

Short Report

The Effect of a High-carbohydrate Diet on TNF- α Levels in Response to LPS after Exhausting Exercise in Rats

Hiromi KITAMURA*, Kumiko MINATO* and Hiromi YANO**

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Abstract

Although strenuous prolonged exercise is associated with suppressed immune function, the effect of a carbohydrate diet on this response is unknown. The aim of the present study was to investigate the effects of feeding a high-carbohydrate diet for a week on immune responses after exhaustive exercise. Female F344 rats were divided into four groups: a high-carbohydrate (CHO) diet exercised group, a high-CHO diet non-exercised group, a standard diet exercised group, and a standard diet non-exercised group. Rats were fed each diet for 7 days, and the exercised rats ran then until exhausted on a treadmill. The non-exercised rats were maintained in a sedentary condition. Then, all rats were injected with lipopolysaccharide (LPS; 1 mg/kg, i.v.) immediately after exercise or in the sedentary condition. Fasting plasma glucose in the high-CHO diet rats was significantly higher than in the standard diet rats (191 ± 5 vs. 163 ± 10 mg/dl, $p < 0.05$). However, exhaustive exercise significantly reduced tumor necrosis factor (TNF) - α response to LPS in both diet groups ($p < 0.05$). Also, an exercise-induced high corticosterone concentration was observed in both diet groups ($p < 0.05$). Furthermore, no significant effect of high-CHO diet was found on spontaneous activity, an index of sickness behavior, before and after the exercise and LPS treatment. Taken together, these results indicated that feeding a high-CHO diet for a week does not induce an increased response of TNF- α to LPS challenge after exhaustive exercise.

Introduction

Strenuous prolonged exercise is associated with suppressed immune function. Endurance athletes have an increased risk for upper respiratory tract infection[1]. Pedersen and Ullum[2] have also suggested that immunosuppression is induced immediately after strenuous exercise. This interesting theory is called "Open Window". In fact, an animal experiment carried out by Bagby et al.[3] showed that exercise markedly suppressed the systemic tumor necrosis factor (TNF), a pro-inflammatory cytokine, in response to lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria. In addition, LPS also induces sickness behavior, such as suppression of spontaneous activity[4].

In contrast, previous studies have reported that the ingestion of carbohydrate (CHO) beverage is beneficial for prolonged exercise performance[5,6]. Furthermore, recent evidence suggested that the ingestion of

* Department of Health and Nutrition, Wayo Women's University Ichikawa, Chiba 272-8533, Japan
E-mail: matsuzaki@wayo.ac.jp

** Department of Health and Sports Science, Faculty of Health Science and Technology
Kawasaki University of Medical Welfare, Kurashiki, Okayama 701-0193, Japan

CHO beverages before and during exercise was associated with maintained immune function[7]. Although it is possible that CHO beverages help to attenuate exercise-induced immunosuppression, it is poorly understood whether prolonged ingestion of a high-CHO diet influences immune responses to strenuous exercise. It was also unknown whether a high-CHO diet accelerates spontaneous physical activity after exhausting exercise and LPS injection.

Hence, the aim of the present study was to examine the effects of a prolonged feeding of a high-CHO diet on the TNF- α response to LPS after exhausting exercise in rats. In addition, spontaneous activity after injection of LPS was examined.

Materials and Methods

Animals

Female 7-week-old F344 rats (n=32, Charles River Lab., Japan) were housed in a controlled environment (22.5 \pm 0.5 $^{\circ}$ C, 12:12-h light-dark cycle) and given water ad libitum. Before the experiments, all rats were run on a treadmill at 15 m/min for 15 min on a 15% grade during a 3-day acclimatization period. The experimental procedures followed guidelines set forth in the Care and Use of Animals in the Field of Physiological Sciences approved by the Council of the Physiological Society of Japan.

Diets

A standard diet [AIN93M (Oriental Yeast, Japan); Protein (P): 14.2% (w/w), Fat (F): 4.0% (w/w), Carbohydrate (C): 72.1% (w/w)] and a high-carbohydrate diet [modified AIN93M; P: 14.2% (w/w), F: 0.0% (w/w), C: 81.1% (w/w)] were used. The energy content per weight was the same for both diets.

Experimental protocol

After adjustment to the housing conditions, rats were divided into four groups: a high-CHO diet exercised group (CHO+Ex, n=5), a high-CHO diet non-exercised group (CHO+R, n=5), a standard diet exercised group (STD+Ex, n=5), and a standard diet non-exercised group (STD+R, n=6). Rats were fed the standard or high-CHO diet for 7 days. Feed intake of the STD groups was adjusted according to that of the CHO groups so that intakes were comparable during the period. All rats were fasted on the eve of the experiment.

The exercised rats ran until exhausted on a treadmill at gradually increasing speeds of 10 to 36 m/min at a 15% grade. Exhaustion was defined as the point at which the rat refused to run despite being given a mild electric shock. The electric shock was used only near the end of the run. Running time in the CHO+Ex group was similar to that in the STD+Ex group (130 \pm 15 and 137 \pm 12 min, respectively). All non-exercised rats were maintained in a sedentary condition for 2h. Immediately after exercise or the sedentary control condition, rats were lightly anesthetized with ether and injected with 1 mg/kg LPS (*E.coli*. 055: B5, Sigma, USA) in the iliac vein[3]. Blood samples were obtained from the abdominal vein under pentobarbital (60 mg/kg) general anesthesia, immediately and 1h after LPS injection. Samples were collected in EDTA-coated tubes and immediately placed on ice. Plasma was obtained by centrifugation of whole blood and stored at -80° C until assayed.

In the second experiment, rats were allowed free access to a running wheel (Yamashitagiken, Japan) and fed a standard (STD: n=5) or high-CHO (CHO: n=6) diet in the same way as the first experiment. After

seven days, all rats were run on a treadmill until exhausted, injected with 1 mg/kg LPS i.v. under anesthesia, and then returned to the cages with running wheels and fed a standard diet for 7 days. Spontaneous activity was recorded daily and evaluated relative to the average for 3 day period immediately prior to the experiment.

Assay for plasma glucose, TNF- α and corticosterone concentrations

Glucose was measured by an enzymatic method. TNF- α was analyzed by enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (BioSource International, Camarillo, USA). The minimum detectable concentration of TNF- α was < 4 pg/ml. Corticosterone was analyzed by a radioimmunoassay at SRL Inc (Japan)[8].

Statistics

Data are expressed as mean \pm SEM. Statistical analyses were performed using an analysis of variance procedure (Stat View for Windows version 5.0). The data on plasma TNF- α , corticosterone and glucose concentrations were tested with one-way ANOVA followed, in appropriate cases, by *post hoc* test with Fisher's protected least-significant difference (Fisher's PLSD) test. The data of running times were tested with Student's *t*-test, and those for spontaneous activity were tested with two-way ANOVA. *P* values < 0.05 were considered statistically significant.

Results and Discussion

To clarify the effect of a high-CHO (C: 85.0%) diet, plasma glucose concentrations in fasting rats were tested after they had been on a high-CHO diet or standard diet for seven days. The fasting glucose concentrations in the plasma from high-CHO diet rats was significantly higher than those from standard diet rats (191 ± 5 vs. 163 ± 10 mg/dl, $p < 0.05$). Accordingly, it was hypothesized that exhaustive exercise-induced TNF- α suppression in response to LPS challenge might be attenuated by a high-CHO diet because a previous study had reported that high plasma glucose concentrations attenuated immunosuppression after exercise[3].

However, it was found that feeding a high-CHO diet for seven days did not attenuate the suppression of TNF- α production in response to LPS after exhaustive exercise (Fig. 1). In fact, plasma TNF- α concentrations in the STD+Ex group were significantly lower than those in the STD+R group ($p < 0.05$). Also, there was a significant difference in cytokine concentrations between the exercised and sedentary groups, despite the high-CHO diet ($p < 0.05$). There were no significant differences between the STD+Ex and CHO+Ex groups in plasma TNF- α concentration. Thus, although the high-CHO diet increased plasma glucose concentrations, the diet did not attenuate immunosuppression in response to LPS after exhaustive exercise. The reason the high-CHO diet did not attenuate immunosuppression might be that high levels of plasma glucose concentration were not maintained during and after the exhausting exercise. Indeed, plasma glucose concentrations were not significantly different between the high-CHO and STD diet groups after exhaustive exercise (55 ± 5 vs. 63 ± 7 mg/dl). During exercise, the ingestion of CHO has been shown to enhance endurance performance[5,6] and ingesting CHO beverages during exercise attenuates a decrease in plasma glucose levels[5,7]. Bishop et al. reported that ingesting CHO beverages during exercise attenuated immunosuppression (i.e. a decrease in LPS-stimulated elastase release from neutrophil), and the decrease in plasma glucose levels[7]. The intake of CHO beverages during exercise may inhibit

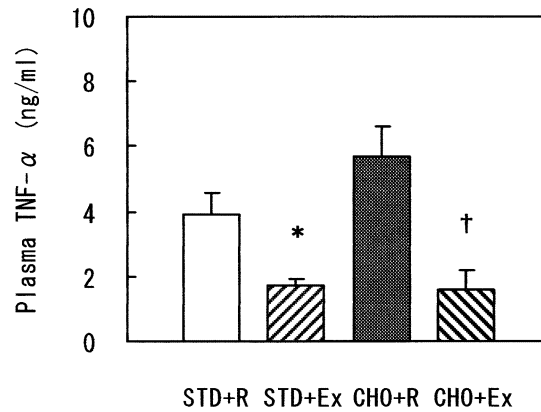


Fig. 1 Effects of a high-carbohydrate diet and exhaustive exercise on plasma TNF- α concentrations in response to LPS. Values were mean \pm SEM. * Significant difference from STD+R group, $p < 0.05$, and † significant difference from CHO+R group, $p < 0.05$. STD, standard diet; R, rest; Ex, exhaustive exercise, and CHO, high-carbohydrate diet.

immunosuppression after exercise.

In the present study, it was found that plasma corticosterone concentrations were significantly increased in both the STD+Ex and CHO+Ex groups after exhaustive running ($p < 0.05$, Fig. 2). There were no significant dietary differences for plasma corticosterone concentration. It is known that corticosterone inhibits TNF- α in response to LPS[9,10]. Indeed, we demonstrated that treatment with dexamethasone, a glucocorticoid agonist, reduced LPS stimulated TNF- α production without exhaustive exercise (data not shown). In addition, strenuous exercise induces an increase in plasma corticosterone concentration[3]. The exercise-induced increase of circulating corticosterone might cause immunosuppression. However, ingestion of CHO beverages during exercise attenuated an increase in plasma glucocorticoid concentration and a fall in plasma glucose levels[7]. It is known that higher glucose levels blunt increases of stress hormones[5,7,11]. Thus, the intake of CHO beverages during exercise might help to maintain immune function, but it seems that eating a high-CHO diet for a prolonged period does not prevent the suppression of TNF- α in response to LPS. To improve immunosuppression during strenuous exercise, it may be necessary to maintain higher glucose levels and then suppress the secretory stress hormones inducing immunosuppression.

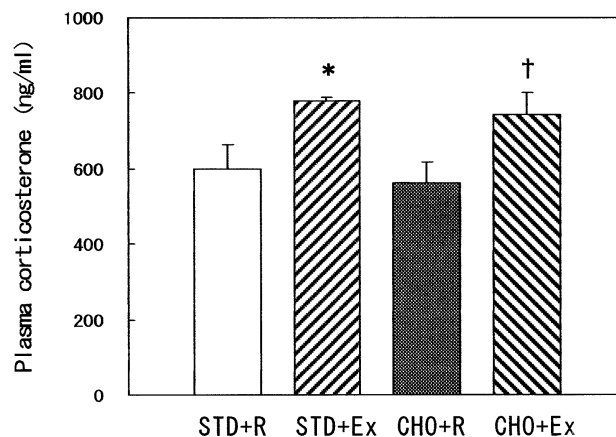


Fig. 2 Effects of a high-carbohydrate diet and exhaustive exercise on plasma corticosterone concentrations. Values were mean \pm SEM. * Significant difference from STD+R group, $p < 0.05$, and † significant difference from CHO+R group, $p < 0.05$.

In addition, to clarify the effect of high-CHO diet on sickness behavior, changes in wheel-running activity were examined before and after LPS injection, since Harden et al reported that LPS injection reduced spontaneous activity[4]. Although it was found that spontaneous wheel-running activity decreased after combined exercise and LPS injection and almost recovered by the sixth day in both STD and CHO groups, no significant differences were found between the groups in spontaneous wheel-running activity after LPS injection (Fig. 3).

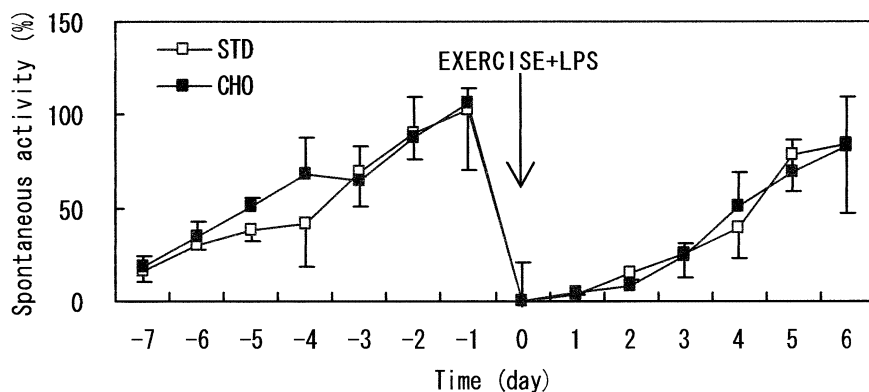


Fig. 3 Changes in spontaneous wheel-running activity before and after exhaustive exercise of rats on a standard and a high-carbohydrate diet. Wheel-running activity per day were expressed as a percentage of the daily average for the three day period just prior to the start of the experiment. Values were mean \pm SEM.

In summary, to clarify the effects of a prolonged feeding of a high-CHO diet prior to exhaustive exercise on immunosuppression, plasma TNF- α concentration were measured in rats on a high-CHO or standard diet after the rats were run until exhausted on a treadmill and then injected with LPS. In addition, spontaneous activity was examined in rats on a high-CHO or standard diet before and after LPS injection. The results indicated that prolonged feeding (7 days) of a high-CHO diet did not induce an increased response of TNF- α to an injection of LPS after exhaustive exercise, and spontaneous activity after LPS injection was not affected by the high-CHO diet.

Acknowledgments

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