原　著

(臨床環境８：78～85，1999)

短期間の運動が血球数およびリンパ球サブセットに及ぼす影響

近藤　弘[1)]　青地克頼[3)]　松原淳子[2)]　佐藤友治[1)]
小林恵美[1)]　今木雅英[1)]　中村武夫[3)]　棚田成紀[3)]

1）大阪府立看護大学医療技術短期大学部
2）(社)関西労働衛生技術センター
3）近畿大学薬学部

Short-term moderate exercise causes transient changes in blood cell count and lymphocyte subpopulations

Hiroshi Kondo[1)] Katsuyori Aochi[3)] Junko Matsubara[2)] Yuji Sato[1)]
Emi Kobayashi[1)] Masahide Imaki[1)] Takeo Nakamura[3)] Seiki Tanada[3)]

1）Osaka Prefectural College of Health Science
2）Kansai Technical Center for Occupational Medicine
3）Faculty of Pharmaceutical Sciences, Kinki University

要約

運動が免疫機能に及ぼす影響について、成人女子学生を対象にトレッドミルによる歩行運動前後の末梢血液リンパ球サブセット値の変化から検討した。総白血球数、リンパ球数、$CD3^+$ 細胞数、$CD4^-8^+$ 細胞数、$CD3^-16^+$ or $CD3^-56^+$ 細胞数は負荷前に比べて負荷直後は有意に増加し、CD4/CD8比は有意に減少した。運動を中止すると、細胞数は負荷前値に回復したが、負荷終了60分経過後の血球数が負荷前値よりも減少傾向を示すものを認めた。ヘモグロビン濃度が最も低値を示した者では、負荷前から負荷直後までのリンパ球サブセット値の変化は著しい傾向を示した。一過性の漸増運動による免疫担当細胞の変化率は細胞種により異なったことから、これらは各種細胞に固有の変化であったことが示唆された。　(臨床環境８：78～85，1999)

Abstract

This study investigated the effect of short-term moderate exercise on the composition of peripheral blood cells and components of the immune system. Eight Japanese female students completed an incremental work-load test on a treadmill at 4.8 km/h without grading. Progression was accomplished by increasing the treadmill speed by 1.2km/h for each subsequent work period. Each work period was 3 min duration followed by 2 min rest. The work-load test was stopped when heart rate of subjects reached 180 bpm. Blood samples were collected before, during and immediately after exercise and 10, 20 and 60 min. after exercise. Lymphocyte subsets were determined by flow cytometry using monoclonal antibodies for total T, helper/inducer T, suppressor/cytotoxic T and natural killer (NK) cells antigen.

This study shows that exercise-induced changes in T lymphocyte percentages do not consistently reflect changes in the absolute numbers of cells. There were sharp reductions in the percentage of total T cells and helper/inducer T cells immediately after exercise. In contrast, the absolute number of total T cells was significantly increased immediately after exercise. The absolute number of helper/inducer T cells slightly increased immediately after exercise, however these changes were not significant. Significant increase in the percentage of NK cells occurred immediately after exercise, and numerical increases in total NK cells were also significant after exercise. The changes in leukocyte count, lymphocyte count and lymphocyte subset count were large for the subject whose hemoglobin concentration was the lowest. Thus, short-term moderate exercise has a detectable influence on peripheral blood cell counts and lymphocyte subsets.

(Jpn J Clin Ecol 8:78 ～ 85,1999)

《Key words》 leukocyte, natural killer cells, T cells, flow cytometry, exercise

受付：平成11年６月23日　採用：平成11年10月25日
別刷請求宛先：近藤　弘
〒583-8555 羽曳野市はびきの3-7-30　大阪府立看護大学医療技術短期大学部臨床検査学科
Received: June 23, 1999 Accepted: October 25, 1999
Reprint Requests to Hiroshi Kondo, Department of Medical Technology, Osaka Prefectural College of Health Science, 3-7-30 Habikino, Habikino Osaka 583-8555 Japan

Ⅰ. Introduction

There is growing interest in the relationship between exercise and the immune system. Several investigations have been conducted on the effect of exercise on the immune system[1~10]. However, studies in exercise immunology are often limited by their experimental design. For example, to investigate the effect of physical exercise on blood cells and the immune system, numerous types of exercise have been utilized such as ergometer exercise[2~7], work-load test on a treadmill[8, 9] and military march[10].

Exercise has been shown to alter the immune system durlig and after exercise. It produces an exercise intensity and duration dependent leukocytosis, increases the circulating lymphocytes, granulocytes, NK cells, T-lymphocytes and causes a reduction in the CD4+/CD8+ratio in peripheral blood[1]. Many investigators have suggested that regular and moderate exercise may improve the ability of the immune system to protect the host against infection[9, 11]. Recently, walking exercise has been promoted as one way to help prevent adult diseases[12].

It was previously reported that a combination of various lifestyle factors was significantly associated with increased NK cell activity[13]. Lifestyle factors such as physical exercise, subjective mental stress and sleeping hours had a relatively large effect on NK cell activity[13]. However, the previous study could not clearly evaluate the effects of physical exercise on the immune system. The response of many components of the immune system to short term exercise is quite transient, and may be over looked unless there is multi-point blood sampling[1].

Although another study reported that lymphocyte subsets changed quickly in response to acute exercise, they did not observe changes in lymphocyte subsets and blood cell counts at multi-points during work-load tests on treadmill.

In the present study, blood cell counts, leukocyte differential counts and lymphocyte subpopulations were investigated, during (from 4 to 7 point), immediately after and 10, 20 and 60 min. after exercise.

Ⅱ. Materials and methods

1. Subjects

Eight female medical technology students from Osaka prefectural college of health sciences participated in this study. None of the subjects were on medication. The basic anthropometrical data are listed in Table 1.

Table 1 Basic anthropometric data and exercise condition paremeters of the subjects

Characteristics	Subjects (N=8)
Age (year)	20.3±0.5
Body weight (kg)	49.9±5.3
Height (cm)	157.8±4.7
Body mass index (kg/m^{-2})	20.0±1.5
Work rate at maximal exercise (watts)	107.3±11.1
Heart rate at maximal exercise (bpm)	180.5±4.8
Maximal heart rate (bpm)	189.8±0.5
Heart rate at maximal exercise (%HRmax)	95.1±2.5

Values are presented as mean ± standard deviation.

The content of the research was clearly explained to all participants, and informed consent was obtained from all.

2. Experimental design

All subjects entered the laboratory, and the experimental procedure was explained to them. After resting in the seated position for at least 5 min, an intravenous catheter (Becton-Dickinson, San Jose CA, USA) was inserted in an antecubital vein. Blood samples were collected in a 2 ml vacutainer tube containing di-potassium ethylenediamine tetraacetic acid (Terumo Corp., Tokyo, JPN) at the following times: pre-exercise period, immediately after each work period during treadmill test, immediately post-exercise, and 10 min, 20 min and 60 min after exercise.

3. The incremental work-load test on a treadmill

Subjects completed an incremental work-load test with 3 min work on a treadmill at 4.8km/h without grading. Progression was accomplished by increasing the treadmill speed by 1.2km/h for each subsequent work period. Each work period was 3 min duration followed by 2 min rest. Heart rate was monitored using electrocardiography and the work-load test was stopped when the heart rate of subjects attained approximately 180 bpm. Testing was conducted using the Series 2000 treadmill and the CENTRA system (Marquette Electronics, Inc., Milwaukee, Wisconsin, USA).

4. Blood cell count and leukocyte differential count

Blood cell counts were determined with an automated blood cell counter (ONYX, Coulter Electronics, Inc., Hialeah, FL, USA). To assess the proportion of T cells or NK cells among lymphocytes, phenotypes of lymphocytes were analyzed using fluorescein labeled monoclonal antibodies (DAKO Inc, Glostrup, Denmark). Phenotypes of lymphocytes were analyzed using a flow cytometer (EPICS-XL, Coulter Electronics, Inc., Hialeah, FL, USA). Lymphocyte subsets were analyzed to determine percent total T (CD3+), helper/inducer T (CD4+CD8-), cytotoxic/suppressor T (CD4-CD8+), natural killer (CD3-CD16+CD56+, CD3-CD16+CD56-, or CD3-CD16-CD56+) (NK), and cytotoxic T (CD3+CD16+CD56, CD3+CD16+CD56-, or CD3+CD16-CD56+) (CTL) cell subsets.

5. Statistical analysis

All values are presented as means ± standard deviation. Two-way repeated measures (time of testing) ANOVAs were used to determine the effects of the exercise on peripheral blood cell counts, leukocyte differential counts and lymphocyte subsets. In the examination of the relationships between heart rates and lymphocyte subset counts, Pearson's correlation coefficients were calculated. The difference in change from pre-exercise between conditions were analyzed using paired t tests with Bonferoni adjustment for multiple comparisons. Probability values of less than 0.05 (two tailed) were regarded as significant. Statistical analysisof data was performed using the StatView (Abacus Concepts, Inc., Berkeley, CA, USA) software program.

Ⅲ. Results

1. Subjects

Physical and physiological characteristics of the subjects under taking the treadmill running protocol are summarized in Table 1. Heart rate at maximal exercise and term of exercise indicate that strength of the exercise can be categorized as moderate[14]. The heart rate increased significantly in response to the exercise ($P<0.05$) and

Table 2 Changes in heart rates, leukocyte counts and leukocyte differential counts

	Sampling times					*F*-values
	Pre-exercise	Immediately post-exercise	10 min post	20 min post	60 min post	
Heart rate (bpm)	82±5	177±14**	91±6	87±7	81±5	2.64*
Total leukocytes ($\times 10^9$/l)	6.21±1.70	9.56±2.30**	7.50±2.28	6.36±1.63	6.15±1.62	4.51*
Lymphocytes ($\times 10^9$/l)	2.04±0.64	3.80±1.02**	2.52±1.00	2.05±0.65	1.96±0.70	7.15***
Granulocytes ($\times 10^9$/l)	3.83±1.38	5.23±2.91	4.60±2.00	3.92±1.43	3.93±1.38	0.99
Monocytes ($\times 10^9$/l)	0.31±0.10	0.52±0.24	0.39±0.25	0.39±0.22	0.26±0.17	1.84

Values are presented as mean ± standard deviation.

*$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. rest.

returned to pre-exercise levels after 60 min recovery (Table 2).

2. Blood cell count, leukocyte differential count and lymphocyte subset

The significant increase in total leukocyte count seen immediately post-exercise ($P<0.01$) (Table 2). At no other time were these concentrations significantly different from those observed at pre-exercise. Granulocyte count and monocyte count increased slightly from pre-exercise levels to the immediate post-exercise levels. However, these changes were not statistically significant.

There were significant decreases in the percentages of total T ($P<0.01$), helper/inducer T cells ($P<0.001$), and the CD4+/CD8+ ratios ($P<0.01$) that occurred immediately post-exercise, while the percentages of suppressor/cytotoxic T cells ($P<0.01$) and NK cells ($P<0.0001$) increased (Table 3). The percentage of CTLs did not change significantly. A significant increase in the absolute number of total T cells ($P<0.05$), suppressor/cytotoxic T cells ($P<0.001$), CTLs ($P<0.01$) and NK cells ($P<0.001$) occurred immediately post-exercise. The absolute number of helper/inducer T cells did not change significantly. There were significant positive correlations between the heart rates and NK cells ($r=0.839$), suppressor/cytotoxicT cells ($r=0.692$), or helper/inducer T cells ($r=0.308$).

As the work rate increased, the leukocyte count, lymphocytes, and granulocytestended to increase (Fig.1). The changes in leukocytes and lymphocytes were large for the subject whose hemoglobin concentration was the lowest. As the work rate increased, the suppressor/cytotoxic T cells, helper/inducer T cells, and the NK cells tended to increase. The CD4+/CD8+ratios tended to decrease. The changes in these parameters were large for the subject whose hemoglobin concentration was the lowest.

As shown in Table 4, there was little change in the erythrocytes, hemoglobin concentration, or hematocrit values immediately after exercise.

Ⅳ. Discussion

This study demonstrates that the significant changes in circulating levels of leukocyte and lymphocyte subset counts occur rapidly in

Table 3 Changes in lymphocyte subset

	Sampling times					*F*-values
	Pre-exercise	Immediately post-exercise	10 min post	20 min post	60 min post	
CD3+ (%)	70.7±5.0	59.4±6.7**	67.5±5.2	70.6±4.6	71.6±6.9	6.16***
CD4-8+ (%)	26.1±5.2	33.8±5.2**	29.6±5.0	27.0±5.1	24.9±4.4	3.99**
CD4+8- (%)	42.3±7.5	30.2±4.8***	36.8±5.8	40.8±6.2	44.0±6.8	6.15**
CD3+16+56-, CD3+16-56+ or CD3+16+56+ (%)	2.5±1.7	3.7±2.4**	3.3±2.1	2.8±1.8	2.3±1.7	0.66
CD3-16+56-, CD3-16-56+ or CD3-16+56+ (%)	8.5±3.2	23.5±5.9***	14.0±3.6	8.4±2.4	5.2±2.3	29.85***
CD3+ ($\times 10^9$/l)	1.47±0.52	2.27±0.72*	1.71±0.73	1.46±0.50	1.41±0.54	2.81*
CD4-8+ ($\times 10^9$/l)	0.54±0.20	1.27±0.28***	0.74±0.28	0.55±0.19	0.49±0.20	15.12***
CD4+8- ($\times 10^9$/l)	0.87±0.33	1.15±0.37	0.93±0.41	0.85±0.33	0.86±0.32	1.03
CD3+16+56-, CD3+16-56+ or CD3+16+56+ ($\times 10^9$/l)	0.05±0.04	0.15±0.11**	0.09±0.08	0.06±0.05	0.05±0.04	2.99*
CD3-16+56-, CD3-16-56+ or CD3-16+56+ ($\times 10^9$/l)	0.17±0.08	0.87±0.22***	0.34±0.13	0.17±0.05	0.10±0.05	53.99***
CD4/CD8 (ratio)	1.71±0.66	0.93±0.24**	1.28±0.33	1.55±0.43	1.81±0.55	4.72**

Values are presented as mean ± standard deviation.

*$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. rest.

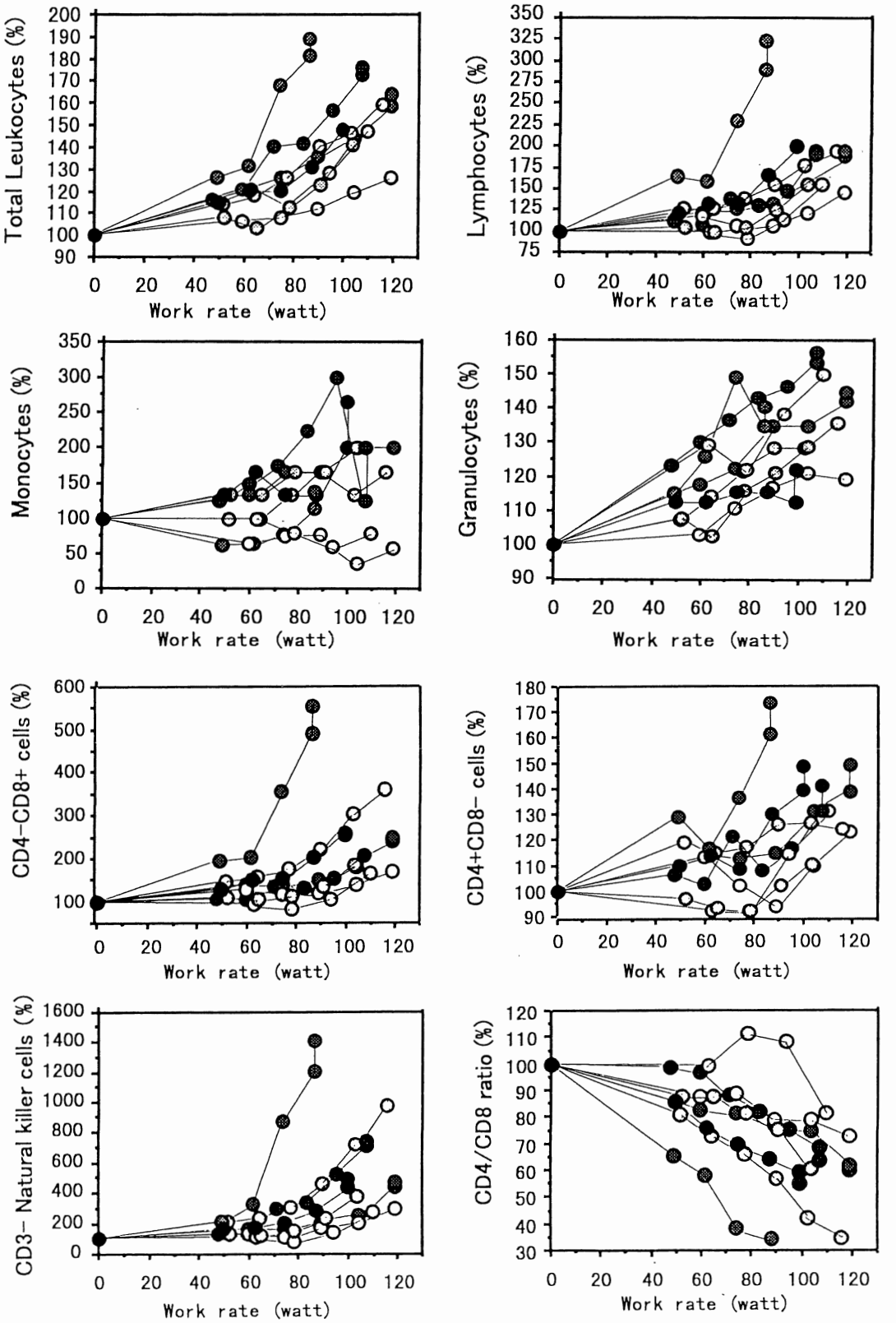

Fig.1 changes in the concentration of circulating leukocytes, leukocyte differential counts and lymphocyte subset

Table 4 Changes in erythrocyte counts, hemoglobin concentration and packed cellvolume

	Sampling times					*F*-values
	Pre-exercise	Immediately post-exercise	10 min post	20 min post	60 min post	
Erythrocytes ($\times 10^{12}$/l)	4.34±0.28	4.56±0.26	4.34±0.27	4.23±0.32	4.12±0.33	2.13
Hemoglobin concentrations (g/l)	12.6±1.8	13.2±1.8	12.8±1.8	12.2±1.6	12.0±1.8	0.56
Packed cell volume (%)	37.8±4.6	39.7±4.4	37.9±4.3	36.7±4.4	36.0±4.9	0.80

Values are presented as mean ± standard deviation.

response to short term moderate exercise.

Physical exercise is known to induce immediate leukocytosis, the magnitude of which is mainly related to the intensity of the exercise[10]. As shown in Fig. 1, observations, from the present study, of the increased circulating leukocyte count in response to short term exercise is consistent with this. The number of circulating total leukocytes (54%), lymphocytes (86%), granulocytes (37%) and monocytes (68%) increased immediately post-exercise. These increases were transient, with the values following exercise returning to near baseline levels within 20-60 min. The increase in leukocytes in response to short time exercise is due mainly to increases in lymphocytes. The prime source of increased lymphocytes observed after short term exercise has been attributed to mobilization of white blood cells from the marginal pool due to hemodynamic redistribution and increase by cortisol and catecholamine secretion[1]. Hemodynamic factors related to exercise that include raised cardiac output, which together with the possible differential localization of leukocytes in storage and low flow areas[15], maybe responsible for the leukocytosis apparent after exercise.

Significant changes in lymphocte subset count were found immediately after exercise. The percentage of total T cells decreased immediately after exercise, although the absolute number of T cells increased. These changes imply that other lymphocytes such as B cells and NK cells except T cells migrated into the circulation[8]. The absolute numbers of total T (54%), suppressor/cytotoxic T (135%), CTLs (200%) and NK cells (412%) increased immediately after exercise. The increased lymphocyte count is attributable to a rise in these cells. These increases are transient, with values following exercise returning to near baseline levels within 20 min. During the recovery period, both helper/inducer and suppressor/cytotoxic T cells tended to decrease, but the CD4+/CD8+ ratio was increased.

Previous investigators[6, 7] found a decrease of the CD4+/CD8+ ratio after exercise despite increases in both helper/inducer and suppressor/cytotoxic T cells, typically due to reduction in percentage of helper/inducer T cells. Our findings showed a similar tendency. Other studies[16, 17] support the view that changes in the CD4+/CD8+ ratio reflect shifts of cell population rather than a down regulation of the immune system.

It was previously reported that mental stress and smoking associated with increase of the NK cells and decrease of the CD4/CD8 cells[18, 19]. The present findings are consistent with previous findings regarding the effects of acute mental stress[18] or cigarette smoking[19] on blood cell counts and lymphocyte subsets. These changes may well be explained by the action of increased plasma catecholamine levels. Previous studies disclosed that catecholamine concentrations increase significantly after exercise[5, 14, 20]. T cells

have surface receptors for sympathetic neurotransmitters, and exercise is known to induce a rapid up-regulation of T cell β-adrenoceptors, especially when the intensity is high[1]. Interestingly, β-adrenergic receptor measurements from human lymphocyte subsets demonstrate that these receptors are predominantly localized at NK cells, in contrast to other lymphocytes[21], and that the binding activity of suppressor/cytotoxic T cells is greater than that of helper/inducer T cells[21, 22]. Therefore, during short-term exercise, the distribution of lymphocyte subsets may be affected mainly by the sympathetic effect of exercise. The circulating lymphocytes are probably being supplied by the marginal pool such as spleen, lungs and walls of high-endothelial venules.

As shown in Figure 1, the change rates of total leukocytes, lymphocytes, lymphocyte subsets and CD4/CD8 ratios differ in the subjects. In a subject who had a tendency of anemia, these changes were largest. So, the effect of exercise on leukocyte redistribution may be associated with hemoglobin concentrations. However, since the sample size in this study was small, to conclusively evaluate the effect of hemoglobin concentration, studies using a larger sample would be necessary.

Exercise produces important shifts in extracellular fluids, which can result in hemo-concentration. However, the increased leukocytes, lymphocytes and lymphocyte subset counts observed in this study, cannot be solely explained from the changed hemo-concentrations induced by short term exercise, because there was little change in erythrocyte count, hemoglobin concentration, or packed cell volume immediately after exercise.

This study has demonstrated that rapid hematological changes occur in response to short term moderate exercise . This response is characterized by increases in the number of NK cells, total leukocyte count, and lymphocyte count, and by a decrease in the CD4/CD8 ratio. These changes are transient in nature and may be associated with hemoglobin concentrations . Further studies are now required to establish the biological significance of transient shifts in blood lymphocyte subsets after moderate exercise.

Acknowledgements

We thank Ms. Kohumi Iuchi for her exellent technical assistance.

References

1) Shephard RJ, Rhind S, et al: Exercise and the immune system natural killer cells, interleukins and related responses. Sports Med 18:340-69, 1994

2) Nielsen HB, Secher NH, et al: Lymphocytes and NK cell activity during repeated bouts of maximal exercise. Am J Physiol 271: R222-R 227, 1996

3) Ndon JA, Snyder AC, et al: Effects of chronic Intense exercise training on the leukocyte response to acute exercise. Int J Sports Med 13: 176-182, 1992

4) Gabriel H, Kindermann W: The acute immune response to exercise: What does it mean?. Int J Sports Med 18: S28-S45, 1997

5) Nieman DC, Henson DA, et al: Effects of brief heavy extertion on circulating lymphocyte subpopulations and proliferative response. Med Sci Sports Exerc 24: 1339-1345, 1992

6) Field CJ. Gougeon R, et al: Circulating mononuclear cell numbers and function-during intense exercise and recovery. J Appl Physiol 71: 1089-1097, 1991

7) Gray AB, Smart YC, et al: Anaerobic exercise causes transient changes in leukocyte subsets and IL-2R expression. Med Sci

Sports Exerc 24: 1332-1338, 1992
8) Fry RW, Morton AR, et al: Acute intensive interval training and T-lymphocytefunction. Med Sci Sports Exerc 24: 339-345, 1992
9) Nieman DC, Nehlsen-Cannarella SL, et al: The effects of acute moderate exercise on leukocyte and lymphocyte subpopulations. Med Sci Sports Exerc 23: 578-585, 1991
10) Burstein GER, Assia E, et al: Changes in white blood cell count during prolonged exercise. Int J Sports Med 8: 253-256, 1987
11) Keast D, Cameron K, et al: Exercise and the immune response. Sports Med 5: 248-267, 1988
12) Porcari JP, Ebbeling CB, et al: Walking for exercise testing and training. Sports Med 8: 189-200, 1989
13) Kusaka Y, Kondo H, et al: Healthy lifestyles are associated with higher natural killer cell activity. Prev Med, 21: 602-615, 1992
14) Kendall A, Hoffman-Goetz L, et al: Exercise and blood lymphocyte subset responses: intensity, duration, and subject fitness effects. J Appl Physiol 69: 251-260, 1990
15) Muir ALM, Cruz BA, et al: Leukocyte kinetics in the human lung: role of exercise and catecholamines. J Appl Physiol 57: 711-719, 1984
16) Keast D, Morton AR: Long-term exercise and immune function. Watson RR, Eisinger M, (eds): Exercise and disease. CRC Publishing, Boca Raton, 1992, pp89 120
17) Espersen GT, Elbach A, et al: Effect of physical exercise on cytokines and lymphocyte populations in human peripheral blood. Acta Pathol Microbiol Immunol Scand 98: 395-400, 1990
18) Kondo H, Morimoto K: Effects of mental arithmetic stress on blood cell counts and the immune system. Environ and Health Prev Med 1: 76-79, 1996
19) Kondo H, Imaki M, et al: Acute effects of cigarette smoking on platelet behabior and immune system in healthy young women. J Anal Bio-Sci 20: 157-166, 1997
20) Shinkai S, Watanabe S, et al: Cortisol response to exercise and post-exercise suppression of blood lymphocyte subset counts. Int J Sports Med 17: 597-603, 1996
21) Landmann R: Beta-adrenergic receptors in human leukocyte subpopulations. Eur J Clin Invest 22 (suppl1): 30-36, 1992
22) Landman RMA, Burgisser E, et al: Beta-adrenergic receptor are different in subpopulations of human circulating lymphocytes. J Receptor Res 4: 37-50, 1984