# Augmented Carbohydrate Oxidation under Moderate Hypobaric Hypoxia Equivalent to Simulated Altitude of 2500 m

Kazushige Goto,<sup>1</sup> Takuma Morishima,<sup>1</sup> Kazumichi Kurobe,<sup>2</sup> Zhong Huang<sup>3</sup> and Futoshi Ogita<sup>4</sup>

<sup>1</sup>Faculty of Sport and Health Science, Ritsumeikan University, Kusatsu, Shiga, Japan

<sup>2</sup>Faculty of Business, Sports Management Course, Hannan University, Matsubara, Osaka, Japan

<sup>3</sup>Department of Sports Sciences, Japan Institute of Sports Sciences, Tokyo, Japan

<sup>4</sup>Department of Sports and Life Science, National Institute of Fitness and Sports in Kanoya, Kanoya, Kagoshima, Japan

Hypoxia itself stimulates glucose uptake mediated by a mechanism independent of insulin. However, whether moderate hypoxia causes similar metabolic effect in humans remains unclear. The present study aimed to determine glycemic regulation following glucose load at a simulated moderate altitude of 2,500 m. Eight healthy young males (mean  $\pm$  standard error: 24  $\pm$  1 years; 171.3  $\pm$  1.6 cm; 66.9  $\pm$  3.7 kg; 22.8  $\pm$  1.0 kg/m<sup>2</sup>) consumed 75 g of glucose solution under either hypobaric condition (560 mmHg) or normobaric condition (745 mmHg). In the hypobaric chamber, the oxygen partial pressure is proportionally reduced with a reduction of atmospheric pressure, consequently leading to the hypoxic condition. Plasma glucose and serum insulin concentrations increased significantly following glucose load in both conditions (P < 0.05). However, no significant interaction (condition × time) or main effect for condition was observed. There were no significant differences in serum glycerol, plasma epinephrine, or plasma norepinephrine concentrations between the two conditions. No significant differences between the conditions were observed in changes in VO<sub>2</sub> or VCO<sub>2</sub>. However, the hypobaric condition showed significantly higher respiratory exchange ratio  $(\dot{V}CO_2/\dot{V}O_2)$  at 90 and 120 min following glucose load (P < 0.05 vs. normobaric condition), suggesting that carbohydrate oxidation following glucose load was enhanced in moderate hypobaric hypoxia. In conclusion, acute exposure to moderate hypobaric hypoxia significantly augmented carbohydrate oxidation following the glucose load, without affecting glucose or insulin responses. Thus, a short-time exposure to moderate hypotaric hypoxia may be beneficial for people with impaired glucose tolerance.

**Keywords:** carbohydrate oxidation; hypoxia; oral glucose tolerance test; respiratory exchange ratio; substrate metabolism

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## Introduction

Training at natural altitudes, or at a simulated altitude using a hypoxic chamber, is widely accepted to improve the endurance capacity of athletes (Dufour et al. 2006). More recently, researchers are focusing on the potential for highaltitude stays and/or training in hypoxic conditions to prevent metabolic syndrome. In an epidemiologic study (Mortimer et al. 1977), coronary heart disease mortality rates were lower in populations living at high altitudes (more than 1,220 m) compared with those living at lower altitudes (below 1,220 m). Serum high-density lipoprotein cholesterol levels were also higher in individuals living at high altitudes (i.e., between 3,000 m and 5,500 m) (Sharma 1990).

In a tightly controlled laboratory setting, Haufe et al.

(2008) reported that 4 weeks of endurance training in hypoxic condition ( $F_1O_2 = 0.15$ ) resulted in greater improvements in glucose tolerance in young males compared with the effects of an identical training regimen under normoxia. Insulin and muscle contractions both trigger the translocation of glucose transport protein (GLUT)-4 to the sarcolemma membrane (Winder 2001). Notably, hypoxia itself stimulates glucose uptake mediated by AMP-activated protein kinase (AMPK) and GLUT4 translocation, via a mechanism independent of insulin (Fryer et al. 2000; Hayashi et al. 2000). These findings led to the hypothesis that postprandial glucose uptake could be augmented under hypoxic condition. Kelly et al. (2010) recently tested this hypothesis by comparing glucose responses following oral glucose tolerance tests (OGTT) at a simulated altitude of 4,300 m (hypobaric condition) versus at sea level (normobaric con-

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e-mail: kagoto@fc.ritsumei.ac.jp

dition). The glucose response to a 75-g glucose load was significantly suppressed at the simulated altitude. However, a simulated altitude of > 4,000 m represents a severely hypoxic environment, and is associated with a high risk of acute mountain sickness or discomfort. Therefore, such altitudes may not be appropriate in large populations for the purposes of promoting health.

Therefore, the purpose of the present study was to investigate whether exposure to moderate hypoxia (equivalent to a simulated altitude of 2,000-2,500 m) is still of benefit for glycemic regulation. We determined the acute effects of exposure to moderate hypobaric hypoxia on metabolic and hormonal responses to a glucose load. In the present study, a hypobaric chamber was utilized (Kelly et al. 2010; Katayama et al. 2010). During the stay in the hypobaric chamber, the oxygen partial pressure is proportionally reduced with a reduction of atmospheric pressure, consequently leading to the hypoxic condition. Our hypothesis was that exposure to moderate hypobaric hypoxia would attenuate elevations of plasma glucose and serum insulin concentrations following a 75-g glucose load.

## Methods

**Subjects** 

Eight healthy young males participated in the present study [mean  $\pm$  standard error (SE): age = 24  $\pm$  1 years; height = 171.3  $\pm$  1.6 cm; weight = 66.9  $\pm$  3.7 kg; body mass index = 22.8  $\pm$  1.0 kg/m<sup>2</sup>]. Subjects had resided at sea level for their entire lives, and were either sedentary or recreationally active. None of athletes were included in the present study.

They were informed of the experimental procedures and possible risks involved in the study, and provided informed consent. The study was approved by the Human Research Committee of the National Institute of Fitness and Sports, Japan.

#### Experimental design

Subjects visited the laboratory twice during the experimental period: each visit was characterized either by simulated hypobaric hypoxia or normobaric normoxia. In the present study, a hypobaric chamber was used to generate hypoxic condition or normoxic condition (Kelly et al. 2010; Katayama et al. 2010).

Subjects ingested a 75-g glucose solution and rested in a chamber maintained at either hypobaric hypoxic condition (560 mmHg, simulated altitude of 2,500 m; hypobaric condition) or normobaric normoxic condition (745 mmHg, normobaric condition). The hypoxic chamber produces hypoxic condition by reducing atmospheric pressure (reduced oxygen partial pressure), and the level of oxygen concentration in the chamber is comparable to approximately 15.0% when applying to normobaric hypoxic condition (Morishima and Goto 2014). The normobaric condition was undertaken during the first visit, and was followed 7 days later by the hypobaric condition. We did not employ a crossover design because of the facility's limited availability. However, results were unlikely to be affected by the order of condition, because the majority of the subjects had a priori experience (several times in other studies) of exposure to hypobaric hypoxia. Subjects were requested to avoid strenuous exercise for 48 h prior to each visit. They were also asked to consume identical meals (breakfast, lunch, and dinner) before each visit.

On the day of the experiment, the subjects visited the laboratory following an overnight fast, and rested for 30 min prior to collection of the first blood sample and measurement of baseline respiratory parameters. Once subjects were situated within the chamber, blood sampling and respiratory measurements were repeated. The subjects then ingested a 75-g glucose solution (225 ml), and rested for 2 h on a chair to monitor the time course of changes in glycemic and metabolic responses. All measurement staff also stayed in a chamber throughout the measurements (except for measurement before entering a chamber).

#### Determination of hormonal responses

Venous blood samples were continuously collected from an antecubital vein on five occasions: before (Pre) and 10 min after entering the chamber (0 min), and at 30, 60, 90, and 120 min postglucose load. Serum and plasma samples were obtained by centrifugation for 10 min, and were stored at -80°C prior to analysis. Serum free fatty acid (FFA) concentrations were measured using a commercially available enzymatic colorimetric assay kit (NEFA-HRII; Wako Pure Chemical Industries, Osaka, Japan). The intra-assay CV was 0.8%. Serum glycerol concentrations were measured using a commercially available kit from Cayman Chemical Company (Ann Arbor, MI, USA). The intra-assay CV was < 5.0%. Plasma epinephrine and norepinephrine concentrations were measured using high-performance liquid chromatography (HLC-725CAII; Tosoh, Tokyo, Japan). The intra-assay CV values were 1.4% for plasma epinephrine and 2.6% for plasma norepinephrine, respectively. Serum insulin concentrations were measured using a commercially available kit (Architect insulin; Abbott Japan, Tokyo, Japan). The intra-assay CV was 1.5%.

Blood lactate concentrations were measured immediately following blood collection using an automatic lactate analyzer (Lactate Pro; Arkray Inc., Kyoto, Japan). Plasma glucose concentrations were measured using an enzymatic method. The intra-assay CV was 0.9%.

## Cardiorespiratory measurements

Oxygen uptake ( $\dot{V}O_2$ ), carbon dioxide output ( $\dot{V}CO_2$ ), and expired minute ventilation ( $\dot{V}E$ ) were measured by Douglas bag method, with a system similar to that used in our previous study (Katayama et al. 2010). Expired gas volume was measured using a dry gas meter (NDS-2A-T; Shinagawa Dev, Tokyo, Japan). The O<sub>2</sub> and CO<sub>2</sub> fractions were analyzed using an automatic gas analyzer (Vmax 29c; Sensor Medics, CA, USA). The respiratory exchange ratio (RER) was determined from  $\dot{V}O_2$  and  $\dot{V}CO_2$  values ( $\dot{V}CO_2/\dot{V}O_2$ ). Heart rate (HR) was recorded using a three-lead electrocardiogram (Tango+; Suntec Medical, Morrisville, NC). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also monitored using the same equipment. Percutaneous oxygen saturation (SpO<sub>2</sub>) was measured using a finger pulse oximeter (PULSOX-Me300; TEIJIN LIMITED, Osaka, Japan) placed on the tip of the right forefinger.

#### Statistical analysis

Data are expressed as means  $\pm$  SE. Two-way [condition (hypobaric condition, normobaric condition) × time (Pre, 0 min, 30 min, 60 min, 90 min, 120 min)] repeated measures analysis of variance (ANOVA) was initially used to determine interaction and main effects. When ANOVA revealed a significant interaction or main effect, the Tukey-Kramer test was used for *post hoc* analysis to assess differences between condition or times. For all tests, *P* < 0.05 was considered to indicate significance.

## Results

## Cardiovascular parameters

Table 1 shows the changes in cardiovascular parameters before and after glucose load. In the hypoxic condition, SpO<sub>2</sub> values decreased rapidly following subjects' entry into the chamber, and remained significantly lower compared with the normobaric condition (P < 0.05 for condition  $\times$  time). HR at baseline was significantly lower in the hypobaric condition than in the normobaric condition (P < 0.05). However, the difference between the two conditions was not observed after entering chamber (at 0 min in Table 1). HR was significantly elevated following glucose load in both conditions (P < 0.05), and there was no significant interaction (condition  $\times$  time) for HR response. Systolic and diastolic blood pressure decreased following glucose load in both conditions. There were no significant interactions (condition × time) for either systolic or diastolic blood pressure, indicating that blood pressure responses were similar in both conditions.

## Circulating metabolites

Fig. 1 shows the time course of changes in plasma glucose and serum insulin concentrations. Plasma glucose and serum insulin concentrations increased significantly following glucose load in both conditions (P < 0.05). However, there were no significant interactions (condition × time) or main effects for condition for either plasma glucose or serum insulin response. When the magnitudes of plasma glucose and serum insulin responses following glucose load were compared with respect to the area under the curve (AUC), AUC values did not differ significantly between the two conditions (for either plasma glucose or serum insulin).

Fig. 2 shows the time course of changes in serum FFA and glycerol concentrations. Serum FFA concentrations decreased rapidly following glucose load in the two conditions (P < 0.05), and the time-courses of changes were similar in both conditions (P > 0.05, for interaction and main effect of condition). Serum glycerol concentrations decreased slightly following glucose load, and changes over time were not statistically significant in either condition. Moreover, there was no significant interaction (condition × time) or main effect of condition for serum glycerol response.

Fig. 3 shows the time courses of changes in plasma epinephrine and norepinephrine concentrations. In the hypobaric condition, plasma epinephrine concentrations slightly, but significantly increased following subjects' entry into the chamber (P < 0.05). However, no significant interaction (condition × time) or main effect of condition was observed for plasma epinephrine response. Similarly, for plasma norepinephrine response, no significant interaction (condition × time) or main effects of condition or time were observed.



Fig. 1. Plasma glucose and insulin concentrations. Time courses of changes following 75-g glucose load are presented. Values are means  $\pm$  SE. \*P < 0.05 vs. Pre.

	Trial	Pre	0 min	30 min	60 min	90 min	120 min
SpO <sub>2</sub> (%)	Normobaric condition	$97.9\pm0.4$	$97.3\pm0.4$	$97.1\pm0.4$	$96.9\pm0.4$	$96.8\pm0.2$	$97.1\pm0.1$
	Hypobaric condition	$97.8\pm0.3$	$92.0\pm0.6^{\ast\dagger}$	$91.1\pm0.8^{*\dagger}$	$91.9\pm0.5^{*\dagger}$	$93.0\pm0.5^{*\dagger}$	$93.6\pm0.3^{*\dagger}$
HR (beats/min)	Normobaric condition	$65 \pm 4$	$67 \pm 4$	$67 \pm 3$	$70 \pm 4$	$73 \pm 4*$	$72 \pm 4*$
	Hypobaric condition	$57\pm2^\dagger$	$65 \pm 4$	$69 \pm 4*$	$72 \pm 4*$	$73 \pm 4*$	$74 \pm 4*$
SBP (mmHg)	Normobaric condition	$120 \pm 3$	$113 \pm 3$	$114 \pm 4$	$114 \pm 4$	$111 \pm 4*$	$109 \pm 3*$
	Hypobaric condition	$117 \pm 3$	$114 \pm 1$	$117 \pm 3$	$115 \pm 3$	$110 \pm 2$	$113 \pm 4$
DBP (mmHg)	Normobaric condition	$79 \pm 2$	$77 \pm 3$	$73 \pm 3$	$69 \pm 3*$	$72 \pm 2$	$72\pm3$
	Hypobaric condition	$78 \pm 2$	$74 \pm 3$	$69 \pm 2*$	71±3	$71 \pm 2$	$72\pm2$

Table 1. Changes in cardiovascular parameters throughout the experimental period.

Values are means  $\pm$  SE. \**P* < 0.05 vs. Pre. †*P* < 0.05 vs. Normobaric condition.



Fig. 2. Serum free fatty acid and glycerol concentrations. Time courses of changes following 75-g glucose load are presented. Values are means  $\pm$  SE. \*P < 0.05 vs. Pre.

## Oxygen uptake and substrate oxidation pattern

Fig. 4 shows the time course of changes in  $\dot{V}O_2$  and RER (calculated as  $\dot{V}CO_2/\dot{V}O_2$ ). No significant interaction or main effects of condition or time were observed for  $\dot{V}O_2$ response. Additionally, no significant interaction or main effect of condition was observed for  $\dot{V}CO_2$  response (P < 0.05, main effect of time). The RER increased gradually following glucose load in both conditions. However, elevation of the RER in response to the glucose load was significantly greater in the hypobaric condition (P < 0.05 for interaction). When comparing the RER response between the two conditions, the RER values at 90 and 120 min postglucose load were significantly higher in the hypobaric condition compared with the normobaric condition (P <0.05). The time course of changes in VE (BTPS) was not significantly different between the hypobaric and normobaric conditions (P > 0.05 for interaction and main effect of condition).

### Discussion

In the present study, hypobaric exposure at a simulated altitude of 2,500 m did not affect the glucose response to a 75-g glucose load. However, the resting RER following glucose load was significantly higher in the hypobaric condition compared with the normobaric condition, suggesting



Time courses of changes following 75-g glucose load are presented. Values are means  $\pm$  SE. \*P < 0.05 vs. Pre.

that carbohydrate oxidation was enhanced under moderately hypobaric hypoxic condition. These results are not consistent with a previous finding by Kelly et al. (2010) of marked attenuation of blood glucose elevation following glucose load at a simulated altitude of 4,300 m. Therefore, the influence of hypobaric exposure on glycemic regulation appears to be dependent on the severity of the hypoxia.

Both exercise training in hypoxia, and prolonged stays at altitude have been shown to improve insulin sensitivity (Schobersberger et al. 2003; Benso et al. 2007; Haufe et al. 2008; Kelly et al. 2010). Schobersberger et al. (2003) demonstrated that a 3-week stay at an altitude of 1,700 m improved homeostatic model assessment of insulin resistance and glycemic control in response to an oral glucose load. Lee et al. (2003) reported that 3 days of mountain hiking at altitude of 2,400 m significantly improved glucose tolerance in sedentary subjects. Furthermore, the same group revealed that a 25 day of hiking activity at altitude of 2,200-3,800 m improved insulin sensitivity in mildly obese populations with drug abuse history (Lee et al. 2004). Brooks et al. (1991) reported that acclimatization to the high altitude for 3 weeks also enhanced glucose utilization at rest and during exercise. More recently, Kelly et al. (2010) compared glycemic regulation following a 75-g glu-



Fig. 4. Resting oxygen uptake and respiratory exchange ratio. Time courses of changes following 75-g glucose load are presented. Values are means  $\pm$  SE. \*P < 0.05 vs. Pre. <sup>†</sup>P < 0.05 vs. the corresponding value in the normobaric condition.

cose load between a simulated altitude of 4,300 m (hypobaric hypoxia) and normobaric condition in healthy subjects. Exposure to severe hypobaric hypoxia markedly lowered glucose elevation following a 75 g glucose load compared with the elevation elicited under normobaric condition, suggesting that insulin sensitivity was acutely improved. Collectively, the influence of exposure to hypoxia on glycemic regulation and metabolic responses is apparent under condition of severe hypoxia (i.e., altitudes  $\geq$ 4,000 m). However, because such high altitudes increase the risk of acute mountain sickness or discomfort, being exposed to them may not be appropriate for the prevention/ treatment of metabolic syndrome in untrained individuals or patients with impaired glucose tolerance. Therefore, the present study aimed to determine whether acute exposure to moderate hypobaric hypoxia (i.e., a simulated altitude of 2,500 m) was still capable of lowering elevations of blood glucose after a glucose load. Contrary to our hypothesis, no differences were observed in glucose response to the 75-g glucose load between the two conditions. Moreover, time courses of changes in serum insulin concentrations were similar, with no difference between the two conditions at any point. Kelly et al. (2010) demonstrated that elevations in serum insulin concentrations following glucose load were significantly attenuated at a simulated altitude of 4,300 m (hypobaric hypoxia). Furthermore, 60-min exposure to moderate hypoxia ( $F_iO_2$ : 14.6%) improved acutely insulin sensitivity in patients with type 2 diabetes (Mackenzie et al. 2011, 2012). However, we were not able to find these effects under moderate hypoxia in young, healthy adults. A main reason for different findings from previous studies would include an experimental setting that we selected moderate hypobaric hypoxia (a simulated altitude of 2,500 m) and young subjects with normal glucose tolerance.

We also monitored the time course of changes in substrate oxidation (fuel utilization) patterns during the 120 min following glucose load. Resting  $\dot{V}O_2$  and  $\dot{V}E$  did not change significantly following glucose load, with no difference between the two conditions. Unexpectedly, HR at baseline was significantly lower in the hypobaric condition. The difference appeared to be transient, and the relatively small number of subjects (N = 8) might be related to the difference. However, we think that the lower HR at baseline in the hypobaric condition did not affect the present results because the difference between the two conditions was not observed immediately before the glucose ingestion. The RER value following glucose load was significantly higher in the hypobaric condition compared with the normobaric condition, indicating that carbohydrate oxidation was augmented at a simulated altitude of 2,500 m. Enhanced carbohydrate oxidation following exposure to moderate hypoxia accords with previous studies demonstrating increased glucose utilization at rest and during exercise under an altitude of 4,300 m (Brooks et al. 1991). We have previously observed that the RER was significantly higher during the post-exercise period, with a simulated altitude of 2,000 m (Katayama et al. 2010). Hypoxia itself stimulates GLUT4 translocation to the plasma membrane (Youn et al. 1994), and it promotes glucose uptake via an insulin-independent pathway (Youn et al. 1991, 1994). Although we are not able to present detailed glucose kinetics (e.g., glucose appearance, disappearance) due to the lack of measurements using a tracer, it is plausible that the glucose supply from liver glycogen was not affected by hypoxia, because there were no differences in plasma epinephrine or norepinephrine concentrations between the conditions. One limitation of the present study pertains to the relatively short duration (2 h) of exposure to hypobaric hypoxia. However, recent data from our laboratory indicated that 7 h of exposure to moderate hypoxia ( $F_iO_2$ : 15.0%) did not affect postprandial blood glucose and insulin responses (Morishima and Goto 2014). Therefore, relatively short exposure duration would not markedly affect the present results.

In conclusion, 2-h exposure to hypobaric hypoxia, at a simulated altitude of 2,500 m, did not affect glucose or insulin responses to a 75-g glucose load. However, carbo-hydrate oxidation following glucose load was significantly augmented under moderate hypobaric hypoxia. From a clinical perspective, exposure to moderate hypoxia may be a realistic method of treating obesity or promoting health.

Because the present findings suggest that the influence of moderate hypoxia on hyperglycemia is minor, at least in healthy subjects, the synergetic effect of moderate hypoxia in combination with exercise needs to be assessed in future studies.

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### **Conflict of Interest**

The authors declare no conflict of interest.

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