Therefore, in our previous study (Maeda and Yasukouchi, 1997), the subjects were divided into two groups based on the degree of AT and the effects of hyperoxic gas breathing were found to be different between the two groups; the blood lactate level was lowest under the conditions of more than 60% O₂ breathing in Higher AT group and 30% O₂ breathing in Lower AT group. From these results, it was concluded that the effects of hyperoxic gas breathing are dependent on the physical fitness of subjects. However, there were significant differences in blood lactate level immediately after an exercise between the two groups and also, not only physical fitness but also the influence of blood lactate level on its oxidation (Jorfeldt et al., 1978; Hatta, 1993) might be involved in these effects. Thus, to discuss the effects of hyperoxic gas breathing on the blood lactate disappearance, it seems necessary to consider the differences in the blood lactate level after exercise between groups.

The purpose of this study was to investigate the

effects of hyperoxic gas breathing on the disappearance of lactate in the blood after a submaximal exercise, considering the differences in the blood lactate level after exercise, and determine the O_2 concentration that effectively induce a lowering of the blood lactate level after exercise in two different physical fitness groups.

Methods

Subjects

Ten healthy male students participated as subjects in this study. They were divided into two groups by difference in daily physical activities. Five males were treated as a Higher AT group and the others treated as a Lower AT group. Table 1 presents physical characteristic of subjects in each group. Only the values of AT were significantly different between the two groups ($p < 0.01$).

Procedure

$\dot{V}O_{2\max}$ and AT were measured before experiment in order to determine individual workload in a climatic chamber at the Kyushu Institute of Design. Subjects wore a T-shirt, short pants and sports shoes. Incremental exercise was done to exhaust subject on an electrically braked bicycle ergometer (Robby Elektronik AB, cycle-ergometer 380b). Oxygen and carbon dioxide concentrations in expired gas were analyzed breath by breath by using automatically metabolic measurement system (Sensor medics, 2900). AT was obtained by the method of

ventilatory threshold (VT) (Wasserman et al., 1973; Miyashita et al., 1989). $\dot{V}O_{2\max}$ and AT measurements were repeated two times at least in each subject.

After catheter was inserted at antecubital vein to collect blood sample, subject entered the chamber and rested on a bicycle ergometer. Fig. 1 presents time schedule of this study. After resting on a bicycle ergometer for ten minutes, he performed three sessions; each session consisted of 5 minutes exercise and 6 minutes recovery. They exercised at a workload of 130%AT and kept pedaling 60 rpm by metronome. Blood samples were collected at 0, 0.5, 1, 2, 3, 4, and 5th min after each exercise, and blood lactate concentration was immediately analyzed by automatic lactate analyzer (Toyobo, Diagluca HEK-30I). Heart rate (HR) and expired gas were measured during experiments. Hyperoxic gas was breathed only during recovery periods. Oxygen conditions of 21, 30, 40, 60 and 80% in inspired gas were employed. Each gas was produced by mixing air with pure oxygen through blender (BIRD, 3800 Microbender), and was kept in Douglas bag through humidifier. Environmental condition was kept at steady state; temperature was about 25°C and relative humidity was about 50%.

As our purpose was concentrated on blood lactate disappearance, data on blood lactate was mainly reported and discussed in this study.

Statistical analysis

Values are reported as mean $\pm$ SE. Factors of subject

Table 1 Physical characteristics of each subject in Higher and Lower AT groups

Higher AT Group					
Subj.	Age (Year)	Body height (cm)	Body weight (kg)	$\dot{V}O_{2\max}$ (ml/min./kg)	AT (% $\dot{V}O_{2\max}$)
1	22	165	60.1	53.8	61
2	21	162	59.5	60.0	59
3	21	163	60.5	50.6	60
4	23	170	65.2	53.9	61
5	22	166	57.7	54.7	61
Mean	21.8	165.2	60.6	54.6	60.4*
SD	0.8	3.1	2.8	3.4	0.9
Lower AT Group					
Subj.	Age (Year)	Body height (cm)	Body weight (kg)	$\dot{V}O_{2\max}$ (ml/min./kg)	AT (% $\dot{V}O_{2\max}$)
1	22	170	57.5	51.4	45
2	22	160	53.0	55.0	52
3	23	173	59.6	45.9	48
4	22	177	52.6	55.4	50
5	23	173	70.3	48.7	49
Mean	22.4	170.6	58.6	51.3	48.8*
SD	0.5	6.4	7.2	4.1	2.6

*: $P < 0.05$. AT: anaerobic threshold

group, oxygen level, session and recovery time analyzed using four-way analysis of variance (ANOVA) as affecting blood lactate disappearance. Three-way ANOVA was employed in each subject group to detect oxygen condition which affect significantly blood lactate disappearance. P values less than 0.05 were considered significant.

Results

Oxygen uptake ($\dot{V}O_2$) during exercise

$\dot{V}O_2$ was obtained only during exercise in the steady state. According to the results of 3-way ANOVA, $\dot{V}O_2$ during exercise at first session was significantly different ($P<0.01$) between the two groups, but was not significantly changed between conditions of breathing gas in each group. Thus, it was indicated that workload was almost the same among all breathing gas conditions in each

group. Fig. 2 presents averages and standard errors of $\dot{V}O_2$ for the last one-minute during exercise in the first session.

Blood lactate concentration (BLA) after exercise

The data of blood lactate (BLA) during recovery periods were analyzed statistically by 4-way ANOVA (Table 2). According to the ANOVA, the values of BLA were significantly affected by factors of subject group ($P<0.01$), oxygen concentration ($P<0.01$), session ($P<0.01$), and time ($P<0.01$), and also by interactions between subject group and session ($P<0.01$).

The changes of BLA after exercise in Higher and Lower AT groups are shown, respectively in Fig. 3 and Fig. 4. The data of BLA was analyzed statistically by 3-way ANOVA in each group to investigate the effect of hyperoxia on BLA disappearance. Table 3 and 4 present

Fig. 1 Time schedule. (Ex.: exercise, Rec.: recovery). Subjects breathed test gas during each recovery period. Blood sample was collecting at rest period and at 0th, 0.5th, 1st, 2nd, 3rd, 4th, and 5th min of recovery period after each exercise.

Fig. 2 Means and standard errors of oxygen uptake during exercise in the first session at each breathing gas condition in Higher and Lower AT groups.

Table 2 The results of 4-way ANOVA on blood lactate

Factor	S.S.	D.F.	M.S.	F
A (Group)	56724.70	1	56724.70	1269.63***
B (Oxygen)	929.65	4	232.41	5.20**
C (Session)	2767.68	2	1383.84	30.97**
D (Time)	2453.43	6	408.91	9.15**
A × B	293.33	4	73.33	1.64
A × C	669.31	2	334.66	7.49**
A × D	11.11	6	1.85	0.04
B × C	72.16	8	9.02	0.20
B × D	86.01	24	3.58	0.08
A × B × C	55.95	8	6.99	0.16
A × B × D	60.03	24	2.50	0.06
A × C × D	177.79	12	14.82	0.33
B × C × D	121.24	48	2.53	0.06
A × B × C × D	57.76	48	1.20	0.03
Error	37529.50	840	44.68	
Total	104998.00	1049		

***: $P<0.001$, **: $P<0.01$.

Fig. 3 The changes in blood lactate concentration during recovery period of each session in Higher AT group. Each mark and bar indicates mean and standard error, respectively.

Fig. 4 The changes in blood lactate concentration during recovery period of each session in Lower AT group. Each mark and bar indicates mean and standard error, respectively.

the results of 3-way ANOVA, respectively, in Higher and Lower AT groups. According to the results, BLA was significantly affected by factors of oxygen concentration ($P<0.01$), session ($P<0.01$) and time ($P<0.01$) in Higher and Lower AT groups, and also by interaction between session and time ($P<0.01$) in Lower AT group. In Higher AT group (Fig. 3), it was observed that BLA obtained above 40% O₂ conditions were lower after the 2nd minute

during recovery in the first session (recovery 1) than that in air condition. At the recovery of third session (recovery 3), the values were lower just after exercise in 60% and 80% O₂ conditions than that in air condition. In Lower AT group (Fig. 4), BLA was slightly lower in 80% O₂ condition at recovery 1, however, at recovery 3 BLA obtained under hyperoxic conditions were almost the same or greater compared with BLA under air condition.

Table 3 The results of 3-way ANOVA on blood lactate in Higher AT group

Higher AT group				
Factor	S.S.	D.F.	M.S.	F
A (Oxygen)	635.67	4	158.92	2.92**
B (Session)	2931.20	2	1465.60	26.88***
C (Time)	1347.52	6	224.59	4.12**
A × B	51.68	8	6.46	0.12
A × C	81.34	24	3.39	0.06
B × C	2200.91	12	183.41	3.36**
A × B × C	84.56	48	1.76	0.03
Error	22896.30	420	54.52	
Total	30229.20	524		

***: $P < 0.001$, **: $P < 0.01$.**Table 4** The results of 3-way ANOVA on blood lactate in Lower AT group

Lower AT group				
Factor	S.S.	D.F.	M.S.	F
A (Oxygen)	587.31	4	146.83	4.21**
B (Session)	505.79	2	252.90	7.26**
C (Time)	1117.02	6	186.17	5.34**
A × B	76.44	8	9.56	0.27
A × C	64.70	24	2.70	0.08
B × C	965.74	12	80.48	2.31**
A × B × C	94.44	48	1.97	0.06
Error	14633.20	420	34.84	
Total	18044.60	524		

: $P < 0.01$. Fig. 5** The rate of mean blood lactate during recovery 3 obtained at each breathing condition to that at air condition. The mark of circle and square indicated values of Higher and Lower AT groups, respectively.

This study also aimed to investigate the most effective oxygen concentration in each group. The data of BLA obtained in each hyperoxic condition was compared with that in air condition by ANOVA (air-30% O_2 , air-40% O_2 , air-60% O_2 , and air-80% O_2). According to ANOVA, the values of BLA were significantly changed at 60% ($P < 0.01$) and 80% ($P < 0.05$) O_2 conditions in Higher AT group, and 30% O_2 condition ($P < 0.05$) in Lower AT group compared with BLA at air condition. Fig. 5 summarize the results of hyperoxic effect in both groups. Fig. 5 shows the ratio of mean BLA value during recovery 3 in each condition to that in air condition. From Figs. 3, 4, and 5, it was confirmed that the effective oxygen concentrations were over 60% in Higher AT group, but the negative effect was observed at 30% in Lower AT group.

Discussion

In our previous study (Maeda and Yasukouchi, 1997), the influence of hyperoxic gas breathing was investigated on the recovery after exercise from the view point of the disappearance of blood lactate aimed to determine the optimum O_2 concentration which allows the most rapid disappearance of lactate in each group. Furthermore, it was demonstrated that the effects of hyperoxic gas breathing after exercise (i.e. the most effective O_2 concentration) varied among the groups with different physical fitness. However, it was also assumed that the oxidation of lactate is known to be stimulated depending on the blood lactate level (Jorfeldt et al., 1978; Hatta, 1993), the above results might have been affected not only by the physical fitness but also by its blood level. Therefore, this study investigated the effects of hyperoxic gas breathing on blood lactate disappearance in considering the differences of blood lactate level after exercise caused by differences of physical fitness.

The lactate level after recovery indicated that the optimum O_2 concentration for lowering its blood level was 60% or 80% for Higher AT group, and air breathing for Lower AT group. A reduction rate for the ratio, total amount of blood lactate for hyperoxic gas breathing to that for air-breathing in each recovery session after exercise was chosen as an indicator for the O_2 effects on the blood lactate. In Higher AT group, the decrease in blood lactate during the recovery session 3, in which the effects of hyperoxic gas breathing were most marked, was 6.87 and 9.36% in the condition of 60 and 80% O_2 breathing respectively, whereas in Lower AT group, a significant decrease in the blood lactate level was not observed for any concentration of O_2 breathing and a significant increase was found for 30% O_2 breathing. The results obtained from Higher AT group were consistent with those from the previous report (Maeda and

Yasukouchi, 1997), but the results obtained from Lower AT group were in contrary.

When workload of 130% AT was converted to the relative workload of $\dot{V}O_{2\max}$, it corresponded to 76.6 to 79.8% $\dot{V}O_{2\max}$ for Higher AT group, which was higher than the workload for the previous study of 70% $\dot{V}O_{2\max}$ (Maeda and Yasukouchi, 1997) and the blood lactate level was also higher than the previous. However, the present results of Higher AT group were consistent to the previous ones (Maeda and Yasukouchi, 1997), suggesting that the disappearance of blood lactate after exercise during which the workload of 70 to 80% $\dot{V}O_{2\max}$ was not affected by its blood level, but only the effects of hyperoxic gas breathing were significant. In Lower AT group, the workload of 130% AT corresponded to $\dot{V}O_{2\max}$ ranging from 58.9 to 67.9%, which was lower than 70% for the previous one. Moreover, the blood lactate level after exercise was also lower. The results more quite different between the conditions of 70% $\dot{V}O_{2\max}$ and 130% AT. It is likely that when the intensity is in the range of 58.9 to 70% $\dot{V}O_{2\max}$, the disappearance of blood lactate might be affected by two factors which were blood lactate level (i.e. workload) and hyperoxic gas breathing.

Fig. 6 shows the relation between the workload during exercise and the blood lactate level after exercise in each group. It includes both data from the present study and the previous one (Maeda and Yasukouchi, 1997). The workload was converted to a relative

intensity of $\dot{V}O_{2\max}$. The close correlation between the workload during exercise and the blood lactate level after exercise was found both in Higher and Lower AT groups ($P < 0.01$). Further, the regression lines for the air breathing and various O_2 breathings, Air-30%, Air-40%, Air-60% and Air-80% were compared in each group. In Higher AT group, no significant differences were found in the slope of regression line for air breathing and all O_2 breathing, but there were significant differences in the intercept between the breathing of air and 60% ($P < 0.01$) or 80% O_2 ($P < 0.05$). Whereas in Lower AT group, significant differences were found in the slope of the regression line between the breathings of air and 30% or 40% O_2 (both, $P < 0.05$), indicating that the effects of O_2 breathing were dependent on the exercise intensity.

As shown in Fig. 6, the intercept of regression line in Higher AT group was largest for the air breathing and decreased as an increase of O_2 concentration of breathing gas, indicating significant effects of hyperoxic gas breathing, especially more than 60% O_2 breathing. On the other hand, significant differences in the slope of regression line were observed between air breathing and 30% or 40% O_2 breathing in Lower AT group. Although there were no significant effects of hyperoxic gas breathing for the exercise intensity of less than ca. 65% $\dot{V}O_{2\max}$, the blood lactate level significantly decreased in the condition of 30% or 40% O_2 breathing for more than 65% $\dot{V}O_{2\max}$.

A few reports showed that when 100% O_2 breathed

Fig. 6 The relation between the workload during exercise and blood lactate concentration after exercise in both groups.

after exercise, significant differences in the blood lactate level were not observed when compared with the conditions of air breathing (Murphy, 1986; Shell et al., 1986). Thus, it has been considered that there are no effects of hyperoxic gas breathing after exercise. However, the present study as well as the previous one (Maeda and Yasukouchi, 1997) revealed that when the subjects were divided into an active group and an inactive one, the disappearance of blood lactate was significantly affected by hyperoxic gas breathing after exercise in both groups and these effects were varied depending on the exercise intensity and differences between groups. These effects are noteworthy since the results from previous related studies were inconsistent. The stimulatory effects of hyperoxic gas breathing after exercise on reducing the blood lactate level are dependent on the physical fitness and the exercise intensity. However, the previous studies were undertaken without considering such factors, resulting in the confusions till now.

From the present results, it was confirmed that the O₂ concentration which allows the most effective decrease in the blood lactate was different between the groups, but the cause remains unknown. Since the disappearance of blood lactate were mediated by three factors; training level, exercise intensity and hyperoxic gas breathing, the cause was inferred from the three factors as well as the previous findings.

It is likely that there are some differences in the oxidative activity of muscle and the transporting capacity of blood circulating system between Higher and Lower AT groups with respect to 1) the enhancement of endurance physical capacity by training, 2) the increase in blood circulating capacity associated with the development of the capillary net (Åstrand and Rodahl, 1986), the decrease in the peripheral vascular resistancy during exercise etc. and 3) the increase in lactate dehydrogenase (LDH) activity (Rusko et al., 1980; Tanada et al., 1993; Karamizrak et al., 1994). The enhancement of the transporting capacity would enable the rapid transfer of the lactate to other organs and the quick oxidation of it (Hatta, 1993). Therefore, it was assumed that oxidation of the lactate accumulated in the working muscles during exercise would be more highly enhanced in the muscles and other organs of Higher AT group than that in Lower AT.

An increase in blood flow induced by a light exercise in the recovery session caused a rapid lowering of blood lactate level (Newman et al., 1937; Belcastro and Bonen, 1975). The lactate oxidation was much stimulated by an increase of blood lactate level (Jorfeldt et al., 1978; Hatta, 1993). These reports suggest that since with the increase of the exercise intensity the lactate level and the blood flow were raised after the exercise, the reduction rate of blood lactate would increase with an increase of the exercise intensity. Thus, the decrease in blood flow

which was caused by the vasoconstriction due to a higher partial pressure of O₂ (Welch et al., 1977) can be highly compensated as the exercise intensity increased (Weltman et al., 1979). In this study, especially in Lower AT group, the influence of exercise intensity was observable; as the exercise intensity corresponded to more than about 65% $\dot{V}O_{2\max}$, the lactate level was reduced by 30% or 40% O₂ breathing. Contrarily, at a lower intensity than the above, the level was increased. On the other hand, the influence of exercise intensity was not observed in Higher AT group. However, there is a possibility that such effects observed in Lower AT group may appear in Higher AT group due to a lower intensity of exercise.

Under the conditions of 30% O₂ breathing, lactate dehydrogenase (LDH) activities in the blood of the exercise-loaded subjects were significantly higher compared to the condition of air-breathing (Nagata et al., 1990), suggesting that hyperoxic gas breathing after exercise might induce an increase of the enzyme activity and the degree of such increase depend on the physical training level of each individual. But, there is no evidence supporting this possibility.

Vascular constriction, which is an adverse effect due to hyperoxic gas breathing was thought to have improved by the increased capacity of the circulation system through regular conducted training and thus, the blood lactate level might have been reduced in the condition of hyperoxic gas breathing in Higher AT group. In addition, it was assumed that some factors such as the differences in oxidative activity of the muscle between the two groups, the increase in LDH activity by hyperoxic gas breathing and the changes in blood flow after exercise depending on the exercise intensity, or some interaction of these might be involved in the different influences on the lactate disappearance of both groups.

The results from previous studies on the effects of O₂ breathing on the blood lactate were inconsistent. However, the factors involved in the effects were elucidated by assigning the subjects into two groups based on their physical fitness. Especially, the fact that those effects were different in the two groups and also affected by the exercise intensity was noteworthy. The finding that the effects of hyperoxic gas breathing are dependent on the workload is thought to be attributed to the differences in blood flow changes after exercise. Considering this in addition to the previous evidence that a light exercise in a recovery session induced a decrease in blood lactate level (Newman 1937, Belcastro and Bonen, 1975) it was expected that some combined effects of hyperoxic gas breathing and light exercise may exist in a recovery session. Therefore, these factors should be considered to make the best "menu" for rapid recovery.

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