

Effect of One Bout General Physical Education on Leucocytes and Immune Cells

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Abstract: Circulating leukocytes are a rich and readily accessible source of information about the health and physiological state of an individual. Blood is a complex tissue, containing a variety of cell types including T-cells, B-cells, monocytes, NK cells and granulocytes, each of which can be further subdivided [1]. The relative proportion of each of these cell types can vary greatly between individuals and with states of health and disease and in response to stimuli. This study was done to observe the effects of one bout general physical education on CD3 (an immune cell) and CBC (complete blood cell) plasma. Twenty normal healthy female subjects were exercised one bout general physical education for a duration 90minutes, at 50-65 % Vo_{2max} and all subjects were informed of the risks and purposes of the study before their written consent was obtained. Blood samples were drawn from the antecubital vein before and immediately after one bout general physical education. Plasma was separated from the cells and stored at $-20^{\circ}C$ until analyzed. Statistical analyses, tables, graph, means \pm SD, t test used for measurement CD3 and CBC response (α was set at 0.05 and 0.01). Means showed the concentration of CD3 decreased but the concentration of CBC increased after exercise. T test showed CD3 and CBC response were significantly ($P \leq 0.05$). The present study has indicated the magnitude of exercise-induced leukocytosis. It was concluded that exercise may induces changes in leukocytes subsets but do not suppress immune function after selected exercise. Recent studies show that several immune cells can be detected in plasma after exercise [2, 3, 4]. In this study immune cells changed. The effect of exercise on subjects was significant that may be transient and related with intensity and duration [2, 5]. The results suggest that the exercise induced changes in lymphocyte subsets but may not induced suppression immune function, so exercise is benefit for all, but we have consider proper intensity, duration and frequency.

Key words: CD3 • Exercise • Neutrophile • Lymphocyte

INTRODUCTION

Individuals will achieve significant improvements in physical working capacity by performing appropriate type of exercise. The change in leukocyte subsets with exercise is dependent on both intensity and duration, with prolonged, high-intensity endurance exercise leading to the greatest degree of cell trafficking [1, 6, 7]. The blood granulocyte count rises strongly (250%) along with monocytes (60%) while lymphocytes egress from the blood compartment (40%). Relatively few studies have been conducted to define the response of these phagocytic cells, especially monocytes, to prolonged intensive exercise. Although more research is needed, investigators have observed that while both moderate and high-intensity exercise are associated with a sustained increase in blood

granulocyte and monocyte phagocytosis and degranulation, only moderate exercise tends to enhance oxidative burst activity, whereas high-intensity exercise often has the opposite effect. Leucocytes the mobile units of the protective system of the body, may circulate freely in the blood, adhere to the vascular endothelium in sites where blood flow is relatively slow and then once again re-enter the circulation in a process of continuous exchange [8, 9, 1]. This process of continuous exchange of leucocytes is influenced by proper stimulation such as sport or exercise [10, 11]. During exercise, leucocytes are recruited to the blood and if muscle damage occurs the cytokine level is enhanced. After prolonged, intense exercise the number of lymphocytes in the blood is reduced and the function of natural killer cells is suppressed[5].The well known phenomenon of leukocytosis is induced by exercise.

The change in peripheral leukocyte number is assumed to be diagnostically informative and may be a prognostic marker, reflecting organ damage and restoration after strenuous physical exercise [12, 13]. The intensity of leukocytosis is proportional to the intensity of work and duration of exercise, independent of gender and subject fitness level.

MATERIALS AND METHODS

This study was conducted at the Department of Physiology Exercise, IAU Varamin-Pishva. Twenty subjects were selected from the college students, according to the following criteria, Normal healthy female subjects with no disease or positive clinical findings, age ranging from 18 to 26 years, weight ranging from 50 to 62 kilograms, non smokers. Subjects were informed of the purpose and risks of participation in the study before written consent was obtained. On the experimental day, a general physical examination of each volunteer was made. The procedure of exercise was explained to all the study participants prior-to exercise. A continuous monitoring of exercise belt velocity was observed throughout the test. Each subject was allowed a 30 minutes rest period before taking pre-exercise blood sample. After taking the first sample, each subject was asked to do exercise (as mentioned below. Then the subjects performed general physical education for 80 minutes (Table 1). Blood samples were taken immediately after exercise During the entire testing session, subjects only consumed water.

Total leukocyte count was determined manually using an improved Neubauer haemocytometer and number of CD3 were enumerated by fluorescence-activated flow cytometry for samples. The results were statistically-evaluated. Whole blood was collected into sodium heparin tubes, diluted with an equal volume of PBS, then layered over Ficollpaque density gradient separation solution and centrifuged at 300 g for 20 min at room temperature. The mononuclear cell layer (PBMC) was removed and washed twice in RPMI-1640 medium supplemented with 2 mM glutamine and gentamycin. Cell viability and cell counts were assessed by Trypan blue exclusion and then cells were labeled with CFSE. After culture, cell suspensions were labeled with CD3 (allophycocyanin), for 15 min in the dark at room temperature. Samples were then washed with 3 ml PBS and re suspended in 200 µl paraformaldehyde solution (2%) and placed in Trucount tubes. Surface marker and

Table 1: Training Program

| Physical activity | Time |
|--------------------|------|
| General warm up | 10 |
| Light running | 5 |
| Specific warm up | 10 |
| Endurance training | 10 |
| Strength training | 10 |
| Agility training | 10 |
| Power training | 10 |
| Speed training | 10 |
| Cool down | 5 |

CFSE analysis was conducted by using a FACSC alibur flow cytometer. The same forward- and side-scatter parameters were used for each trial as established for human peripheral leucocytes. Standard gating procedures were used to select mainly lymphocytes and to differentiate between labeled and unlabeled cells. Fluorescent staining was used to further characterize T lymphocytes (CD3). Data were analyzed by using Flow Jo version 3.3 software. Statistical analyses, tables, graph, means ± SD, t test used for measurement CD3 and CBC response (α was set at 0.05 and 0.01).

RESULTS

The pre exercise and post exercise total leukocyte counts and CD3 are given in Tables 2. While Tables 2, 3 and Figure 1 show the differences, percent variation and significance of these differences.

Neutrophils, lymphocytes and platelets in blood compared to rest, the total number of circulating leukocytes increased significantly. Lymphocyte numbers changed. There appeared to be a re-distribution of lymphocytes and a change in activation/adhesion status. Circulating numbers of monocytes and eosinophile did not differ, the percentage distribution of the circulating monocyte population did not change.

Table 2: Changes of CBC and CD3

| Variables | Med ± Sd | |
|-------------|-------------|--------------|
| | Before Ex. | After Ex. |
| CD3 | 57±5.7 | 50.4±5.98 |
| Neutrophile | 54.6±7.59 | 48.2±9.13 |
| Lymphocyte | 39.65±5.65 | 46.1±9.46 |
| Monocyte | 4.05±2.48 | 3.85±1.69 |
| Eozinophile | 1.7±1.56 | 1.65±1.15 |
| Platelate | 271.95±39.1 | 327.45±39.81 |

*: p ≤ 0.05
 **: p ≤ 0.01

Table 3: p value of CBC and CD3

| Variable | P | t |
|-------------|---------|-------|
| CD3 | 0.00* | 6.91 |
| Neutrophile | 0.01* | 2.82 |
| Lymphocyte | 0.007** | -3.05 |
| Monocyte | 0.76 | 0.31 |
| Eozinophile | 0.9 | 0.13 |
| Platelate | 0.00* | -9.02 |

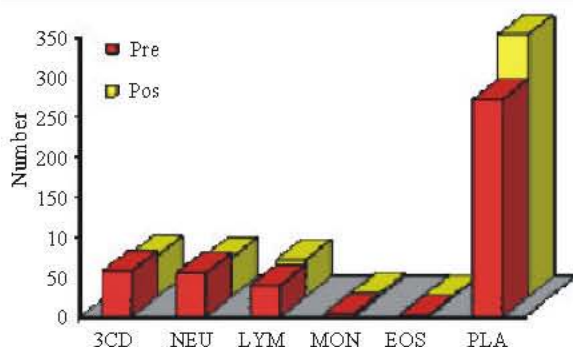


Fig. 1: Changes of CD3 and CBC

DISCUSSION

Circulating cytokine concentration are elevated in response to strenuous exercise and other forms of physical stress. Blood leucocytes are a first line of defense against invading pathogens and a major source of immune inflammatory mediators. Changes in leukocyte distribution, function and antigen expression in response to physical exercise are well described [9, 14, 15] and the intention of this study was to investigate possible interactions between circulating leukocytes and immunological events in skeletal muscle. Leukocytosis after exercise is a common finding in most studies and is usually attributed to an increase in the number of neutrophils and is explained as the release of these cells from the marginated pool. Previous studies have interpreted leukocytosis as an indication/result of muscle inflammation. The present study has indicated the magnitude of exercise-induced leukocytosis. It was concluded that exercise may induces changes in leukocytes subsets but do not suppress immune function after selected exercise. Recent studies show that several immune cells can be detected in plasma after exercise. In this study immune cells changed. The effect of exercise on subjects was significant that may be transient and related with intensity and duration. The results suggest that the exercise induced changes in lymphocyte subsets but may not induced suppression immune function. Number of CD3 may increased during exercise but decreased after exercise to base in rest.

Performance un custom exercise induced stimulated CD3. The effect of exercise on subjects was significant that may be transient and related with intensity and duration and this exercise induced changes in lymphocyte subsets. The increase in total leukocyte count reported by these researchers was slightly more because the exercise stress was more severe than the present study and another reported that moderate exercise elicits lower changes in cell concentration than strenuous exercise [16, 17, 18]. Nieman have reported that exercise-induced leukocytosis was evident even after three hours of recovery [4] while Suzuki observed the persistence of exercise induced leukocytosis for one hour after termination of exercise [2, 15, 4]. The present study has clearly indicated that the magnitude of exercise-induced leukocytosis depends upon the intensity and duration of exercise [19]. Precautions must be taken while drawing blood samples for such routine investigations as total leukocyte count.

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