

# The Influence of Ingestion Glucose Beverage Before Graded Exercise to Exhaustion on Saliva IgA Concentration in Hypoxia and Normoxia Environment

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## Abstract

In addition to exercise, hypoxia environment might induce higher immune stress. The present study was to investigate the ingestion of glucose before graded exercise to exhaustion in hypoxia environment on immune responses. Eight healthy college students (age:22.1±0.4 yrs; weight: 71.6±2.3kg; height:171.4±2.3cm ) participated in this study. Subjects were ingested either 75g/250ml glucose (G) or placebo (P) drink 30 min before exercise on a cycle ergometer in the hypoxia (H) or normoxia (N) chamber. Exercise intensity was initiated at 75W and was increased 25W every 3 min until volitional fatigue. Saliva samples were collected at fasting, before exercise, immediately after exercise, and 1 hour after exercise. The results showed that the saliva IgA concentrations were slightly increased after the ingestion of glucose but there were no significant difference between the trials. There were no difference in salivary flow rate and adjusted IgA concentrations. The current data suggested that the ingestion of glucose before a single bout graded exercise to exhaustion did not alter salivary immune response in hypoxia environment.

**Keywords:** Hypoxia, Saliva IgA, Saliva flow rate

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## Introduction

Saliva immunoglobulin A (IgA) has been considered as the first line of defense to microorganisms in the lumen of the respiratory tract and gut [1]. A decreased saliva IgA has been thought to be correlated with an increase in the incidence of upper respiratory tract infection (URTI) when human subjects perform intensive prolonged exercise [2, 3]. To reduce the phenomenon, ingestion of carbohydrate beverage has been reported effectively attenuate exercise-induced immune suppression [2, 4-6].

Exercise in hypoxia environment has been widely use for endurance training. It has been known that training in hypoxia might induce various immune stresses [7]. In addition, exercise

in hypoxia environment was found to increase the rate of carbohydrate utilization at the similar exercise intensity [8]. The carbohydrate availability was decreased when exercise in hypoxia which might induce higher immune stress. Therefore, the current study was to examine the effect of glucose drink before exercise in hypoxic and normoxic environment on saliva IgA concentration.

## Methods

### Subjects

Eight healthy, active male college students voluntarily participated in this study (age:22.1±0.4yrs; weight: 71.6±2.3kg; height:171.4±2.3cm ). Subjects were given their informed consent before fully understanding the procedure and possible risk of the study. The protocol was approved by Ethical Committee of National Taiwan Sports University (Taichung).

### Design

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Subjects were asked to complete four experimental trials in a randomized order. Subjects ingested either glucose drink (75g/250ml)(G) or placebo drink (P) 30min before exercise on a cycle ergometer either in the hypoxia (H) or normoxia (N) environment. Salivary samples were collected for evaluating the immune responses.

### Protocol

Subjects were asked to report to the laboratory at 8-9 in the morning after 10 h overnight fasting. After weighing, subjects were asked to drink either glucose drink (75g/250ml) or placebo drink before going into the chamber (Colorado Altitude Training, Boulder, Colorado). The chamber was adjusted either in hypoxia (15% O<sub>2</sub>) or normal air condition (20.9% O<sub>2</sub>). Subjects then rested for 30 min before exercise on a cycle ergometer (Lode, Netherland). Ten min before exercise, subjects were asked to enter the chamber for a standardized warm up. Then, subjects were asked to exercise at 70 rpm pedal speed on the cycle ergometer. The exercise intensity initiated from 75W and increased 25W every 3 min until volitional fatigue. The expired gas samples were analyzed using a breath-by-breath gas analyzer (Cortex, Metabmax 3B, Germany). The heart rate was monitored throughout the exercise period (Polar WM21, Finland). After exercise, subjects were rested in normoxia environment for an hour. Saliva samples were collected for 2 min at fasting, before exercise, post exercise and 1 hour post exercise.

### Sample collection and analysis

Subjects were asked to dribble unstimulated saliva into a sterile, pre-weighing bijou tube for 2 minutes. Then the tubes were weighing again for calculating the saliva flow rate. The density of saliva was assumed to be 1g/ml (Chicharro et al 1998). Saliva samples were then frozen at -20°C for later analysis. Saliva IgA concentrations were analyzed using sandwich ELISA method [9]. Briefly, plates were coated with rabbit anti-human IgA (Sigma I-8760). Saliva samples were assayed at a dilution of 1/500 and were performed in triplicate against the rang (0-600ug/l) of human IgA standards (Sigma, I-2636). Standards and diluted saliva samples were incorporated into the pre-coated anti-IgA micro-well plate and were incubated for 90min at room temperature. Following a wash step, peroxidase conjugated goat anti-human IgA (Sigma, A4156) was added and was incubated for further 90min at room temperature. The plate then washed again. A 100 ul of ABTS solution was then added to each well. The plate was incubated at room temperature for 30 minutes before absorbance was measured at 405 nm (Bio Rad, USA).

### Statistical analysis

All collected data was presented as Mean±S.E.M. Changes in saliva IgA concentrations and saliva flow rate were analyzed by a two-way ANOVA with repeated measures. The difference between baseline and other time points in saliva IgA concentrations were analyzed by post hoc Tukey's test. A P value less than 0.05 was considered statistically significant.

## Results

Subjects' maximal oxygen uptake (VO<sub>2max</sub>), exhaustion time (ET), maximal heart rate (HR) and respiratory ration (RER) at exhaustion during 4 trials were shown in Table 1. There were no differences in VO<sub>2max</sub>, ET, HR and RER between trials (p>0.05). There were no significant differences in saliva IgA concentrations at each correspondence time between trials (Fig 1, p>0.05). The IgA concentrations were slightly increased at post-exercise compare to fasted state in NP, HP and HG trials but not significant. Saliva flow rate was no difference between trials (Fig 2, p>0.05). Normoxia trials were showed a trend of decline in saliva flow rate at post-exercise compared to that of at pre-exercise. We adjusted the saliva IgA concentrations with saliva flow rate (Fig 3), there were no differences found between trials (p>0.05). When we set saliva IgA concentrations at fasted state as 100%, the data showed the trend of increased during post-exercise when compared to fast (Fig 4, p>0.05). Similarly, when fast saliva flow rate set as 100%, the data showed a trend of decline of saliva flow rate at post-exercise (Fig 5, p>0.05). There were no differences in saliva flow rate changes during the trials (Fig 6, p>0.05).

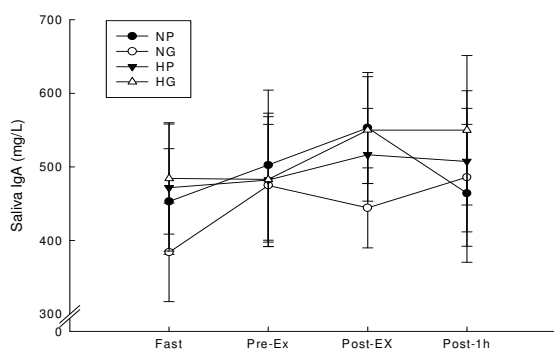


Figure 1. Saliva IgA concentrations during the four experimental trials.

NP: normoxia with placebo trial; NG: normoxia with glucose trial; HP: hypoxia with placebo trial; HG: hypoxia with glucose trial.

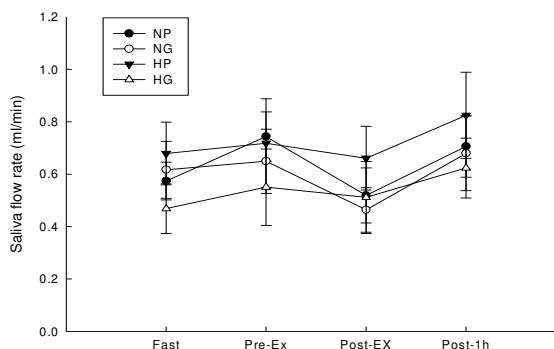


Figure 2. Saliva flow rate during the four experimental trials.

NP: normoxia with placebo trial; NG: normoxia with glucose trial; HP: hypoxia with placebo trial; HG: hypoxia with glucose trial.

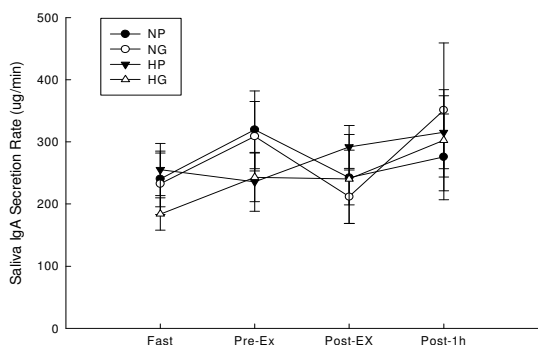


Figure3. Saliva IgA secretion rate during the four experimental trials. NP: normoxia with placebo trial; NG: normoxia with glucose trial;HP: hypoxia with placebo trial; HG: hypoxia with glucose trial.

Table 1. Subjects’ maximal oxygen uptake ( $VO_{2max}$ ), exhaustion time (ET), maximal heart rate (HR) and respiratory ration (RER) at exhaustion during 4 trials. NP: normoxia placebo group; NG: normoxia glucose group;HP: hypoxia placebo group; HG: hypoxia glucose group

	NP	NG	HP	HG
$VO_{2max}$ (mi/kg/min)	43.5±3.7	43.0±2.5	40.0 ±2.8	40.1 ±1.6
ET (min)	22.2±1.4 (17-25)	21.7±1.4 (16-24)	20.6 ± 1.1 (16-25)	21.2 ± 1.0 (17-25)
HR (beats/min)	183.1±4.4	185.6±4.9	181.0±3.8	183.3±3.6
RER	1.12±0.05	1.13±0.06	1.07±0.06	1.08±0.08

**Discussion**

The main findings of the current study were the ingestion of glucose 30 min before graded exercise to exhaustion did not significantly alter saliva IgA concentrations and saliva flow rate in hypoxic and normoxic environments. Previous studies have shown similar results that the saliva IgA concentrations unchanged immediately after a single bout of exercise [10, 11]. To our knowledge, the present study was the first study to examine the effect of carbohydrate ingestion before exercising on mucosal immune function in the acute exposure of hypoxic environment.

Studies on saliva IgA concentrations immediately after exercise have shown inconclusive results; some reported increased [12, 13], unchanged [10, 11], or decreased [14-16] in saliva IgA concentrations. The current study showed a large scale of variation between subjects (Fig 1 & Fig 4). As a result, no differences were found between trials, although there was a trend of increased IgA concentration after exercise. In addition to large variation, the exercise duration might also affect the current results. The exercise protocol of previous studies mostly were prolonged strenuous exercise and last for over 1.5h – 4h [4, 17]. Subjects exercised for 17-25 min in hypoxia trials and 16-25 min in normoxia trials. Although subjects reached volitional fatigue during the graded cycling test, the

exercise duration were merely reached one of third exercise duration when compared to the previous studies. Therefore, this might be the reason that we could hardly find the changes after exercise. It might be also explained the huge variation between subjects were found in the current study.

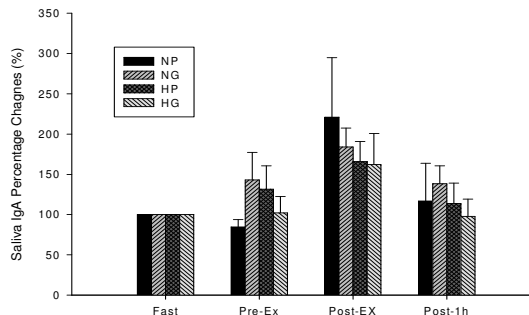


Figure 4. Saliva IgA concentration percentage changes compared to Fast state during four experimental trials. Fast state set as 100%. NP: normoxia with placebo trial; NG: normoxia with glucose trial; HP: hypoxia with placebo trial; HG: hypoxia with glucose trial.

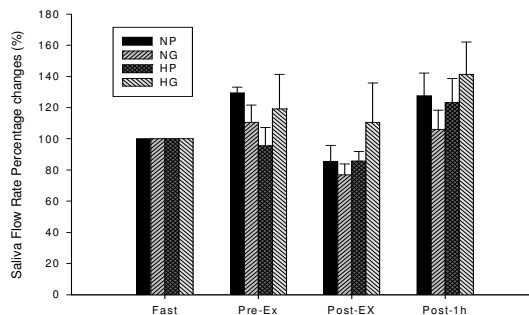


Figure 5. Saliva flow rate percentage changes compared to Fast state during four experimental trials. Fast state set as 100%. NP: normoxia with placebo trial; NG: normoxia with glucose trial; HP: hypoxia with placebo trial; HG: hypoxia with glucose trial.

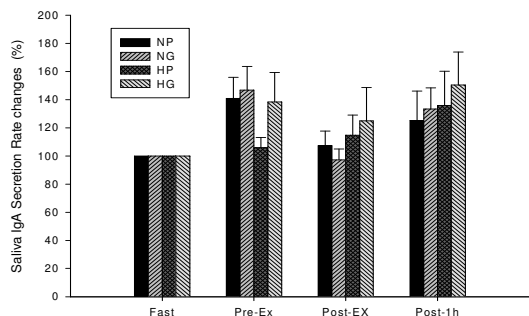


Figure 6. Saliva IgA secretion rate percentage changes compared to Fast state during four experimental trials. Fast state set as 100%. NP: normoxia with placebo trial; NG: normoxia with glucose trial; HP: hypoxia with placebo trial; HG: hypoxia with glucose trial

Saliva flow rate were thought to be an independent salivary defensive factor to oral health [18]. Several studies have demonstrated that the saliva flow rate was decreased after strenuous prolonged exercise [19-21]. The current study showed a trend of decreased saliva flow rate after exercise in NP, NG and HP trials which was agreed with previous results. Ingestion of carbohydrate before exercise was thought to be reduced exercise-induced immune stress [2, 4-6]. The current study did not find any differences in saliva between glucose and placebo trials. Similar results were reported by Li & Gleeson (2005). They found ingestion of carbohydrate before prolonged exercise did not induce different effects on oral immunity. However, they reported a lower stress hormone responses when subjects ingestion of carbohydrates during the second bout of exercise in a day. Our results were agreed carbohydrate ingestion prior exercise in hypoxia or normoxia environment did not alter oral immunity. However, we did not measure stress hormones, it was difficult to speculate the outcome, especially when subjects exercised in hypoxia environment. In addition, the current exercise protocol was 16-25min only which did not hugely challenge muscle glycogen storage. The carbohydrate availability was not depleted. This might be one of the reasons that we could not found the difference in saliva IgA concentration changes. The current data suggested that the ingestion of glucose beverage before a short single bout of graded exercise to exhaustion in hypoxia environment did not hugely alter salivary immune function including saliva IgA concentration and saliva flow rate.

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