



Influence of physical exercise on interleukin-17, cortisol and melatonin levels in serum, whole blood and mitogen activated peripheral blood mononuclear cells

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ABSTRACT

The purpose of this study was to assay the effect of two months exercise and two months silent on the levels of interleukin-17 (IL-17), melatonin and cortisol in serum, whole blood (WB) and peripheral blood mononuclear cells (PBMCs) cultures. Thirteen male non-athletic health volunteers participated in a two months moderate exercise program (running %50-%65 VO₂ max). The blood samples were collected in three stages, 24 hours before to start exercise, 48 hours and two months after the last session of the training. WB and PBMCs were cultured with mitogens phytohemagglutinin and lipopolysaccharides for 48 hours. The serum and supernatants of WB and PBMCs were analyzed for IL-17, melatonin and cortisol by enzyme-linked immunosorbent assay. Red blood cells (RBC) variables were also measured. IL-17 secretion by PBMCs in the post-exercise stage (51.14±5.43 pg/ml) compared with pre-exercise (36.74±6.98 pg/ml) was increased. But, the amount of melatonin produced by PBMCs in the post-exercise (7.94±0.35 pg/ml,) and 2-month silent (6.05±0.27 pg/ml) stages compared with pre-exercise (9.16±0.19 pg/ml) were decreased. Regardless of the effect of the exercise, PBMCs had more ability to produce IL-17 than WB. As well as, level of cortisol in WB was higher than in serum and PBMCs culture. Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration RBCs were also increased in post-exercise stage. The other measured parameters were not changed during exercise and recovery. Moderate exercise caused to higher in vitro production of IL-17 and lower production of melatonin by PBMCs.

Keywords: Cortisol, Exercise, Interleukin-17, Lymphocyte, Melatonin

INTRODUCTION

The inflammation is a physiological response of the immune system to microbial agents or tissues damages. Several factors such as cytokines, lymphocytes and some hormones orchestrate inflammation. There are some controversial reports that exercise could have anti/pro-inflammatory effects and can protect/vulnerable us against the development of several chronic diseases [1-4].

IL-17 is a cytokine produced by lymphocytes T helper-17, epithelial, endothelial, and fibroblastic cells. IL-17 induces inflammatory process by effect on a wide range of cells in order to produce inflammatory cytokines such as tumor necrosis factor, interleukin-1 (IL-1), interleukin-6 (IL-6) and prostaglandin-E₂. In addition, IL-17 production, it is mainly induced by IL-6 production that some researchers have reported that certain chronic diseases are associated with excessive production of IL-17 [3, 5]. A study shows that combined exercise for 8 weeks significantly reduces the production of IL-17 in plasma and peripheral mononuclear blood cells (PBMCs) culture [6]. Meanwhile, another study reported that an hour of exercise significantly increases IL-17 in men [7].

Melatonin, N-acetyl-5-methoxytryptamine, mainly is produced from pineal gland but its production is not limited to pineal gland and can be produced by blood lymphocytes and bone marrow [8-10]. Melatonin as an immune system regulatory agent seems to inhibit inflammation during exercise according to the intensity of exercise. Plasma melatonin levels increase after exercise in a transient and short-term process. But a decrease or no change in melatonin amount have also been reported after exercise [2, 11].

Cortisol is a steroid hormone and is produced during a stress response and could have anti-inflammatory effects. During exercise, catecholamine's are produced which could reduce production of cortisol and some inflammatory cytokines like IL- 1 and tumor necrosis factor [12, 13].

This study was designed due to existence of controversial reports about the effect of exercise on the inflammatory system factors. Therefore, the chronic effect of moderate exercise (running) on the secretion of IL- 17, melatonin and cortisol in serum, whole blood (WB) and PBMCs cultures samples were investigated. The routine red blood cells parameters were also determined. The samples were collected in different stages of pre-exercise, post-exercise and two- month silent after the exercise (recovery) from volunteers.

MATERIALS AND METHODS

Subjects

This study was a part of a larger study to explore the effect of exercise on immune systems. All the volunteers who participated in this study filled out a questionnaire assessing their physical activities, medical histories and demographic characteristics. The participants did not have any altitude exposure, intake of iron supplements or other medications. Thirteen healthy male university students (age 19-23 years) were selected and attended in this study. The entire subject participating in the research signed an informed written consent form approved by the Ethics Committee of Hamadan University of Medical Sciences.

Exercise protocol

Before beginning the protocol, two primary exercise sessions were performed to detect the heart rate of every one and each participant himself measured his heart rate that might be not less than 130 and not more than 150. The exercise protocol (running) was used with the average intensity of 50%-65% maximum oxygen consumption. The participants took part in the exercise every other day for two months at 19:30 to 19:55. Each exercise session included: 5 minutes warm-up and 20 minutes of moderate-intensity exercise (running). According to the age and resting heart rate, exercise intensity and minimum and maximum heart rate for each individual was calculated by Karvonen formula [14]. The highest minimum and the lowest maximum heart rate were chosen for all participants.

Blood collection

Blood were collected in three different times from all participants. Twenty four hours prior to the exercise protocol (pre- exercise), forty eight hours after the last session of the exercise (post- exercise) and end of two months rest after exercise (2- month silent). Fasting venous sterile blood samples were taken from the cubital vein with the each participant at rest. Blood samples were collected in two specimen containers, one containing ethylenediamine tetra acetic acid (EDTA) as anticoagulant, and the other without anticoagulant. Sera were isolated and frozen at -80 °C for subsequent enzyme-linked immunosorbent assays (ELISA) analysis, and the bloods containing anticoagulant were used to culture WB and PBMCs and red blood cells (RBC) tests. [15].

In vitro production of IL- 17, melatonin and cortisol by the WB culture activated with mitogens

To determine *in vitro* production of IL- 17, melatonin and cortisol by WB, one ml of fresh blood containing anticoagulant was suspended in one ml complete RPMI1640 medium (Gibco-BRL, Australia) containing 100 U/ml penicillin G (Hayan, Iran), 10% FCS (Gibco-BRL, Australia), 100 µg/ml streptomycin (Hayan, Iran) and 5 µg/ml PHA (Sigma, Germany), 25 µg/ml LPS (Sigma, Germany), and incubated for 48 h in a CO₂ incubator at 37 °C. Thereafter, the supernatants were collected and frozen at -80°C until IL- 17, melatonin and cortisol detection [16, 17].

In vitro production of IL- 17, melatonin and cortisol by PBMCs culture activated with mitogens

Briefly, four ml of fresh blood containing anticoagulant was diluted with 8 ml Hank's solution. PBMCs were isolated by Ficoll- Paque and washed twice with Hank's solution. 2×10^5 cells were cultured as monolayer culture in 1ml RPMI1640 medium, supplemented with materials like for the WB culture and incubated for 48 h in a CO₂ incubator at 37 °C. The supernatants were collected and frozen at -80°C until IL- 17, melatonin and cortisol measurements [16].

IL- 17, melatonin and cortisol measurement by ELISA

Serum and supernatants levels of IL- 17, melatonin and cortisol were determined by ELISA according to the manufacturer's instructions (Boster Biological Tech.Co. USA for IL- 17, Chongqing Biospes Co. China for melatonin and DiaPlus inc. USA for cortisol). The standard curves were generated by Smart Magellan™ data analysis software and the amount of IL- 17, melatonin and cortisol were calculated.

RBC parameters

RBC count, hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular Hb (MCH) and mean corpuscular Hb concentration (MCHC) of the samples were determined by using standard laboratory procedures and an Sysmex-KX21N cell counter analyzer [15].

Statistical analysis

To compare the data of IL- 17, melatonin and cortisol from different stages within each samples the General Linear Models (GLM) procedure as a repeated measurement were used. Paired- sample T- test was also used to determine the differences in the mean values ($P < 0.05$). To analysis production of IL- 17, melatonin and cortisol between different samples serum, WB and PBMCs Kruskal- Wallis and Mann-Whitney tests were used for this analysis. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for Windows, Version 16). Results were expressed as mean \pm standard error.

RESULTS

Thirteen male university students with main demographic characteristics include: mean age= 22.38 \pm 0.89 years, mean weight=71.54 \pm 2.50 kg, mean height=176 \pm 2 cm, mean heart rate at rest=63.38 \pm 0.33 beat, mean maximum heart rate=197.62 \pm 0.89 beat and mean heart rate at exercise=130.46 \pm 0.53 beat were recruited for this study. Three different samples of blood serum, WB and PBMCs were collected at three different stages, twenty hours before exercise, forty eight hours after two months exercise and after two months rest.

Figure 1 shows that there was a significant increase in the production of IL- 17 by PBMCs in post-exercise status (51.14 \pm 5.43 pg/ml) compared with its level in pre-exercise time (36.74 \pm 6.98 pg/ml, $P = 0.017$). But, after two months rest the potential of activated PBMCs by mitogen to produce IL- 17 has returned to pre- exercise stage. Levels of IL- 17 in serum and WB cultures were not changed in before and after exercise and after two months recovery.

Final results for each sample in different stages showed that *in vitro* production of melatonin from PBMCs were decreased significantly after exercise (7.94 \pm 0.35 pg/ml, $P = 0.038$) and 2- month silent (6.05 \pm 0.27 pg/ml, $P = 0.010$) compared with pre- exercise state (9.16 \pm 0.19 pg/ml) (Figure 2).

According to data, it seems that exercise and resting did not change the levels of cortisol in different samples and in different stages (Figure 3).

Figure 4 shows the total levels of IL- 17, melatonin and cortisol in three samples of serum, WB and PBMCs cultures supernatant regardless the effect of exercise. Statistical analysis showed that the level of IL- 17 in supernatant of PBMCs (36.75 \pm 7.48 pg/ml) were increased compared with its level in the supernatant of WB culture (19.42 \pm 3.90 pg/ml, $P = 0.012$). Levels of cortisol in different samples were also varied (serum: 16.51 \pm 1.12 μ g/ml, WB: 27.09 \pm 3.25 μ g/ml and PBMCs: 9.40 \pm 0.56 μ g/ml, $P < 0.001$). There was no statistical difference between the other samples in different times.

RBC variables analysis in different stages of pre-exercise, post-exercise and 2- month silent showed that MCV, MCH and MCHC were increased due to exercise (Table 1).

Table 1: Comparison the effect of exercise and resting on RBC variables

Parameter	Pre-exercise	Post-exercise	2-month-silent	P values
RBC count ($\times 10^{12}/L$)	5.15 \pm 0.22	5.08 \pm 0.19	5.27 \pm 0.14	0.795
Hb concentration (g/L)	15.00 \pm 0.36	15.25 \pm 0.43	15.36 \pm 0.43	0.434
PCV (%)	46.08 \pm 1.08	45.75 \pm 1.02	46.09 \pm 1.07	0.538
MCV(fl)	81.46 \pm 2.24	83.17 \pm 2.52	[†] 81.82 \pm 2.68	<0.001
MCH(pg/cell)	27.50 \pm 0.49	29.09 \pm 0.58	[†] 27.27 \pm 1.09	<0.001
MCHC(g/L)	32.54 \pm 0.27	33.80 \pm 0.13	[†] 33.50 \pm 0.27	<0.001

Results are presented as mean \pm SEM. Red blood cell= RBC; hemoglobin=Hb; packed cell volume=PCV; MCV=mean corpuscular volume; MCH= mean corpuscular Hb; MCHC= mean corpuscular Hb concentration. Test values are expressed as International System of Units.

[†]Significant

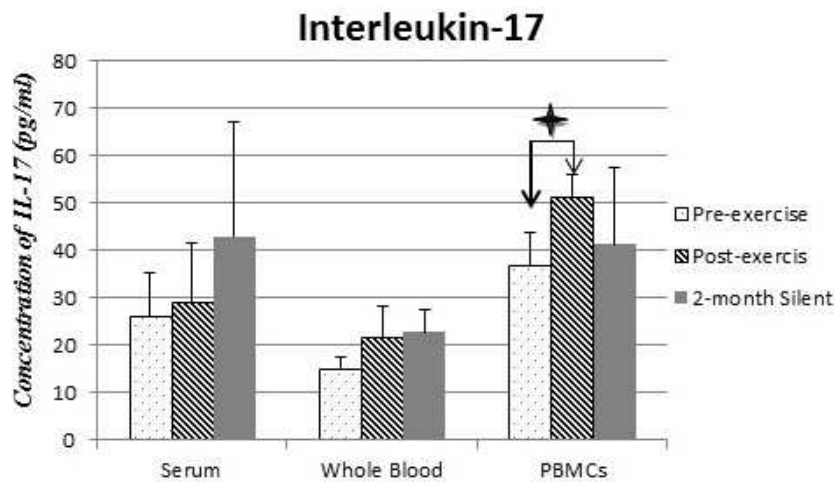


Figure 1: Effect of exercise and resting on IL- 17 production. One ml WB and 2×10^5 PBMCs from each participant in each stage were cultured in one ml RPMI in the presence of mitogens PHA (5 $\mu\text{g/ml}$) and LPS 25 $\mu\text{g/ml}$ for 48 hr. Serum and the supernatant of each sample were assayed for IL- 17. Final results for each sample in different stages showed that *in vitro* production of IL- 17 from PBMCs were increased significantly ($P=0.017$) from 36.74 ± 6.98 pg/ml, in pre- exercise to 51.14 ± 5.43 pg/ml, in post- exercise. There was no statistical difference between the other samples in other stages. * $P < 0.05$

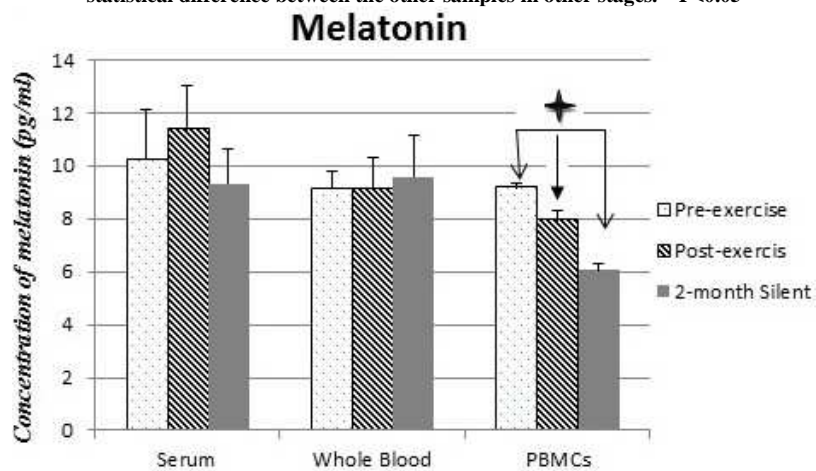


Figure 2: Comparison the effect of exercise and resting on melatonin production. One ml WB and 2×10^5 PBMCs from each participant in each stage were cultured in one ml RPMI in the presence of mitogens PHA (5 $\mu\text{g/ml}$) and LPS 25 $\mu\text{g/ml}$ for 48 hr. Serum and the supernatant of each sample were assayed for melatonin. Final results for each sample in different times showed that *in vitro* production of melatonin from PBMCs were decreased significantly after exercise (7.94 ± 0.35 pg/ml, $P=0.038$) and 2- month silent (6.05 ± 0.27 pg/ml, $P=0.010$) compared with pre- exercise state (9.16 ± 0.19 pg/ml). There was no statistical difference between the other samples in other stages. * $P < 0.05$

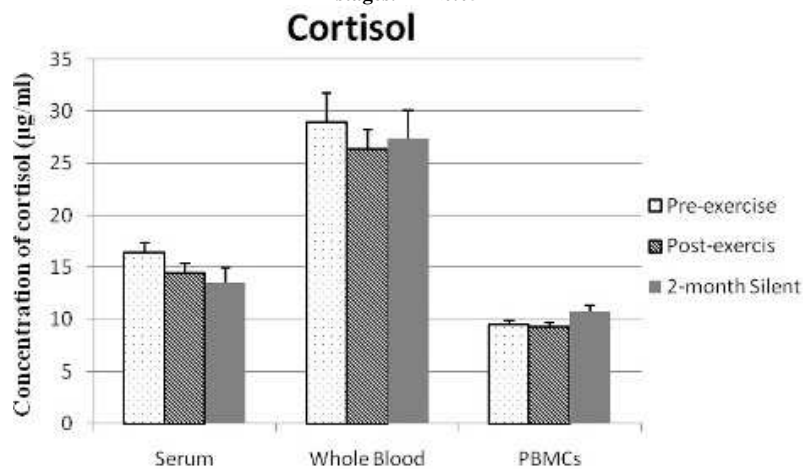


Figure 3: Comparison the effect of exercise and resting on cortisol production. One ml WB and 2×10^5 PBMCs from each participant in each stage were cultured in one ml RPMI in the presence of mitogens PHA (5 $\mu\text{g/ml}$) and LPS 25 $\mu\text{g/ml}$ for 48 hr. Serum and the supernatant of each sample were assayed for cortisol. Final results for each sample in different times showed that there was no statistical difference between the level of cortisol in different samples and in different stages

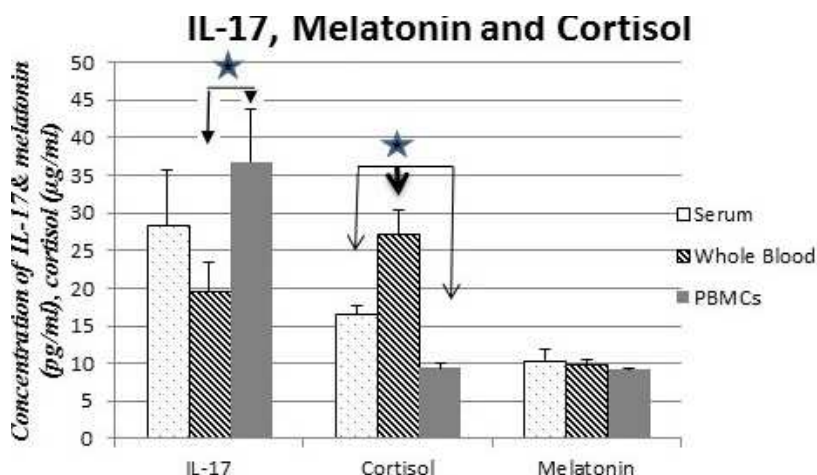


Figure 4: Different levels production of IL- 17, melatonin and cortisol in serum, WB and PBMCs culture supernatants. Statistical analysis showed that the level of IL- 17 in supernatant of PBMCs cultures (36.75 ± 7.48 pg/ml) were increased compared with its level in the supernatant of WB culture (19.42 ± 3.90 pg/ml, $P=0.012$). Levels of cortisol in different samples were also variable (serum: 16.51 ± 1.12 µg/ml, WB: 27.09 ± 3.25 µg/ml and PBMCs: 9.40 ± 0.56 µg/ml, $P<0.001$). Kruskal- Wallis and Mann- Whitney tests were used for this analysis. There was no statistical difference between the other samples. * $P<0.05$

DISCUSSION

The main findings of the present study were significant increase in the production of IL- 17 by PBMCs in the post-exercise stage compared with the pre- exercise status. Furthermore, melatonin secretions in the supernatant of PBMCs cultures were continuously decreased in post-exercise and in two months rest (recovery stage) in comparison with the pre-exercise status.

High production of IL- 17 by PBMCs of the participants in the post- exercise stage may support the recent findings by our pioneers that exercise caused an increase in the blood levels of IL- 17 [3, 5]. It is supposed that IL- 6 increases dramatically following long- lasting endurance exercise. This response may stimulate the production of IL- 17 in the blood. Meanwhile, the expression of IL- 17 in the blood is transient. Because of in our study, the blood collection in the post-exercise stage was performed forty eight hours after the end of the last session training. It seems that the high level of IL- 17 in the blood was not able to be detected. However, PBMCs were still capable to release IL- 17 to the culture when were stimulated with mitogens. Although, the precise role of this increase in IL- 17 release by PBMC during exercise is not yet well established and further works are needed in this area [3, 5, 18]. Following exercise, inflammatory responses will begin and researches show that melatonin can have anti-inflammatory roles and repair the skeletal muscle damages [2]. Accumulating evidences suggest that the blood melatonin levels increase immediately after exercise and rebound back to pre-exercise levels after one hour. In contrast, half-life within cells is believed to be longer and is determined by how rapidly it is used as a free radical scavenger. Therefore, because exercise produces free radicals, melatonin's intracellular half-life was shortened as it was rapidly used as a free radical scavenger [19]. This may be able to explain why the melatonin release from PBMCs in the post- exercise and two- month silent were decreased.

Cortisol is a glucocorticoid hormone and is an indicator for evaluate of stress from physical or psychological stimuli. In the recent years, cortisol measurement has been widely used to assess physical stress response to exercise [12]. The results of cortisol evaluation in different stage and different samples of the present study did not show any significant change at the levels of cortisol. This finding is accordance with other studies like Jeon et al who found that long-term regular aerobic exercises had no effect on cortisol [20]. Furthermore, it can be considered that the *in vitro* production of cortisol by PBMCs is a novel finding in the present study.

Regardless of the effect of exercise or sedentary, the cumulative abilities of the blood, WB and PBMCs samples in the production of IL- 17, melatonin and cortisol were analyzed. The results of this analysis showed that PBMCs produce more IL- 17 in comparison with WB culture. The reason could be due to higher concentration of lymphocyte Th17 population in the PBMCs compared with the blood. [6, 18]. In addition, the production of high level of cortisol in WB culture compared with serum and PBMCs predicts the possibility production of cortisol by RBC or by neutrophils that need to be more investigated [21, 22].

RBC parameters evaluation showed that RBC count, Hb and PCV were not changed during exercise and after two months of recovery. In contrast, MCV, MCH and MCHC were increased due to exercise and returned to pre-

exercise levels after two months silent. The increase in MCV suggests that intraerythrocyte osmolality was increased. This high osmolality probably leading to swelling of the RBC cells induced by a shift of water from the diluting cell counter solution into the red cells prior to the MCV measurement [23].

CONCLUSION

Chronic effects of moderate exercise cause to higher production of IL- 17 and lower production of melatonin by PBMCs cultures. Therefore, this imbalance between IL- 17 and melatonin production would be in favor of inflammation increase.

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