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Original Article

Effects of Exercise Intensity on Circulating Leukocyte Subpopulations

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Abstract

Objectives: The purpose of this study was to examine the relation between exercise intensity and immune function.

Methods: Ten healthy young males underwent a constant work rate exercise of three levels, 90%, 80% and 70% ventilatory threshold (VT) work rate, for 20 min on a bicycle ergometer. These work rates were calculated for each individual based on his VT work rate obtained by the incremental exercise tests. Blood samples were collected before and after the exercise, and immune function indices were measured.

Results: Compared with the obtained $\dot{V}O_2$ at VT ($VT\dot{V}O_2$) in the incremental test, the $\dot{V}O_2$ with the exercise of 70% VT work rate was at a similar level and the one with the exercise of 90% or 80% VT work rate had a significantly greater value. The numbers of leukocytes and neutrophils significantly increased in the 90% and 70% VT work rate groups. In 80% VT work rate group, the CD4/CD8 ratio was significantly depressed. The $CD16^+CD57^-$ (%), natural killer cell populations, had a tendency to increase at 80% VT work rate, and also the $CD16^+CD57^+$ (%) had a similar tendency at 90% or 80% VT work rate.

Conclusions: This study shows that moderate exercise reaching or exceeding the VT level acutely affects T cell and NK cell subsets.

Key words: lymphocyte, exercise stress, acute effect, ventilatory threshold, exercise intensity

Introduction

Previous studies have demonstrated the influence of exercise training on immune function including populations of leukocytes, lymphocytes, T cells and NK cells (1–5). A common finding is that the numbers of neutrophils and the proportion of lymphocyte subsets change acutely with moderate exercise. They return to normal (baseline) after several hours (6–8). However, Hoffman-Goetz et al. reviewed that, following severe exercise, the blood lymphocyte levels even fall below normal and the duration of this suppression depends on the intensity and duration of the exercise (9).

As an indicator for workload intensity of exercise, anaerobic threshold (AT) was advocated by Wasserman et al. (10). Ventilatory threshold (VT) among AT is a well-known one in addition to lactate threshold. VT can be measured without drawing blood and can be determined by a modified V-slope technique (11). VT has been studied to evaluate exercise tolerance in both healthy people and patients in training (12).

The exercise intensity using the AT level is moderate, because AT occurs in the range of 40% to 78% $\dot{V}O_2$ max (13) or 50% heart rate reserve in many healthy subjects (14). The training method using the VT level has not been standardized for short or long-term protocols. A protocol for long-term exercise is desired to improve immune function in addition to physical fitness.

The purpose of this study was to clarify the relation between exercise intensity using the AT level and the acute effect of immune functions, especially for NK lymphocyte subsets, which is crucial in defense against tumor and infection.

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Materials and Methods

Subjects

Ten healthy young untrained males aged 18–23 years (average±SD: 21.1±1.9) participated in this study. All subjects, with mean heights and weights of 170.1±5.9 cm and 65.6±7.6 kg,

respectively, were students at Fukui Institute of Technology for Medicine. They were free of cardiac, pulmonary, metabolic, musculoskeletal disorders and any other diseases. Subjects gave informed consent institutionally reviewed for participation in this study.

Measurements

The experiments were organized into two series. In the first series, each subject performed an incremental exercise test using the cycle ergometer (Aerobike 232C, Combi Co., Ltd., Japan) to determine VT. After 4 min of unloaded pedaling, work rate was increased by 20–30 W·min^{-1} to volitional tolerance when subjects kept pedaling at a rate of 50–60 rpm. During each test, oxygen uptake ($\dot{V}O_2$), carbon dioxide output ($\dot{V}CO_2$) and minute ventilation ($\dot{V}E$) were measured by analyzing expired breath, with a computerized gas analysis system (Aeromonior AE-280, Minato Med Co., Ltd., Japan). The modified V-slope method, which is from a plot of $\dot{V}CO_2$ as a function of $\dot{V}O_2$, was used to determine VT. Work rate at VT in the incremental exercise test was defined as VT work rate.

In the second series, each subject performed three kinds of constant work rate exercise by pedaling the cycle ergometer for 20 min. The 90%, 80% and 70% VT work rates were calculated for each individual based on his VT work rate obtained by the incremental exercise tests. The criterion for selecting work rates was as follows: if the 100% VT work rate obtained from incremental exercise test was used for the constant work rate exercise, the $\dot{V}O_2$ in a constant work rate exercise would exceed the level of $\dot{V}O_2$ at VT ($VT\dot{V}O_2$) in the incremental exercise test. During each constant exercise in addition to the incremental tests, $\dot{V}O_2$ was measured. $\dot{V}O_2$ for the final minute in each constant exercise was compared with $VT\dot{V}O_2$. All exercise began in the afternoon and each exercise was performed at one day or more intervals. Subjects refrained from any type of moderate or heavy exercise on the test day. Before each exercise there was at least 10-min seated rest.

Blood analysis

A blood sample was drawn from the antecubital vein immediately before and after each exercise for analysis of immune functions. The number of total blood leukocytes and differentials were estimated by standard methods.

The immunophenotyping was performed by flow cytometry using a fluorescence-activated cell sorter analyzer (FACS; Becton-Dickinson, U.K.). Lymphocyte subsets were determined using monoclonal antibodies as follows: percentage of T helper/inducer cells ($CD4^+$), T suppressor/cytotoxic cell ($CD8^+$) and NK cell ($CD16^+CD57^-$, $CD16^+CD57^+$, $CD16^-CD57^+$). These three subsets ($CD16^+CD57^-$, $CD16^+CD57^+$, $CD16^-CD57^+$) are related to NK cell subpopulation (15). These analyses were carried out in Otsuka Tokyo Assay Laboratory.

Statistical analysis

Results were presented as means±standard deviations (SD). $VT\dot{V}O_2$ in the incremental test and $\dot{V}O_2$ with the exercise of three work rates were statistically compared using paired t tests. Cellular parameters before and after exercise were also compared using the paired t test. We considered differences significant at $P<0.05$.

Results

The VT determined by incremental exercise test, $\dot{V}O_2$ was 15.3±1.7 ml·min^{-1}·kg^{-1} and VT work rate was 80.7±19.8 W. $\dot{V}O_2$ for the final minute averaged on three constant work rates for each individual is presented in Table 1. $\dot{V}O_2$ with the exercise of 90% VT and 80% VT work rate were, on average, significantly higher than $VT\dot{V}O_2$ ($P<0.01$ and $P<0.05$, respectively). $\dot{V}O_2$ with the 70% VT work rate was not significantly different from $VT\dot{V}O_2$.

Table 2 shows the influence of physical activity on blood cells. Immediately after 90% and 70% VT work rate, the number of leukocytes significantly increased, compared with those before exercise ($P<0.005$ and $P<0.05$, respectively). However, there were no changes after the 80% VT work rate. In addition, the numbers of erythrocytes and hematocrit level increased significantly only after the exercise of 90% VT work rate ($P<0.001$). An increase in the number of neutrophils occurred as a result of the exercise of 90% and 70% VT work rate ($P<0.05$). Although the number of leukocytes and neutrophils

Table 1 Average $\dot{V}O_2$ for the final one minute exercise at each constant work-rate

Work rate	$\dot{V}O_2$ (ml·min^{-1}·kg^{-1})
90%VT	17.8±2.1**
80%VT	16.4±1.5*
70%VT	14.7±1.7

* $P<0.05$, ** $P<0.01$, compared with the $VT\dot{V}O_2$ (15.3±1.7 ml·min^{-1}·kg^{-1}) determined by the incremental exercise test for each participant.

Table 2 The influence of each work-rate exercise on the blood cells of subjects

	90%VT work rate		80%VT work rate		70%VT work rate	
	before	after	before	after	before	after
Total leukocytes (10^3·μl^{-1})	5.73±1.58	6.45±1.72***	6.45±2.17	6.96±2.22	5.73±1.55	6.09±1.55*
Erythrocytes (10^4·μl^{-1})	504.3±27.9	521.6±28.5****	507.1±28.3	517.7±30.8	510.6±28.1	517.2±25.9
Hematocrit (%)	45.4±2.1	46.8±2.2****	45.9±2.1	46.5±2.2	46.0±2.4	46.3±2.3
hemoglobin (g·dl^{-1})	15.7±0.8	16.3±0.8****	15.8±0.8	16.1±0.8	15.9±0.7	16.1±0.7***
Neutrophils (10^3·μl^{-1})	3.15±1.21	3.51±1.40*	3.80±1.64	4.06±1.83	3.07±0.79	3.29±0.84*
Lymphocytes (10^3·μl^{-1})	2.03±0.61	2.05±0.61	2.00±0.69	2.20±0.62	2.07±0.62	2.20±0.61
Monocytes (10^3·μl^{-1})	0.30±0.08	0.34±0.10	0.35±0.15	0.41±0.20	0.33±0.13	0.33±0.11

* $P<0.05$ *** $P<0.005$ **** $P<0.001$, determined by paired t test for each subject.

Table 3 Effects of each constant work-rate exercise on the lymphocyte subsets

	90%VT work rate		80%VT work rate		70%VT work rate	
	before	after	before	after	before	after
$CD4^+CD8^-$ ($10^3 \cdot \mu l^{-1}$)	1.06±0.37	0.98±0.29	0.99±0.32	0.90±0.31*	1.17±0.22	1.10±0.25
$CD4^+CD8^-$ (%)	35.5±4.6	33.3±5.1	35.4±5.4	31.1±2.7*	35.7±3.0	34.7±4.9
$CD4^-CD8^+$ ($10^3 \cdot \mu l^{-1}$)	1.03±0.32	1.06±0.32	0.98±0.37	1.00±0.33	1.09±0.25	1.11±0.35
$CD4^-CD8^+$ (%)	34.9±5.6	35.9±6.6	34.5±6.6	36.0±7.4	33.2±4.9	34.4±5.8
$CD4^+/CD8^+$	1.06±0.29	0.97±0.31	1.08±0.32	0.90±0.23*	1.10±0.24	1.15±0.31
$CD16^+CD57^-$ ($10^3 \cdot \mu l^{-1}$)	0.14±0.05	0.16±0.07	0.13±0.04	0.17±0.10	0.18±0.10	0.17±0.07
$CD16^+CD57^-$ (%)	5.6±3.2	6.5±3.9	5.3±2.4	6.7±4.2	7.1±5.8	6.2±3.0
$CD16^-CD57^+$ ($10^3 \cdot \mu l^{-1}$)	0.24±0.21	0.25±0.20	0.27±0.25	0.28±0.25	0.23±0.18	0.26±0.21
$CD16^-CD57^+$ (%)	8.8±6.5	9.3±6.6	9.7±7.3	10.2±8.1	7.5±4.3	8.1±5.0
$CD16^+CD57^+$ ($10^3 \cdot \mu l^{-1}$)	0.36±0.18	0.43±0.25	0.31±0.18	0.39±0.14	0.38±0.27	0.40±0.20
$CD16^+CD57^+$ (%)	13.4±5.2	16.2±7.3	12.6±7.4	15.9±7.5	13.3±7.4	14.3±6.2

* $P<0.05$, determined by paired t test for each subject.

per unit volume before the exercise of 80% VT work rate were higher than those before the exercise of 90% and 70% VT work rate, there were no significant differences between them. No significant changes in the number of lymphocytes and monocytes occurred. The percentage of lymphocytes, neutrophils and monocytes did not change with any exercise.

As shown in Table 3, in the 80%VT work rate group, the CD4/CD8 ratio was depressed significantly ($P<0.05$), secondary to the decrease in the $CD4^+$ (%) ($P<0.05$) along with a slight increase in the $CD8^+$ (%) ($P<0.1$). In the 90% VT work rate group, $CD4^+$ (%) cells were slightly decreased ($P<0.1$). There were no changes in $CD4^+$ (%) or $CD8^+$ (%) in the 70% VT work rate group. The $CD16^+CD57^-$ (%) tended to increase in the 80% VT work rate group ($P<0.1$), and the $CD16^+CD57^+$ (%) also did in the 90% and 80% VT work rate group ($P<0.1$). In the 70% VT work rate group, no significant changes in these cells occurred. Lymphocyte subsets, except for the number of $CD4^+$ cells in the 80% VT work rate group, did not change.

Discussion

In this study, we first demonstrated that $\dot{V}O_2$ at an exercise intensity of 70% VT work rate was at the same level as $VT\dot{V}O_2$ in the incremental exercise test. However, $\dot{V}O_2$ with exercise of 80% or 90% VT work rate was higher than $VT\dot{V}O_2$. If the 100% VT work rate obtained from the incremental exercise test had been used for the constant work rate exercise, the $\dot{V}O_2$ in a constant work rate exercise would exceed the level of $VT\dot{V}O_2$ in the incremental exercise test. The percentage of VT taken for exercise depends on the protocol of the incremental exercise test, especially the work rate (W/min). An easy method to obtain the $VT\dot{V}O_2$ level of constant work rate exercise from $VT\dot{V}O_2$ in the incremental exercise test is needed.

This study shows that exercise above the VT level for 20 min induces a decrease in the CD4/CD8 ratio with a significant decrease in the number and percentage of the $CD4^+$ cells and a tendency for increases in the percentage of natural killer cells.

Exercise at the 90% VT work rate elicited a significant increase in the number of erythrocytes and the hematocrit level. These changes were considered to be a result of hemoconcentration. Apparently, the exercise at the 90% VT work rate was stronger than that of the 80% or 70% VT work rate for all subjects.

Some investigators reported that the number of leukocytes increases during exercise, and that the extent of the increase varies (5, 16–20), suggesting that the number of leukocytes is related in a complex rather than a simple manner to both the intensity and the duration of exercise. In the present study, the number of leukocytes significantly increased at the 70% and 90% VT work rates with increases in neutrophil counts, without an increase in lymphocyte counts. However, the number of leukocytes or neutrophils after exercise at the 80% VT work rate did not increase significantly although the average increase was similar to those of the 90% and 70% VT work rates. Since the number of leukocytes or neutrophils per unit volume at rest before the exercise at the 80% VT work rate in a few subjects was higher than those for the 70% or 90% tests under variable conditions, the increase occurring during the exercise may not be apparent. Alternatively, the nonsignificant increase might be due to the limited number of subjects for the 80% test. Therefore, it is suggested that exercise greater than or at VT might elicit an increase in leukocyte and neutrophil counts.

As for the change of lymphocyte subsets after exercise, previous studies on the relation between exercise and the CD4/CD8 reported, in common, that severe or moderate exercise training causes the number or percentage of $CD4^+$ cells to be decreased (3, 5, 21). The decrease in $CD4^+$ (%) may be caused by the relative increase in the number of the NK cells and the decrease in the number of $CD4^+$. However, in this study, the decrease in the number or the percentage of $CD4^+$ cells did not occur after exercise of 90% VT work rate, though it occurred after exercise of 80% VT work rate. It is suggested that this might be due to the insufficient number of subjects because the decrease in $CD4^+$ cells after exercise at the 90% VT work rate showed the same tendency as that of the 80% VT work rate. By increasing the number of subjects, further investigations are needed to examine the effects of exercise on the decrease in the number of $CD4^+$ cells.

However, NK function is crucial in defense against tumors and infections. The number and cytotoxicity of NK cells was reported to increase after severe or moderate exercise (1, 7, 22, 23). In the present study, after exercise at the 80% or 90% VT work rate, the increase in $CD16^+CD57^-$ (%) or $CD16^+CD57^+$

(%) appeared to be induced as an acute effect, although no significance was noted ($P<0.1$). The NK cell activity or counts after severe or moderate exercise have been reported to decrease to normal (baseline) or below (1, 2, 5). Pedersen et al. (23) suggested that, during moderate as well as severe exercise the immune system is enhanced, but severe exercise is followed by immunodepression. In addition, Castell et al. (24) reported that the decrease in NK cells was maintained within at least 16 h after a marathon race. If the decrease continues over several days, there is a danger of infection or other diseases.

Several studies reported a relation between long-term exercise training and immune function (25–30). Nieman et al. (28) reported that moderate exercise training for 6 weeks was associated with elevated NK cell activity. Rhind et al. (31) reported that moderate endurance training for 12 weeks was associated with sustained alterations in immune function, both at rest and when exercising. However, Nieman et al. (32) reported moderate exercise training (walking) for obese woman was unrelated to any significant changes in resting immune function. It is very important to know the effective intensity of exercise training for a long term.

In summary, the present findings suggest that constant exercise intensity, which approaches or exceeds the VT level, causes a decrease in CD4/CD8, and an increase in leukocytes and neutrophils. NK cells had a tendency to increase as an acute effect.

In other words, exercise below the level of VT may not induce a change in CD4/CD8, the number of leukocytes, neutrophils or NK cells.

When patients with poor health condition or with immunodeficiency are requested to exercise, it is necessary to ensure the patient's immune status does not fall even within several hours or days of exercise training. The findings of the present study showed that exercise below the level of VT might be acceptable for these patients from a viewpoint of immune function. However, it is necessary to investigate the relevant exercise protocol over long periods to reinforce immune function.

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