【Short Communication】

LIPOPOLYSACCHARIDE-INDUCED TUMOR NECROSIS FACTOR (TNF)- α PRODUCTION DEPENDS ON EXERCISE INTENSITY IN RATS

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I . Introduction

Acute prolonged and/or high-intensity exercises suppress immune response^{1~3)}. In fact, we and others reported that strenuous exercise reduces plasma tumor necrosis factor (TNF)- α levels in response to lipopolysaccharide (LPS), which is a component of the cell wall of gram-negative bacteria^{4,5)}. The relationship between exercise intensity and the suppression of TNF- α in response to LPS is not fully understood.

It is known that catecholamines inhibit LPS-induced TNF- α production⁶⁾ and its release depends on exercise intensity⁷⁾. Recently, we reported that the β -adrenergic receptor blocker almost completely reversed strenuous exercise-induced suppression of TNF- α in response to LPS⁴⁾. Accordingly, we made a hypothesis that the suppression of LPS-induced TNF- α production after exercise depends on exercise intensity via catecholamines. Here we studied the effects of different exercise intensities on LPS-induced TNF- α production in rats.

II . Methods

A . Animals

Female 10-week old F344 rats (Charles River Laboratories Japan, Kanagawa, Japan) were used in this experiment. The rats were housed in a controlled environment (20 $\pm$ 1 $^{\circ}$ C, 12:12-hour light-dark cycle) and allowed unrestricted access to standard rat chow (Oriental Yeast Co., Tokyo, Japan) and tap water. Before the experiment, all rats were run on a treadmill at 15 m/min for 15 min on a 15 % grade during a 3-day acclimation period. The institutional animal care and usage committee of the Wayo Women's University approved the experiment. The experimental procedures followed the 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.

B . Experimental design

The rats were randomly assigned to four groups; sedentary (Rest; n=18), low-intensity exercise (L-Ex; n=8), moderate-intensity exercise (M-Ex; n=8) and high-intensity exercise (H-Ex; n=7) groups. L-Ex, M-Ex and H-Ex rats ran at a speed of 10, 21 and 26m/min, respectively. All exercised rats ran on

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a treadmill for 30min on a 15% grade. The running speed of rats were equivalent to exercise intensities of 50%, 70% and 80% maximal oxygen uptake ($\dot{V}O_2$ max), as determined in our previous study⁸⁾. All rested rats were kept in a sedentary condition for 30min with no access to food and water. Immediately after exercise or resting periods, all rats received i.v. injections of LPS (*Escherichia coli* 055:B5, 1 mg/kg, Sigma-Aldrich Japan, Tokyo, Japan) under the mild anesthesia with ether.

Blood samples were collected from abdominal vein under systemic anesthesia with sodium pentobarbital (60mg/kg i.p.) immediately after exercise or resting periods and 1 hour after LPS injection. Plasma was separated and kept frozen at -20°C until later measurement of stress hormones and TNF- α was taken.

C . Assay for Stress Hormones and TNF- α

The blood samples taken immediately after exercise or resting periods were measured for plasma stress hormones at a commercial laboratory (SRL, Inc. Tokyo, Japan). Plasma adrenaline, noradrenaline and dopamine were measured using automated HPLC⁹⁾. Plasma corticosterone was determined by RIA¹⁰⁾. The blood samples taken 1 hour after LPS injection were measured for plasma TNF- α using a commercially available ELISA kit (BioSource International, Camarillo, CA).

D . Statistics

Data are expressed as mean $\pm$ standard error of the mean (SEM) and analyzed using an analysis of variance (ANOVA) procedure by Fisher's protected least-significant difference (PLSD) test for post hoc analyses (by StatView for Windows Ver. 5.0, SAS Institute Inc. Cary, NC). $P < 0.05$ was considered as statistically significant.

III . Results and discussion

Although previous investigations indicated that exhaustive exercise inhibits the augmentation of LPS-induced TNF- α production^{4,5)}, the relationship between exercise intensity and the suppression of LPS-induced TNF- α production has not been clar-

Fig. 1. Effects of different exercise intensities on plasma levels of LPS-induced TNF- α . Data are expressed as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using an analysis of variance (ANOVA) procedure. Fisher's protected least-significant difference (PLSD) test was used for post hoc analyses. p values of < 0.05 were considered statistically significant. ** $p < 0.01$, as compared with Rest and L-Ex groups.

ified. In this study, we found no difference between Rest and L-Ex groups in plasma levels of LPS-induced TNF- α (Fig. 1). In contrast, plasma levels of LPS-induced TNF- α in both M-Ex and H-Ex groups were significantly lower than Rest and L-Ex groups ($p < 0.01$, respectively). There was no significant difference in plasma levels of LPS-induced TNF- α between M-Ex and H-Ex groups. Thus, more than moderate exercise ($> 70\% \dot{V}O_2$ max) was shown to be effective in the suppression of LPS-induced TNF- α production.

Prolonged and/or exhaustive exercise increases stress hormones^{7,8)}, which induces immunosuppression^{6,11)}. In this study, we clarified whether moderate- and high-intensity exercise also increases these hormones or not. Plasma stress hormones levels are summarized in Table 1. No significant differences in plasma corticosterone levels among the four groups were found. Bagby et al.⁵⁾ reported one possibility that glucocorticoids would play a role in the exercise-induced reduction in TNF- α in response to LPS. However our previous study found that exhaustive exercise-induced suppression of plasma TNF- α after LPS injection did not reverse with the administration of the glucocorticoids receptor antagonist RU486⁴⁾. We suggest that corticosterone is not in-

volved in the exercise mediated suppression of TNF- α production.

In contrast, plasma adrenaline and dopamine levels in M-Ex and H-Ex groups were significantly higher than Rest and L-Ex groups. In addition, high plasma noradrenaline levels were also observed in H-Ex group. From these results, catecholamines were found to be induced by moderate- and high-intensity exercise. Therefore exercise-induced catecholamines are responsible for the moderate- and high-intensity exercise-induced suppression of LPS-induced TNF- α production. In fact, it was reported that pre-treatment of rats with the β -adrenergic receptor blocker completely reversed an exhaustive exercise-induced suppression of plasma TNF- α in response to LPS⁴). Although we did not measure plasma interleukin (IL)-6 levels, Starkie et al.¹²) indicated the possibility of IL-6 inhibiting endotoxin-induced TNF- α production. Exercise-induced suppression of LPS-induced TNF- α production might be influenced by not only catecholamines but also IL-6.

Our study showed that moderate-intensity exercise attenuated the production of pro-inflammatory cytokine in response to pathogens. Numerous reports have shown that moderate exercise increases the anti-viral¹³) and phagocytic functions¹⁴). Thus, moderate exercise might attenuate inflammation and maintain other immune functions. On the other hand, Dhabhar et al. have suggested that under conditions of acute

stress, just as the stress response prepares the nervous, neuroendocrine, and other systems for fight or flight, it may also prepare the immune system for challenges (e.g., wounding or infection) which may be imposed by the stressor¹⁵). Therefore, our observations suggest the postponement of acute inflammation required for elimination of bacteria and regeneration of tissues from wounds and injuries that compromises physical performance. Further studies are required to examine the cellular mechanisms by which pro-inflammatory cytokine is attenuated.

IV . Conclusions

Moderate- and high-intensity exercise was shown to suppress LPS-induced TNF- α production. Thus inflammatory reaction against bacterial invasion may be modified depending on exercise intensity via catecholamines.

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Table 1. Plasma stress hormones levels in resting and different exercised groups.

Group	Corticosterone (ng/ml)	Adrenaline (ng/ml)	Noradrenaline (ng/ml)	Dopamine (ng/ml)
Rest	973 $\pm$ 44	2.8 $\pm$ 0.4	0.4 $\pm$ 0.1	0.04 $\pm$ 0.01
L-Ex	990 $\pm$ 50	4.6 $\pm$ 1.1	0.7 $\pm$ 0.2	0.03 $\pm$ 0.00
M-Ex	1145 $\pm$ 26	7.2 $\pm$ 0.7 *	0.5 $\pm$ 0.0	0.10 $\pm$ 0.01 **
H-Ex	1093 $\pm$ 245	8.3 $\pm$ 1.4 **	3.9 $\pm$ 1.3 **††	0.13 $\pm$ 0.03 **

Data are expressed as means $\pm$ SEM. * p <0.05, ** p <0.01, as compared with Rest and L-Ex groups. †† p <0.01, as compared with M-Ex groups.

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