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Substrate utilization during exercise and recovery at moderate altitude

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Abstract

Recent studies have shown that exercise training at moderate altitude or in moderate hypoxia improved glycemic parameters. From these data, it has been supposed that endurance exercise in moderate hypoxia affects substrate utilization and that exposure to moderate hypoxia in combination with exercise may be utilized as part of metabolic or diabetes prevention program. However, the influence of exercise at moderate hypoxia on circulating metabolites and hormones in terms of substrate utilization is unclear. The purpose of this study was to elucidate the influence of exercise in moderate hypoxia on substrate utilization. We determined cardiorespiratory, metabolic, and hormonal parameters during exercise and postexercise recovery at a simulated moderate altitude of 2000 m, and then we compared these variables with values obtained at sea level. Seven men participated in this study; subjects reported to the laboratory on 4 occasions. Two maximal exercise tests were performed to estimate peak oxygen uptake at the simulated 2000-m altitude and sea level on different days. Afterward, submaximal exercise tests were carried out at a simulated altitude of 2000 m or sea level, separated by 1 week. Subjects performed submaximal exercise at the same relative exercise intensity (50% peak oxygen uptake) at a simulated altitude of 2000 m and at sea level for 30 minutes. The tests were performed in random order, and subjects were blinded to the respective altitudes. Venous blood samples and expired gases were obtained before, during exercise (15 and 30 minutes), and during postexercise recovery periods (15, 30, 45, and 60 minutes). The respiratory exchange ratio during exercise and recovery at moderate altitude was greater than at sea level. The epinephrine and norepinephrine concentrations during exercise and recovery were higher ($P < .05$) at moderate altitude than at sea level. Free fatty acids and glycerol concentrations during recovery were lower ($P < .05$) at moderate altitude than at sea level. These results suggest that carbohydrate utilization is increased during exercise and postexercise recovery period in moderate hypoxia as compared with normoxia. It is also suggested that moderate hypoxia influences the changes in circulating metabolites and hormones in terms of substrate metabolism during exercise and the recovery.
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1. Introduction

Some recreational activities, such as hiking or cross-country running, are performed at moderate altitude (1500-2500 m) [1]. Many visitors to places of moderate altitude have known or unknown arterial hypertension, diabetes mellitus, obesity, and disturbances in lipid metabolism [2]. Recent studies [2-4] have found that the homeostasis model assessment index of insulin resistance and glucose response to oral glucose tolerance tests after exercise training at moderate altitude (1700~2400 m) or in normobaric moderate hypoxia (inspired O_2 fraction; F_{IO_2} = 0.15) improved more than training in normoxia in healthy men and patients with metabolic syndrome. From these studies, it has been supposed that exposure to moderate hypoxia in combination with endurance exercise could affect substrate utilization and that exercise in moderate hypoxic conditions may be utilized as part of a metabolic or diabetes prevention program [3-5].

It was reported that carbohydrate utilization increased during exercise at high altitude compared with sea level [6,7], although a few studies showed no influence of high altitude or severe hypoxia [8,9]. A change in the regulation of metabolic pathways to favor greater dependence on carbohydrate utilization at high altitude would aid in maintaining homeostasis by optimizing the energy yield

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per unit O_2 [10-13]. Considering the practical use and safety for metabolic or diabetes prevention program, exposure to high altitude or severe hypoxia may not necessarily be suitable. Therefore, it is important to elucidate substrate utilization during exercise at moderate altitude. However, investigations of substrate utilization have only been performed at high altitudes (3000~4300 m) or under conditions of severe normobaric hypoxia (F_{IO_2} = 0.12), and the influence of exercise at moderate altitude on circulating metabolites and hormones in terms of substrate utilization is unclear.

Physical exercise affects substrate utilization not only during exercise but also during postexercise recovery, and the substrate utilization during recovery is also of concern to individuals who have metabolic disturbances [14]. Many studies have investigated substrate utilization during exercise, whereas a few studies have focused on metabolic event during postexercise recovery [14-16]. It is assumed that hypoxic circumstances affect substrate utilization during recovery period as well as during exercise. As far as we know, however, there are no available data with regard to the substrate utilization during postexercise recovery in hypoxic condition.

The purpose of the present study, therefore, was to elucidate the influence of exercise under conditions of moderate hypoxia on substrate utilization. We determined cardiorespiratory, metabolic, and hormonal parameters during exercise and postexercise recovery at a simulated altitude of 2000 m (moderate hypoxia) and then compared these variables during exercise and recovery at sea level.

2. Methods

2.1. Subjects

Seven healthy men participated in this study. The mean values and standard error (SE) for age, height, and body mass were 22.9 ± 0.5 years, 171.1 ± 2.7 cm, and 62.2 ± 2.7 kg, respectively. Subjects lived their entire lives at sea level and were either sedentary or recreationally active. They were informed of the experimental procedures and possible risks involved in this study, and informed consent was obtained. The present study was approved by the Human Research Committee of the Research Center of Health, Physical Fitness and Sports, Nagoya University.

2.2. Experimental procedures

At a preliminary visit to the laboratory, all subjects were familiarized with the apparatus and testing procedures in a hypobaric chamber. Afterward, subjects reported to the laboratory on 4 additional occasions.

For the first and second occasions, a maximal exercise test was performed in a chamber that simulated altitude of 2000 m (600 mm Hg) or sea level (742 mm Hg). Each test was performed at least 3 days apart. Subjects performed an incremental exercise test by using a mechanically braked bicycle ergometer (824E; Monark, Stockholm, Sweden). The barometric pressure in the chamber was lowered to 600 mm Hg over 10 minutes and then held at that level. The maximal exercise test began within the first 10 minutes. The maximal exercise test began at an initial power output of 60 W, and the workload was increased 30 W every 2 minutes until exhaustion. The pedaling rate was kept at 60 rpm with the aid of a metronome. During the test, expired gases were collected into a Douglas bag during the last 30 seconds of each intensity level until exhaustion. The highest value obtained for oxygen uptake ($\dot{V}O_2$) during the maximal exercise test was used as the peak oxygen uptake ($\dot{V}O_{2peak}$). The order of altitude and sea level trials was randomized, and the subjects were blinded to the respective altitudes.

On the third and fourth occasions, submaximal exercise tests were carried out at a simulated altitude of 2000 m or sea level, separated by 1 week. The tests were performed in random order, and the subjects were blinded to the altitude. The subjects were fed the same 3 meals (breakfast, lunch, and dinner) on the day before each experimental day. After overnight fasting, that is, 12 hours, subjects arrived at the laboratory. They rested for 30 minutes, and venous blood samples from an indwelling cannula in the antecubital vein were taken in normoxia (baseline). Then, subjects moved to another experimental room and were brought into the chamber. Similar to the procedure of maximal exercise test, the barometric pressure in the chamber was lowered to 600 mm Hg over 10 minutes and then held at that level. For the submaximal exercise test at sea level, pressure was changed to 742 mm Hg over 10 minutes and then held at that level. The submaximal exercise test began within the first 10 minutes. Subjects performed submaximal exercise at the same relative exercise intensity (50% $\dot{V}O_{2peak}$) in each condition (simulated altitude of 2000 m or sea level) for 30 minutes (exercise). The pedaling rate was kept constant at 60 rpm. After exercise, subjects rested for 60 minutes after each condition (recovery). Venous blood samples and expired gases were obtained before, during exercise (15 and 30 minutes), and recovery periods (15, 30, 45, and 60 minutes). Expired gases were collected for 3-minute periods at rest (baseline and recovery) and for 1-minute periods during exercise (exercise) by means of a Douglas bag.

2.3. Measurements

2.3.1. Cardiorespiratory measurements

Oxygen uptake, carbon dioxide output ($\dot{V}CO_2$), and expired minute ventilation ($\dot{V}E$) were measured using the Douglas bag method. The system was similar to our previous studies [17,18]. Expired gas volume was measured with a dry gas meter (NDS-2A-T; Shinagawa Dev, Tokyo, Japan), and gas analyses were performed by means of O_2 and CO_2 analyzer (Vmax29c; Sensor Medics, Tokyo, Japan). The respiratory exchange ratio (RER) was determined from $\dot{V}O_2$ and $\dot{V}CO_2$ measurements. Heart rate (HR)

was recorded by a 3-lead electrocardiogram (Tango+; Suntec Medical, Morrisville, NC), and arterial oxygen saturation (Sa_{O_2}) was measured by a finger pulse oximeter (Biox 3740; Ohmeda, Madison, WI) placed on the tip of the right forefinger.

2.4. Blood analysis

Blood samples for the measurements of hormones and metabolites were stored frozen at −85°C until analyses. Serum free fatty acid (FFA) concentrations were measured using an enzymatic method (NEFA-HRII; Wako Pure Chemical Industries, Osaka, Japan). The interassay and intraassay coefficients of variation (CVs) were 3.9% and 1.0%, respectively. Serum glycerol concentrations were measured using an enzymatic colorimetric method with kits (Wako Pure Chemical Industries, Osaka, Japan). These interassay and intraassay CVs were less than 5.0%. Whole blood lactate concentrations were measured immediately after blood collection. Blood lactate concentrations were determined using an automated blood analyzer (Lactate Pro LT-1710; Arkley, Kyoto, Japan). Plasma glucose concentrations were analyzed using an enzymatic method; the interassay and intraassay CVs were 1.0% and 0.6%, respectively. Plasma epinephrine (Epi) and norepinephrine (NE) concentrations were measured using high-performance liquid chromatography with kits (HLC-725CAII; Tosoh, Tokyo, Japan). Sensitivity of these assays, and interassay and intraassay CVs were 6.0 pg/mL and 3.0% and 1.3% for Epi, and 6.0 pg/mL and 2.4% and 1.2% for NE, respectively. Serum insulin concentrations were measured using a commercially available kit (Architect insulin; Abbott Japan, Tokyo, Japan). The insulin assay sensitivity was 1.01 μU; and the interassay and intraassay CVs were 4.8% and 1.9%, respectively. Serum growth hormone (GH) concentrations were measured using immunoradiometric assay with kits from SRL (Tokyo, Japan). The GH assay sensitivity was 0.04 ng/mL; the interassay and intraassay CVs were 4.7% and 2.3%, respectively. A detailed description of given procedures can be found elsewhere [19].

2.5. Statistical analysis

All values are expressed as mean ± SE. For all data, the assumption of normal distribution was verified using a Kolmogorov-Smirnov test. The changes in each parameter at altitude or sea level during the experiment were analyzed using 1-way analysis of variance with repeated measurements. If there was a significant main effect, post hoc, pairwise comparisons were made using the Holm's sequential Bonferroni procedure. The comparisons of parameters between sea level and moderate altitude were achieved using paired t test if the distribution was regular. When the distribution was not regular, Wilcoxon test (nonparametric test) was used. The SPSS (11.5; SPSS, Tokyo, Japan) and the StatView (5.0; SAS Institute, Tokyo, Japan) statistical packages were used for these analyses. A $P < .05$ was considered to be significant.

3. Results

3.1. Baseline parameters

The $\dot{V}O_{2peak}$ at a simulated altitude of 2000 m tended to be lower than that at sea level, although differences did not reach statistical significance (2.83 ± 0.11 L/min at 2000 m, 2.92 ± 0.12 L/min at sea level, $P = .06$). There were no significant differences in any of blood or cardiorespiratory parameters under baseline resting states at sea level on the 2 submaximal exercise trial days.

3.2. Cardiorespiratory variables

Change in RER is shown in Fig. 1. Respiratory exchange ratio increased during exercise in each condition, and RER at 30 minutes during exercise at moderate altitude was significantly ($P < .05$) higher compared with sea level. During postexercise recovery period, RER tended to be higher at moderate altitude; and there was a significant ($P < .05$) difference between the 2 conditions at 60 minutes during postexercise recovery period. Changes in cardiorespiratory parameters are shown in Table 1. The $\dot{V}O_2$, $\dot{V}CO_2$, and $\dot{V}E$ increased significantly ($P < .05$) during exercise and then returned to baseline levels during the recovery period in both conditions (Table 1). There were no significant differences in these variables between moderate altitude and sea level. At simulated moderate altitude, Sa_{O_2} decreased significantly ($P < .05$) during exercise. Similarly, Sa_{O_2} during exercise at sea level also showed a small but significant ($P < .05$)

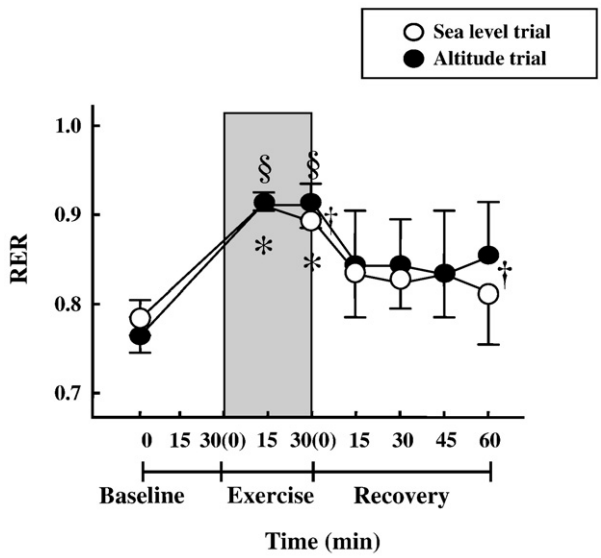

Fig. 1. Respiratory exchange ratio during exercise and recovery at simulated 2000-m altitude and sea level. Values are means ± SE. *$P < .05$ vs baseline at sea level. §$P < .05$ vs baseline at altitude. †$P < .05$ between moderate altitude and sea level.

Table 1
Changes in cardiorespiratory parameters throughout the experimental period

Variables	Trials	Baseline	Exercise		Recovery			
			15 min	30 min	15 min	30 min	45 min	60 min
$\dot{V}O_2$ (L/min)	Sea level	0.22 ± 0.02	1.41 ± 0.04*	1.46 ± 0.09*	0.24 ± 0.01	0.24 ± 0.02	0.22 ± 0.01	0.21 ± 0.01
	Altitude	0.22 ± 0.01	1.41 ± 0.05‡	1.42 ± 0.05‡	0.25 ± 0.01	0.21 ± 0.01	0.23 ± 0.02	0.22 ± 0.01
$\dot{V}CO_2$ (L/min)	Sea level	0.18 ± 0.02	1.29 ± 0.05*	1.30 ± 0.09*	0.20 ± 0.02	0.20 ± 0.03	0.18 ± 0.02	0.17 ± 0.03
	Altitude	0.17 ± 0.02	1.29 ± 0.05‡	1.30 ± 0.17‡	0.21 ± 0.02	0.18 ± 0.01	0.19 ± 0.03	0.19 ± 0.02
$\dot{V}E$ (L/min)	Sea level	7.8 ± 0.9	37.2 ± 2.0*	37.2 ± 2.3*	8.8 ± 0.7	8.2 ± 0.8	8.6 ± 0.8	8.2 ± 1.2
	Altitude	8.4 ± 0.7	38.0 ± 2.0‡	38.6 ± 1.9‡	9.6 ± 0.9	9.0 ± 1.8	9.0 ± 1.0	8.7 ± 0.7
SaO_2 (%)	Sea level	98.4 ± 0.2	97.3 ± 0.3*	97.3 ± 0.3*	97.6 ± 0.3	97.6 ± 0.2	97.7 ± 0.2	98.0 ± 0.2
	Altitude	98.5 ± 0.2	91.7 ± 0.9†,‡	92.4 ± 0.4†,‡	94.3 ± 1.3†,‡	94.0 ± 1.2†,‡	93.9 ± 0.6†,‡	94.7 ± 0.4†,‡
HR (beats/min)	Sea level	56.5 ± 1.7	121.6 ± 3.3*	126.0 ± 2.9*	71.9 ± 2.4*	68.4 ± 2.1*	65.0 ± 2.8*	67.0 ± 3.0*
	Altitude	59.4 ± 2.0	130.0 ± 6.1‡	133.1 ± 4.8‡	84.3 ± 3.2†,‡	78.9 ± 3.2†,‡	72.9 ± 3.2‡	71.7 ± 2.3‡

Values are mean ± SE.
* $P < .05$ from baseline at sea level.
† $P < .05$ sea level vs altitude.
‡ $P < .05$ from baseline at altitude.

decrease. During the recovery period, SaO_2 retuned to baseline levels at sea level, whereas it remained significantly lower than baseline ($P < .05$) at moderate altitude. Heart rate was increased during exercise in both conditions, but there were no significant differences between moderate altitude and sea level. During the recovery period, HR at moderate altitude was higher than that at sea level ($P < .05$, at 15 and 30 minutes).

3.3. Circulating metabolites

Fig. 2 shows changes in blood lactate and plasma glucose concentrations during the experiment. Lactate concentrations increased significantly ($P < .05$) during exercise at moderate altitude and at sea level. During the exercise and recovery period, lactate concentrations tended to be higher at moderate altitude than at sea level ($P < .05$, at 60 minutes during the recovery). Plasma glucose concentrations during exercise decreased significantly ($P < .05$) at 15 minutes in both conditions. Thereafter, values returned to baseline levels in both conditions. There were no significant differences in plasma glucose concentrations during exercise and postexercise recovery between simulated moderate altitude and sea level.

Serum FFA and glycerol concentrations during exercise and the recovery period are shown in Fig. 3. At each condition, FFA concentrations tended to decrease at 15 minutes during exercise and return to baseline levels at 30 minutes during exercise. During the recovery period, significant ($P < .05$) increases in FFA concentrations compared with baseline appeared in both conditions. Free fatty acid concentrations at 15 minutes during the recovery period at moderate altitude were significantly ($P < .05$) lower than that at sea level, and this difference was maintained throughout the recovery period. Glycerol concentrations increased significantly ($P < .05$) from baseline during exercise under both conditions. During the postexercise recovery period, glycerol concentrations at moderate altitude returned to baseline levels, whereas they remained significantly higher ($P < .05$) at 15 minutes during the recovery period at sea level. Accordingly, glycerol concentrations were lower at moderate altitude than at sea level during the recovery period ($P < .05$, at 15 and 45 minutes).

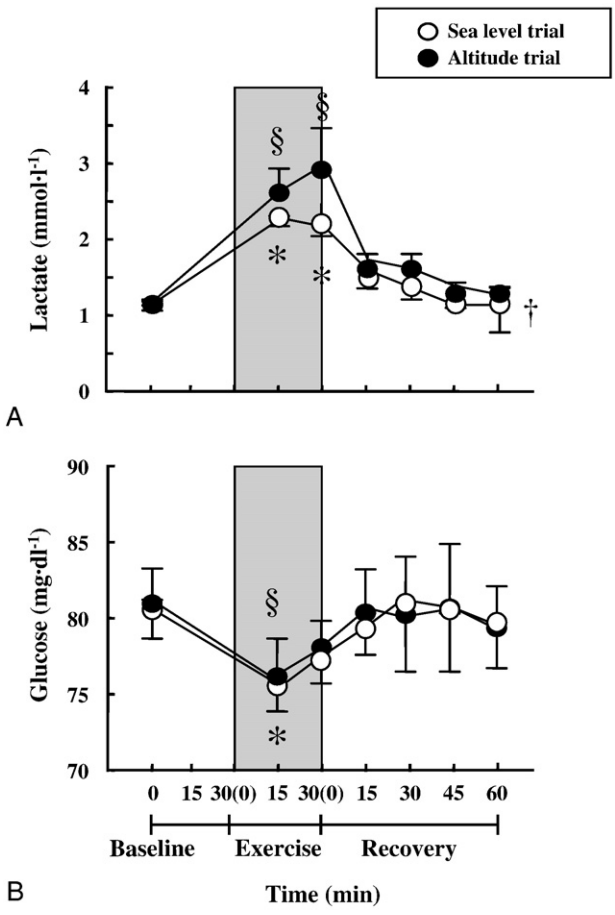

Fig. 2. Blood lactate (A) and glucose (B) concentrations during exercise and recovery at simulated altitude of 2000 m and sea level. Values are means ± SE. *$P < .05$ vs baseline at sea level. $^{\S}P < .05$ vs baseline at altitude. $^{\dagger}P < .05$ between moderate altitude and sea level.

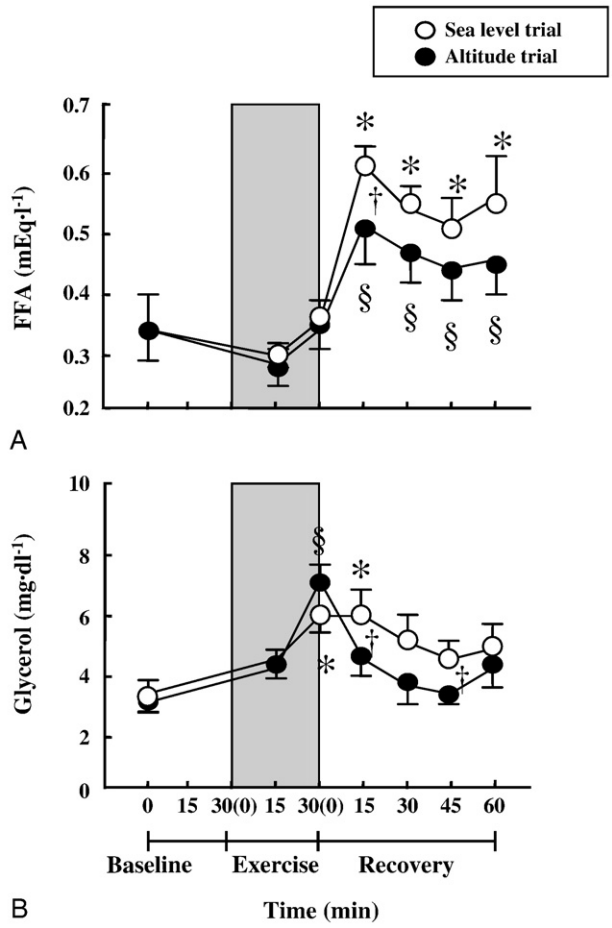

Fig. 3. Serum FFA (A) and glycerol (B) concentrations during exercise and recovery at 2000 m altitude and sea level. Values are means ± SE. *$P < .05$ vs baseline at sea level. §$P < .05$ vs baseline at altitude. †$P < .05$ between moderate altitude and sea level.

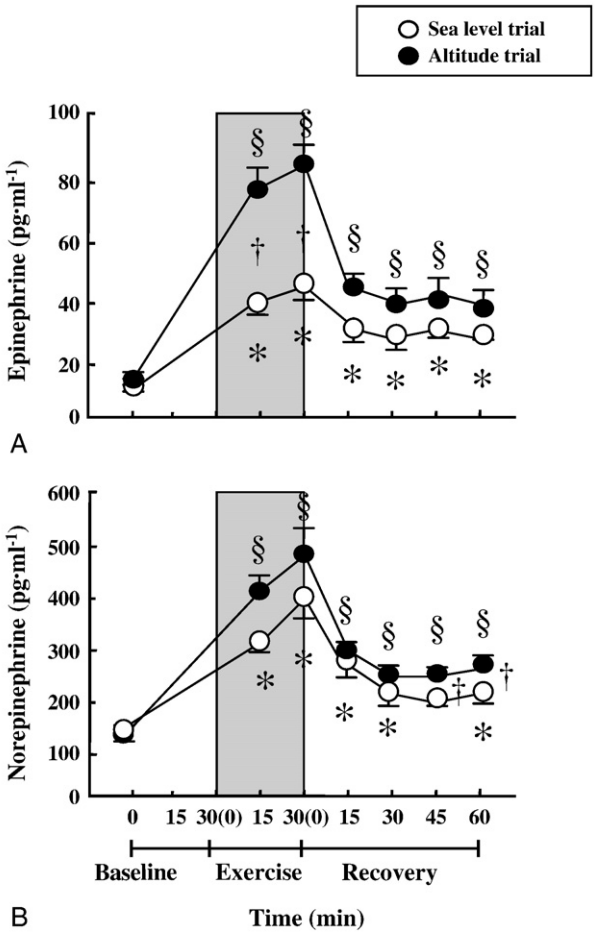

Fig. 4. Plasma Epi (A) and NE (B) concentrations during exercise and recovery at simulated moderate altitude and sea level. Values are means ± SE. *$P < .05$ vs baseline at sea level. §$P < .05$ vs baseline at altitude. †$P < .05$ between moderate altitude and sea level.

3.4. Circulating hormones

Changes in plasma Epi and NE concentrations are shown in Fig. 4. Epinephrine levels increased significantly ($P < .05$) during exercise at both moderate altitude and sea level. Epinephrine concentrations during exercise at a simulated altitude were significantly higher ($P < .05$) than those at sea level. Epinephrine levels decreased after exercise in each condition, but were still significantly higher ($P < .05$) during the recovery period compared with baseline under both conditions. Epinephrine concentrations tended to be higher at moderate altitude during postexercise recovery period compared with sea level, although differences were not significant. A significant ($P < .05$) increase from baseline in NE concentrations also appeared during exercise in each condition. Norepinephrine levels during exercise tended to be higher at moderate altitude than those at sea level, although differences were not significant. Norepinephrine concentrations in each condition were reduced after exercise, but remained significantly higher ($P < .05$) compared with baseline in both conditions. During the recovery period, NE levels were significantly higher at the simulated moderate altitude than at sea level ($P < .05$, at 45 and 60 minutes).

Fig. 5A shows changes in serum insulin concentrations throughout the experiment. At sea level, insulin concentrations decreased during exercise, but differences were not significantly different from baseline. Values returned to baseline levels during recovery. Similarly, insulin concentrations during exercise at moderate altitude slightly decreased during the first 15 minutes of exercise, but returned to baseline levels at 30 minutes of exercise. During recovery, insulin concentrations tended to be higher at moderate altitude than at sea level, but differences were not significant. Serum GH concentrations increased significantly from baseline ($P < .05$) with exercise at both sea level and moderate altitude, but no significant differenced were observed at any point between conditions (Fig. 5B).

4. Discussion

The primary findings of this study were that (1) RER during exercise and postexercise recovery period at moderate

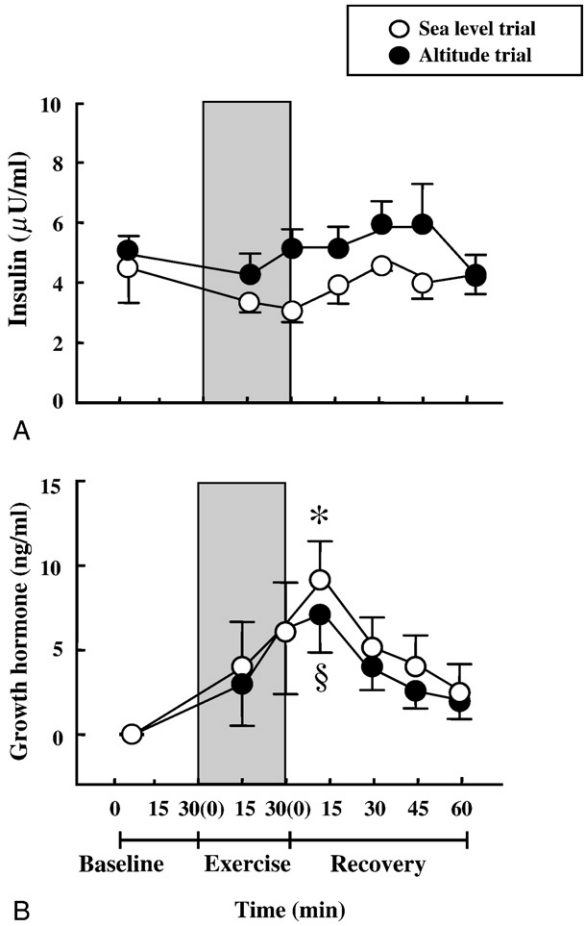

Fig. 5. Serum insulin (A) and GH (B) concentrations during exercise and recovery at simulated moderate altitude and sea level. Values are means ± SE. *$P < .05$ vs baseline at sea level. §$P < .05$ vs baseline at altitude.

altitude was higher than that at sea level, (2) FFA and glycerol concentrations during the recovery at moderate altitude were lower than those at sea level, and (3) Epi and NE concentrations during exercise and recovery were higher at moderate altitude than at sea level.

At moderate altitude, RER values during exercise and recovery were slightly but significantly higher compared with those at sea level (Fig. 1), suggesting an increase in carbohydrate utilization in moderate hypoxia. This result corresponds with the data by Friedmann et al [20] who showed higher RER values during exercise in moderate normobaric hypoxia (F_{IO_2} = 0.15) in endurance-trained athletes. In the present study, insulin concentration at moderate altitude was not suppressed more at moderate altitude, although Epi and NE concentrations were higher at moderate altitude as compared with sea level. In addition, lactate concentration tended to be higher at moderate altitude. On the other hand, there was no difference in plasma glucose concentration between moderate altitude and sea level. These results could provide supportive mechanistic evidence for increased carbohydrate utilization in moderate altitude. Some investigators proposed that a change in the regulation of metabolic pathways to favor greater dependence on carbohydrate utilization at altitude would aid in maintaining homeostasis by optimizing the energy yield per unit O_2 [10-13]. Our results support this proposition. On the other hand, at higher altitude (3000 m) or severe hypoxia (F_{IO_2} = 0.12), several studies revealed no differences in RER during exercise at the same relative intensity between both hypoxia and normoxia [8,9]. RER values are influenced by several factors. RER values during exercise are related to the preceding 24-hour diet and overnight fast [21,22]. In this study, subjects ate the same diet 24 hours before each submaximal exercise at moderate altitude and sea level and exercised at the same time of the day. Thus, this factor should not have affected the results of this study. Indirect calorimetry has been criticized for accuracy during exercise because of hyperventilation, which may cause an overestimation of tissue CO_2 production [20,21,23]. An increase in lactate (H^+), which stimulates ventilation, may disturb the validity of the calculation of substance oxidation because of an enhanced CO_2 and thereby lead to an overestimation of RER [24]. However, there were no significant differences in $\dot{V}_E$ during exercise and recovery between simulated moderate altitude and sea level in this study. Moreover, values of RER were less than 1.0 throughout the experimental period at each condition. Thus, it is likely that RER, as an index of substrate utilization, could be at comparable levels throughout the experimental period in the present study.

Adipose tissue lipolysis is brought about through increased sympathetic system activity, as well as other hormones, such as increased GH and decreased insulin concentration [25,26]. In the present study, plasma Epi and NE concentrations during exercise and postexercise recovery at moderate altitude were higher than those at sea level (Fig. 4). From these results, it is likely that lipolysis is increased at moderate altitude. However, there were no differences in FFA and glycerol concentrations during exercise between moderate altitude and sea level (Fig. 3). Moreover, the increases in FFA and glycerol concentrations at simulated moderate altitude during postexercise recovery period were lower than those at sea level. These results indicate that lipolysis during postexercise recovery may be diminished at simulated moderate altitude, and the diminished lipolysis in this study agrees with a previous one that reported a decrease of adipose tissue lipolysis after prolonged exposure to hypobaric hypoxia [25]. Although the mechanisms of the lower FFA and glycerol concentrations during recovery at moderate altitude are unclear, there are several possible considerations for the decreased lipolysis at moderate altitude. Firstly, insulin concentration during recovery period tended to be higher at moderate altitude than at sea level (Fig. 5A). It is well known that inhibition of lipolysis occurs by increased insulin concentration [25,26], and thus, higher insulin concentrations during postexercise recovery at moderate altitude may diminish lipolysis of adipose tissue. Secondly, it has been

supposed that GH secretion, which is induced by exercise, may play a role in lipolysis in the postexercise recovery period [26-28]. However, there were no significant differences in serum GH levels during exercise between moderate altitude and sea level (Fig. 5B). This result indicates that GH secretion was not the reason for the differences in lipolysis between moderate altitude and sea level. Thirdly, lactate concentration concerns lipolysis and FFA mobilization. High concentrations of arterial lactate concentration have been shown to inhibit lipolysis and FFA mobilization [29]. In the present study, greater increases in lactate concentrations during exercise and recovery at moderate altitude were seen, although the differences between conditions were small (Fig. 2A). Therefore, the decreased lipolysis at simulated moderate altitude may be partly due to an increase in glycolysis.

Recently, it was reported that significant improvements of the homeostasis model assessment index of insulin resistance and glucose response to oral glucose tolerance tests resulted after exercise training at moderate altitude or in normobaric moderate hypoxia [3,4,9]. From the result of their study, it is suggested that short-term living at moderate altitude with activities may be developed as a potential natural remedy for the prevention and treatment of type 2 diabetes mellitus [3,5]. The results of the current study, which showed that exercise at moderate altitude was shifted toward increased carbohydrate utilization, may support an improved glucose tolerance after short-term altitude exposure. Further investigations are needed to clarify whether exercise training at moderate altitude improves glucose tolerance in patients with type 2 diabetes mellitus.

4.1. Technical considerations and limitations

Previous studies demonstrated that carbohydrate utilization was increased during exercise, conducted at the same absolute work rate, at high altitude (~4300 m) compared with sea level [6,7]. However, the interpretation of findings from these studies is complicated by the use of a higher relative intensity of exercise at altitude, which would tend to increase reliance on glucose as a fuel [13,21,30]. In the present study, subjects exercised at the same relative exercise intensity, that is, 50% $\dot{V}O_{2peak}$, at moderate altitude and sea level. Therefore, the effect of a difference of relative exercise intensity was excluded when we examined changes in substrate utilization at moderate altitude and sea level.

The concentration and turnover of blood metabolites and hormones under hypoxic condition should be noted. Concentrations of FFA and glycerol are taken as indicators of FFA and glycerol turnover because there is often a positive correlation between concentration and turnover rates. However, this relationship may vary under hypoxic conditions, and thus the mechanism for changing fatty acid utilization in acute hypoxia is more complex than concentration-driven muscular uptake [7]. Further research is necessary to clarify fatty acid production and consumption during exercise and postexercise recovery period at moderate altitude using direct leg arteriovenous difference measurement. In the present study, the subjects were men only. Sex difference in substrate utilization during exercise at high altitude (4300 m) has been revealed [10,13,21], although the changes during postexercise recovery at altitude are unclear. Therefore, it is speculated that substrate utilization during exercise and recovery period at moderate altitude in women differs from that in men. We set endurance exercise for 30 minutes and a simulated altitude for 2000 m. The effects of endurance exercise at moderate altitude on substrate utilization could differ considerably depending on the duration of exercise and magnitude and pattern of hypoxic exposure. Therefore, more studies are needed to elucidate the changes in substrate utilization during exercise and postexercise recovery at different durations of exercise, different levels of moderate altitude (e.g., 1000-, 1500-, or 2500-m altitude), and different patterns of hypoxic exposure (e.g., prolonged or intermittent).

In conclusion, moderate-intensity exercise at a simulated moderate altitude (2000 m) causes a higher RER during exercise and recovery compared with values at sea level during exercise at the same relative exercise intensity. In addition, lower FFA and glycerol concentrations and higher plasma Epi and NE concentrations appeared during exercise and postexercise recovery at moderate altitude. These results suggest that carbohydrate utilization is increased during exercise and postexercise recovery period in moderate hypoxia as compared with normoxia. It is also suggested that moderate hypoxia influences the changes in circulating metabolites and hormones in terms of substrate metabolism during exercise and the recovery.

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