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The Effect of Two Months Adaptive Aerobic Exercise on CD95 Expression on Peripheral Blood Lymphocytes

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Abstract: Many studies have recently shown that exercise-mediated lymphocyte apoptosis occurs in peripheral lymphocytes. However, many other studies did not confirm this notion. We aim to study the effect of two months adaptive aerobic exercise on CD95 expression on lymphocytes in healthy women. 24 healthy inactive women with 20.98± 3.14 average age were divided into 2 groups; the test group was performed 8-wk adaptive aerobic exercise completed a long moderate-intensity exercise consists 30 minutes training in day and control group completed 8-wk inactivity. Blood was collected immediately before and after 8-wk, after exhaustive exercise, and 24h after exhaustive test to determine CD95 expression on lymphocytes. CD95 expression on lymphocytes was determined by employing flow cytometry. No significant difference was detected in the amount of lymphocyte apoptosis between first and second stage in control group. In contrast, we found that the level of CD95 was significantly decreased after two months of adaptive exercise. Moreover, we observed that CD95 expression did not changed after exhausting exercise in both control and experimental groups. *In* conclusion, our data show that adaptive exercise reduces CD95 expression on lymphocytes, indicating that health activity might lead to decrease apoptosis of peripheral blood lymphocytes in active person in compare with inactive person.

Key words: Apoptosis · Lymphocyte expression of CD95 · Adaptive exercise

INTRODUCTION

Apoptosis or programmed cell death has been known as one of the most important key regulators of cell proliferation, survival and death [1]. Nowadays, the response of immune system to exercise and sport has evolved into a topic which is attracted by health care and sport professionals. Alternations in immune system parameters are often seen in many athletics [2, 3]. Therefore, a better understanding immune system response to exercise and sports may help both athletic and heath professional.

CD95 or FAS is a surface molecule which is expressed on a apportion of lymphocytes and its expression is increased during apoptosis, indicating that CD95 is a marker for apoptosis in lymphocytes [4]. Many studies have demonstrated that intensive and exhaustive exercises activate lymphocytes and finally lead to apoptosis of these activated cells. For example, Kruger *et al.*, have shown that exercise enhances the number of CD95-expresssing lymphocytes in payer's patches and lung of mice significantly increased. Consistently, they reported that CD95-deficient mice showed a remarkable lower T-lymphocytes apoptosis in

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spleen, lung and the bone marrow after exercise, indicating that CD95 plays a crucial role in exercise-mediated apoptosis [5]. Moreover, another study reported that intensive exercise enhances CD95 expression on lymphocytes of healthy volunteers, but moderate exercise did not have any effect on CD95 expression [6].

Accumulating evidence imply that adaptive exercise not only have various beneficial effects on skeletal, muscle and heart but also it may enhance the function of immune system [7, 8]. Regular training promote resistant to upper respiratory infection, whereas intensive training increase the risk of infection [9, 10]. Accordingly regular training protect against malignancy, but exhaustive exercise is somehow associated with cancer risk [11]. Overall, mounting evidence indicate that regular training have many advantages on immune system. However, its mechanism at least partially, remains to be explored.

Several studies have reported that interleukin (IL)-6 has been increased during and after exercise. Thus, IL-6 level has been shown to be 1000 fold increased after marathon [12]. In addition, the level of other inflammatory cytokines such as tumor-necrosis factor (TNF-αλπηα), IL-1 have been shown to be increased at least 2-3 fold after strenuous exercise [13]. Furthermore, many reports have shown that the number of lymphocytes decreases following intensive exercise, which could be as the result of induction of apoptosis in these cells [14]. Herein, we investigate whether two months of adaptive exercise could have any effect on CD95 expression in lymphocytes of health women.

MATERIAL AND METHODS

Volunteers: 24 healthy inactive women with 20.98± 3.14 average age were divided into 2 groups: A month before starting an exercise program participants include the descriptive variables of height, weight, age, body fat percentage and Vo2max are measured in both experimental and control groups. The heart rate during exercise was measured to ensure that same intension training program by marking gauge using Polar heart rate. The characteristic of volunteers is shown in table 1.

Exercise Protocol: Exercise bouts took place between two months, just at the end of the dark cycle one session strenuous exercise were performed at 80% maximal oxygen uptake on treadmill for 10 min in both of them according to the previous study[15]. The experimental group was run at this intensity for 30 min/day, 3 days/week for 8 week. They performed 8-wk moderate-intensity adaptive aerobic exercise consists 30 minutes running in day and control group completed 8-wk inactivity. Our strenuous exercise consisted of treadmill running. The first day after two month's adaptive exercise, one session strenuous exercise were performed at 80% maximal oxygen uptake on treadmill for 10 min in both of the groups. In addition, running time of control group dramatically increased when compared to experimental group. Blood was collected at rest, immediately after 8-wk, 30 min after exhaustive exercise, and 24h after exhaustive test to determine CD95 expression on lymphocytes cells.

Table 1: Central tendency and dispersion indices of the variable in the sample group.

	Variable	average	St.d
Age	*(year)*	*21.50*	*3.02*
High	*(cm)*	*161.54*	*0.546*
Weight	*(kg)*	*59.86*	*7.165*
BMI	*(kg/m^2)*	*23.09*	*2.87*
VO_{2max1}	*(ml/kgl)*	*20.290*	*6.66*
VO_{2max2}	*(ml/kgl)*	*22.931*	*5.09*

Simple Collection and Processing: Blood sample were taken before, after two months of adaptive exercise, immediately after intensive exercise and 24 h later. All samples were taken in special tubes containing EDTA as anticoagulant. Blood samples were stained with 10 µl of either anti-human CD95-PE or isotype antibody (both from Biolegend, CA) for 30 min on ice and kept in dark. Then washed with FCM buffer (PBS containing 1% BSA) and analyzed by flow cytometry (FACS Calibur; Becton-Dickinson). Data were analyzed by FCS Express software. Lymphocytes were gated based on their forward and side scatters and then CD95 expression was analyzed on them.

RESULTS AND DISCUSSION

In order to gate the lymphocyte population, samples were ran by flow cytometry and lymphocytes were gated based on their forward and side scatter. As demonstrated in figure 1, lymphocytes possess a small side scatter and forward scatter and show a typical population. When we examine the expression of CD95 on lymphocytes of control group we found that this antigen is highly expressed on lymphocytes and did not change after two months (Figure 2). Interestingly, we found that two months of adaptive training reduced CD95 expression on lymphocytes compared to the control group (Figure 2).

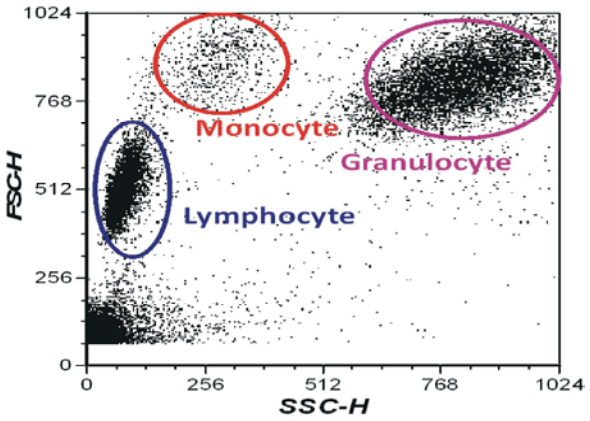

Fig. 1: Gating of lymphocyte population by flow cytometry. Whole blood cells were ran by flow cytometry and lymphocyte populations were gated based on their forward scatter (FSC) and side scatter (SSC).

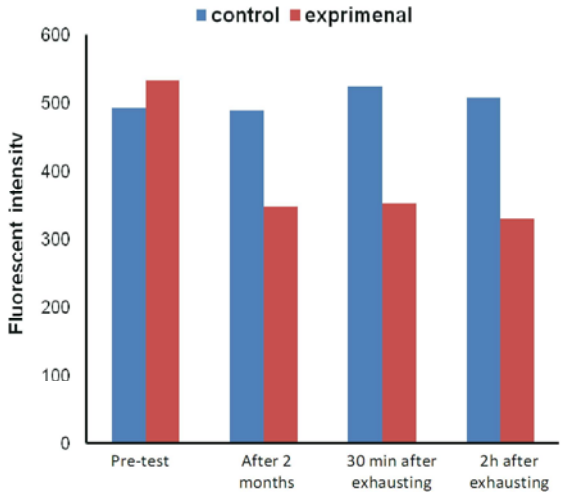

Fig. 2: CD95 expression on lymphocyte in both the control and experimental groups. Whole blood cells were stained with either isotype antibody or mouse anti-human CD95-PE for 30 min on ice, then washed and fixed with 1% paraformaldehyde. Cells were analyzed by flow cytometry and the fluorescence intensity of CD95 expression was measured with FCS express software. CD95 expression was examined on the lymphocytes of the both control and experimental groups at the time points indicated in the figure. Fluorescence intensity of CD95 expression on lymphocyte populations of all participants was evaluated.

However, another study has previously shown that resistant exercise enhances apoptosis and CD95 expression in lymphocytes. They have also concluded that resistant exercise induces apoptosis in an intensity-dependent manner [16]. We could explain this discrepancy as we used a moderate protocol for adaptive exercise and our protocol may have some beneficial effects on immune system by reducing CD95 expression on lymphocytes. CD95 has been shown to be associated with apoptosis and is a signal of initiating of apoptosis [4]. Thus, reduction in the level of CD95 expression implies that adaptive light training apoptosis might strength the immune system against pathogens. Supporting of this notion, a previous report has demonstrated that regular training reduces the chance of occurring malignancy [11]. Our data are not in agreement with a previous report showing that expression of CD95 is influenced by acute exercise in healthy children and adolescents [17]. However, another study conducted by Simpson RJ, *et al*., have recently demonstrated that apoptosis of peripheral lymphocytes doesn't appear to contribute to exercise-mediated lymphocytopenia [14]. We have to note that the type and duration of the training protocols as well as the gender and race of the participants may influences on the level of CD95 expression and apoptosis of lymphocyte.

Finally, when we applied exhaustive exercise for control group we observed that CD95 expression significantly upregulated on lymphocytes 30 min after exhaustive exercise. However, even though the expression of CD95 on lymphocytes reduced 24h latter, but it did not reach to the steady-state (Figure 2). Our data are in the line with other studies demonstrating that CD95 expression on lymphocytes increased during and just after the intensive exercise an then resorted to the steady-state 24h latter [17, 18]. On the other hand, we observed the CD95 expression on lymphocytes of experimental group did not significantly enhance 30 min after exhaustive exercise implying that adaptive exercise may prevent of CD95 uprgulation on lymphocytes (Figure 2). Accumulating evidence indicate that light and regular training enhance immune system function [2, 10], whereas intensive exercise suppress the immune system and increase lymphocytes apoptosis [9, 17]. Our data show that adaptive exercise may reduce apoptosis-related molecules, CD95. However, we have to consider that other makers and molecules associated with apoptosis such as Bcl-2, mitochondrial membrane potential need to be examined after two months of adaptive exercise.

In conclusion, we are showing for the first time that adaptive exercise reduces CD95 expression on lymphocytes of healthy individual. Thus, light and regular training may prevent induction of apoptosis in lymphocyte.

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Conflict of Interest Disclosure: The authors declare that they have no competing financial interests.

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