Chronic Effect of Training on Neutrophil Functions in Humans

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Abstract

We performed this study to evaluate the chronic effect of training on neutrophil functions in humans. Twenty-six university students (14 males and 12 females) with a mean age of 19.1 ± 0.8 years were divided into the athlete group and the control group based on answers given to a written questionnaire. The capacity of circulating neutrophils to ingest bacteria (phagocytosis) and to produce superoxide (nitroblue tetrazolium reduction) was measured under resting conditions in the absence of training activities. In addition, we measured hematological and serum biochemical parameters.

The present analyses revealed that the frequency of subjective symptoms concerning susceptibility to infections (sum of males and females) was higher in the athlete group than in the control group ($p < 0.05$). In the investigation of males, hemoglobin and serum protein levels of the athlete group were lower than that of the control group ($p < 0.01$, $p < 0.05$, respectively). Although total and differential leukocyte counts, and phagocytic activity of neutrophils were not significantly different between the groups, superoxide productivity of neutrophils in the male athlete group was higher than that in the control group ($p < 0.05$).

Since significant differences were observed in hemoglobin and serum protein levels in the male athletes, the training is considered to have been intense. An increase was noted in the neutrophil superoxide production along with these changes, but no significant difference was observed in the phagocytic activity. Therefore, there is the possibility of superoxide overproduction, which may lead to tissue damage.

Key words: training, neutrophil, superoxide, serum proteins, hemoglobin

Introduction

Individuals engaged in sports are increasing due to the surging interest in health and fitness¹. Some individuals involved in sports expect that resistance to infections would be increased. In fact, a reduction in the incidence of colds has been reported in individuals who have trained over a long period². However, increased susceptibility to infections due to intense training has also been suggested³.

A number of investigations, many of which focused on lymphocyte functions^{4–6}, have been carried out to elucidate the relations between exercise and immunological competence. There have been, however, relatively few studies on the neutrophils that are known to play an important role in initial protection against infections⁷. Moreover, these studies on the chronic effect of training on the neutrophil function reveals considerable disagree-

ment; i.e. some reported enhancement of neutrophil function by training⁷, others reported no marked change⁸, and others reported decreases in neutrophil function⁹. These discrepancies might be attributable to the effects of serum factors, since the neutrophil functions were examined after their isolation from whole blood in these studies. Therefore, measurement under as physiologic conditions as possible is considered to be important. Furthermore, the Janus-faced function of superoxides might have led to the discrepancies. The excessive release of superoxides, which are produced by neutrophils as bactericidal agents, was also reported to cause tissue damage¹⁰. Although an evaluation of the balance between phagocytic activity and the superoxide-producing activity is considered to be important, little information has been reported.

We, therefore, measured the superoxide production and phagocytic activity of neutrophils simultaneously using the nitroblue tetrazolium (NBT) reduction test^{11–13} without isolating neutrophils from whole blood. We also evaluated chronic effects of training on these neutrophil functions, in addition to various hematological and serum biochemical parameters in young students who were divided into athlete/non-athlete groups using a questionnaire.

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Materials and Methods

Subjects

The subjects were 26 students belonging to the School of Human Sciences, Faculty of Sports Science, Waseda University in Saitama Prefecture (14 males and 12 females) with a mean age of 19.1 ± 0.8 years. Informed consent was obtained from all subjects. The students were asked not to be involved in exercise for at least 48 hours before blood sampling. Blood samples were taken in the early morning after an overnight fast.

Questionnaire

The subjects were asked to complete a questionnaire concerning susceptibility to infections (subjective symptoms regarding whether or not each subject was apt to catch a cold), medical history, training activities, smoking and drinking alcohol. No medical histories of serious diseases or illnesses under medical treatment were found in our subjects.

Subjects were divided into the athlete group (belonged to a sports club at the university or answered that they were regularly involved in intense training activities, $n=10$, 7 males and 3 females) and the control group (answered that they only periodically did moderate exercise or did not have any exercise habits, $n=16$, 7 males and 9 females) based on the questionnaire. In the athlete group, all subjects had an exercise habit for more than 6 months. Classification of intense training activity/moderate exercise was performed on the basis of Borg's ratings of perceived exertion (RPE) scale¹⁴⁾ at the interview of the students (Table 1).

Hematological parameters

Peripheral venous blood samples were obtained by antecubital venipuncture. The items examined were as follows; hemoglobin concentration (Hb, cyanmethemoglobin method), total leukocyte count (WBC, Mélangeur method) and the classification (May-Giemsa stain method) in whole blood.

Serum biochemical parameters

Serum samples for biochemical tests were separated from venous blood by centrifugation at $1,000 \times g$ for 10 min after allowing the blood to clot at room temperature for 30 min. The items examined were as follows; lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ -glutamyl transpeptidase (γ -GTP), creatine kinase (CK), total protein (TP), albumin (ALB), triglyceride (TG), total cholesterol (T-CHO), high density lipoprotein cholesterol (HDL), blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA), blood glucose (GLU), iron (Fe) in serum (measured with biochemical assay kits prescribed for a Clinical Chemistry Analyzer CL-7100, Shimadzu Co., Ltd., Tokyo).

Assays of neutrophil functions

Phagocytic activity and superoxide production of neutrophils were measured using the NBT reduction test (*in-vitro*)¹¹⁾. A spontaneous test and stimulated (by the foreign agent) test were performed. *Staphylococcus aureus* 209P was used as the foreign agent. Phagocytic activity was determined from the phagocytic ratio and the number of ingested particles per cell. Superoxide production was evaluated by the NBT reduction ratio and NBT reduction score^{12,13)}.

Table 1 Profiles of the athlete group and control group

A. Athlete group

Name (initial)	Gender	Subclass ^s	Exercise
K.T.	Male	1	Baseball
N.T.	Male	1	Baseball
S.A.	Male	1	Soccer
O.J.	Male	1	Kendo
M.T.	Male	1	Rugby football
K.Y.	Male	1	Rugby football
O.K.	Male	2	Tennis
H.M.	Female	1	Golf, training
O.Y.	Female	1	Kendo
T.A.	Female	2	Tennis

B. Control group

Name (initial)	Gender	Subclass	Exercise
S.K.	Male	4	—
Y.K.	Male	3	Tennis
N.N.	Male	4	—
K.K.	Male	4	—
W.H.	Male	4	—
Y.T.	Male	4	—
I.M.	Male	4	—
S.E.	Female	4	—
F.K.	Female	4	—
K.M.	Female	4	—
S.T.	Female	3	Jazz dancing
S.A.	Female	4	—
I.R.	Female	3	Tennis
O.Y.	Female	3	Tennis
Y.H.	Female	4	—
I.A.	Female	4	—

^s (Subclass)

1: Belonged to a sports club at the university

2: Involved in intense training activity
(subjective training intense score =15 or more)

3: Moderate exercise
(subjective training intense score <15)

4: No exercise habit

Statistical analysis

Statistical values were shown as the mean $\pm$ SD. Differences in variables among the groups were compared using the Kruskal-Wallis test or t test. Comparison of frequency was performed using Fisher's exact probability test. The interrelationship between variables was calculated by Pearson's correlation coefficient. P values of less than 0.05 were considered significant.

Results

Evaluation of questionnaire

Differences in frequency of subjective symptoms concerning susceptibility are shown in Fig. 1. Of the subjects in the athlete group (sum of males and females), 60% answered that they were apt to catch a cold, whereas only 18.8% of subjects in the control group (the same as the above) gave this answer ($p<0.05$). A significant difference was not found in the case of separation by gender.

The differences in the frequency of smoking habit and alcohol-drinking habit are shown in Table 2. A significant difference was not found between the groups.

Fig. 1 Difference in frequency of subjective symptoms concerning susceptibility to infections in the athlete group (males: n=7, females: n=3) and the control group (males: n=7, females: n=9).

Table 2 Habit of smoking and alcohol intake in the groups

	Males		Females	
	Athlete (n=7)	Control (n=7)	Athlete (n=3)	Control (n=9)
Smokers	2 (28.6%)	2 (28.6%)	0 (0.0%)	0 (0.0%)
Alcohol intake (25 cc/day)	4 (57.1%)	2 (28.6%)	0 (0.0%)	0 (0.0%)

Hematological parameters

The mean values of hematological parameters are summarized in Table 3. Hb of the male athlete group was significantly lower than that of the control group ($p<0.01$). No significant difference in Hb was, however, observed in the female groups. Total and differential leukocyte counts were not significantly different between the groups.

Serum biochemical parameters

The mean values of serum biochemical parameters are summarized in Table 4. In the investigation of males, CK was significantly higher ($p<0.01$) while TP and ALB were significantly lower ($p<0.05$) in the athlete group than those of the control group. No serum biochemical parameters were significantly different between the female groups.

Table 3 Hematological parameters in the athlete group and the control group

	Males		Females	
	Athlete (n=7)	Control (n=7)	Athlete (n=3)	Control (n=9)
Hb (g/dl)	13.7±1.2**	15.2±0.8	11.9±1.3	12.1±0.8
WBC ($\times 10^3/\text{mm}^3$)	5.6±1.2	5.9±2.1	5.4±1.4	6.3±1.3
Neutrophils (%)	58.2±7.1	60.0±8.3	51.0±7.2	63.3±8.1
Lymphocytes (%)	33.8±8.0	33.0±7.0	42.1±3.7	32.6±7.9
Monocytes (%)	3.4±1.3	3.1±2.0	3.9±1.1	3.0±1.0
Eosinophils (%)	4.6±1.7	3.1±2.0	3.0±1.0	2.1±1.0

Statistical difference between the athlete and the control groups (** $p<0.01$).

Abbreviations: Hb: hemoglobin, WBC: leukocyte count, in whole blood, respectively.

Neutrophil functions

The mean values of parameters of neutrophil functions are shown in Table 5. In the investigation of males, stimulated NBT reduction activities (representing O_2^- productivity of neutrophils) of the athlete group were higher than those of the control group, in both the reduction score and ratio ($p<0.05$, respectively). In the investigation of females, however, the stimulated NBT reduction score of the athlete group was lower than that of the control group ($p<0.05$).

Phagocytic activities were not significantly different between the groups.

Correlation between significant parameters

The correlation between significant parameters in blood, serum components and significant neutrophil functions in male subjects is shown in Table 6. A significant correlation was found between TP and stimulated NBT reduction score ($r=-0.577$, $p<0.05$). In female subjects, no significant parameters in blood and serum components (Tables 3–4) or significant correlations among the indicators were identified.

Discussion

A previous study suggests that neutrophil dysfunction caused by intense training is one of the factors for increased susceptibility to infections in male athletes⁹⁾. The reason why we can clearly demonstrate the paradoxical function of neutrophils was that the cells' functions were measured under physiological conditions as described in the text. We observed that the activity of superoxide production by neutrophils in the male athletes was significantly higher than that in male non-athletes, whereas phagocytic activity was not different between the groups (Table 5). The increase in the superoxide production by neutrophils, therefore, may indicate overproduction of superoxides which leads to tissue damage such as exercise-induced muscle inflammation, rather than enhancement of resistance against infections. Although enhanced CK levels in athletes is attributed to training-induced muscle damage, a recent investigation revealed that neutrophil-derived free radicals are closely involved in the inflammatory process in muscles after training¹⁵⁾.

As shown in Table 6, the neutrophil superoxide production in males showed a significant negative correlation with TP and a negative correlation with ALB. Serum proteins including albumin are known to be primary components of free radical scavengers¹⁶⁾. The above negative correlations may indicate consumption of serum proteins for disposal of overproduced superoxides in the male athletes. Sufficient intake of proteins to prepare enough radical

Table 4 Serum biochemical parameters in the athlete group and the control group

	Males		Females	
	Athlete (n=7)	Control (n=7)	Athlete (n=3)	Control (n=9)
LDH (U/l)	482±89	475±87	513±90	418±49
AST (U/l)	24.8±7.8	23.0±3.4	22.3±2.1	19.3±4.9
ALT (U/l)	12.5±2.7	14.9±8.8	11.0±1.7	9.7±5.0
ALP (U/l)	195±44	211±52	171±37	140±32
γ-GTP (U/l)	8.3±2.0	13.6±5.8	7.0±2.7	9.3±7.3
CK (U/l)	185±92**	98.1±35.3	93.3±32.7	64.7±13.2
TP (g/dl)	7.6±0.4*	8.0±0.3	7.9±0.2	7.7±0.4
ALB (g/dl)	4.9±0.2*	5.2±0.2	5.0±0.2	5.0±0.2
T-CHO (mg/dl)	154±22	164±26	200±45	181±30
HDL (μg/dl)	63.0±16.9	58.1±6.6	87.7±28.9	68.1±13.9
BUN (mg/dl)	16.6±4.2	13.3±2.0	13.5±2.4	11.7±1.9
CRE (mg/dl)	1.1±0.1	1.1±0.2	0.9±0.1	0.9±0.1
UA(mg/dl)	5.6±1.6	5.8±0.7	4.5±0.2	4.6±0.8
GLU (mg/dl)	89.0±6.8	86.7±3.9	84.0±10.0	79.1±8.7
Fe (mg/dl)	89.5±40.9	109±45	104±66	88.0±44.4

Statistical difference between the athlete and the control groups (*p<0.05, **p<0.01).

Abbreviations: LDH: lactate dehydrogenase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, γ-GTP: γ-glutamyl transpeptidase, CK: creatine kinase, TP: total protein, ALB: albumin, TG: triglyceride, T-CHO: total cholesterol, HDL: high density lipoprotein cholesterol, BUN: blood urea nitrogen CRE: creatinine, UA: uric acid, GLU: blood glucose, Fe: iron, in serum, respectively.

Table 5 Neutrophil functions in the athlete group and the control group

	Males		Females	
	Athlete (n=7)	Control (n=7)	Athlete (n=3)	Control (n=9)
<i>Spontaneous NBT reduction test</i>				
reduction score	58.8±10.1	48.9±15.4	39.0±3.0	49.1±25.6
reduction ratio	0.3±0.1	0.2±0.1	0.2±0.1	0.3±0.2
<i>Stimulated NBT reduction test</i>				
reduction score	76.3±8.6*	61.6±13.0	49.0±3.0*	56.4±16.7
reduction ratio	0.5±0.1*	0.3±0.1	0.2±0.1	0.3±0.1
<i>Phagocytic activity</i>				
particles/cell	11.1±3.4	9.6±2.1	7.9±2.0	7.5±4.2
phagocytic ratio	0.8±0.1	0.8±0.1	0.9±0.1	0.8±0.1

Statistical difference between the athlete and the control groups (*p<0.05).

scavengers may be necessary for prevention of tissue damage caused by superoxides.

CK was significantly increased while Hb, TP and ALB were significantly reduced in the male athletes (Tables 3–4), and these findings were in agreement with those of previous studies^{17–21}. Training was considered seriously intense in the male athletes. In addition to sufficient intake of proteins, improving the intensity and content of training may be important.

Table 6 Correlation between significant parameters in male subjects

	Hb	CK	TP	ALB
Hb				
CK	–0.367			
TP	0.535	–0.239		
ALB	0.535	–0.171	0.811**	
NBT score#	–0.291	0.315	–0.577*	–0.471

#: indicates stimulated NBT reduction score.

Other abbreviations: see Tables 2 and 3.

*p<0.05, **p<0.01

In the female athletes, however, the activity of superoxide production by neutrophils was significantly lower than that in the female non-athletes. Estrogen, a female hormone, is known to be a potent scavenger of superoxides produced by neutrophils²², and it may explain the difference between males and females. We obtained unpublished information that the level of training intensity in the male athletes was higher than that in the female athletes. This may also be a cause of the difference in the superoxide production activity between the male and female athletes.

In conclusion, it is suggested that long-term continuation of excessive training may cause overproduction of superoxides by neutrophils in male athletes. Sufficient supplementation of proteins and other free radical scavengers may be useful for its prevention. Further studies to clarify the interactions between serum components and neutrophil function are needed.

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