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Association of immune parameters with stress hormone levels in elite sportsmen during the pre-competition training period [1] [2]

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Abstract

This study was performed to determine stress hormone levels and association with immune parameters. Eleven elite basketball players (who mean age of 21.45±1.63 years) and ten elite cyclists (who mean age of 20.00±1.49 years) volunteered for study. Peripheral venous blood samples were drawn at rest during the pre-competition period. Total erythrocyte, leukocyte numbers, Hb and Hematocrit were performed with coulter hematology analyzer. Determination of lymphocyte subpopulations ($CD3^+$, $CD4^+$, $CD19^+$, $CD45^+$) was performed by flowcytometry. Growth hormone and cortisol concentrations were determined by radioimmunoassay kits. Significance of changes in leukocyte subsets and stress hormones were analysed by using Mann Whitney U test. Assosiations between cell counts and hormone levels were analysed by using Spearman's correlation analysis.

In professional basketball players Hb and Hct values were higher than the cyclists. Total leukocyte number and granulocyte rate were high in the basketball players and lymphocyte and monocyte rate were high in the cyclists. Growth hormone and cortisol levels were high in the cyclists. $CD4^+$ Thelper/inducer cell numbers were significantly high in the basketball players although $CD19^+$ B cells numbers were high in the cyclists. There were no significant differences in $CD3^+$ total T cells numbers in two groups.

Keywords: Training; cortisol; growth hormone; immune system; lymphocyte subgroups

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Introduction

Physical exercise causes physiological stress. Response of the organism to exercise, as all stressors, systematically triggers neuroendocrine and immune events. This interaction allows the system to turn back to homeostasis state. Neuroendocrine and immune systems contribute to the adaptation to increased physiological demand during exercise. These systems also adapt to chronic loading (Frugala, 2011).

Acute and chronic exercises alter the number and functions of natural killer cells (NK), neutrophils and monocytes, which are the cells of natural immune system. It is known that lymphocytosis is observed before and just after the exercise depending on duration and intensity of exercise. The number of T cells and less frequently B cells are decreased below pre-exercise levels in the first step of recovery and return back to the normal resting levels within 24 hours. Mobilization of subgroups of T and B cells is usually influenced by catecholamines. Although many studies reported that mitogen- and antigen-stimulated T cell proliferation is decreased following acute exercise, there are signs of T cell subgroup activation stimulated by acute exercise in response to in vivo and mitogen and antigen stimulation. T and B cell functions may become sensitive to increased training load in well-trained athletes. Number of circulating type 1 T cells may decrease with decrease in proliferative response of T cells, and stimulated B cell immunoglobulin synthesis may be reduced. This depression in immune system is associated with the level of circulating stress hormone (Walsh, 2011).

It is known that utilizing exercises activate stress hormones. Adrenocorticotropin (ACTH) concentration is remarkably increased during long-term heavy exercise and leads to increase in cortisol release. Basal cortisol level may increase both after long-term exercise and during heavy exercise period (Wittert, 1996; Viru, 2001).

Leukocytosis is the main response to some physiologic (stress and exercise) and pathologic (endotoxemia and fever) conditions. Dynamic exercise leads to mobilization of immune cells from marginal pools to peripheral blood. This typically comprises early granulocytosis and monocytosis followed by neutrocytosis after exercise. The magnitude of these changes depends on type, duration and intensity of the exercise (Nieman,1999; Nieman,2001).

The underlying mechanism of exercise-related immune changes is multifactorial and includes neuroendocrinological factors, such as Epinephrine, Norepinephrine, Growth Hormone, Cortisol and β-endorphin, and certain physiological factors (Rhind,1999).

Distribution of lymphocyte subgroups is associated with the level of stress hormones. Epinephrine, total leukocyte and lymphocyte count and $CD3^+$, $CD4^+$, $CD8^+$ and $CD3\text{-}CD16^+/56^+$ are important determinants of subgroup distribution (Baj,1994; Dressendorfer, 2002). The association between Norepinephrine and lymphocyte subgroups is less strong. Cortisol is a long-acting hormone and allows lymphocytosis follow neutrocytosis and lymphopenia in post-stress period (Mignini, 2008). Cortisol is negatively associated with leukocytes, $CD14^+$monocytes and $CD19^+$ B- and $CD4^+$T-cell subgroups but positively associated with granulocytes. Hyperthermia has been suggested to pave a way for exercise-stimulated immune cell re-distribution with circulating epinephrine, norepinephrine and cortisol by causing sympathoadrenal activation (Consitt, 2001, Nielsen, 1996, Nieman, 1994, Rhind, 1999).

Method

11 elite basketball players with mean age of 21.45±1.63years, mean height of 195.09±5.72cm, mean body weight of 97.36±8.88kg, mean training age of 11.08±4.75years and 10 elite cyclists with mean age of 20.00±1.49years, mean height of 178.60±4.88cm, mean body weight of 70.20±5.33kg, mean training age of 6.02±3.78 years were voluntarily participated in the study. Venous blood samples of training subjects were collected in the morning at rest.

Hematological analyses: Total erythrocyte and leukocyte counts and hemoglobin, and hematocrit values were assessed by coulter hematology analyzer. Lymphocyte subgroups ($CD3^+$, $CD4^+$, $CD19^+$, $CD45^+$) were identified by flow cytometer method.

Hormone analyses: Growth hormone and cortisol levels were assessed by radioimmunoassay method.

Statistical analysis: SPSS software was used for statistical analysis. All data were presented as mean ± SD. Mann Whitney U test was used for the comparisons. Regression analysis was done by Spearman's correlation analysis.

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Results

In this study, it was found that stress hormone levels were significantly higher in cyclists and this associated with low $CD4^+$ T helper/inducer cell count.

Table 1. Demographic characteristics of subjects

Variables	Basketballers (n=11)	Cyclists (n=10)
Age (years)	21.45±1.63	20.00±1.49
Height (cm)	195.09±5.72	178.60±4.88
Body weight (kg)	97.36±8.88	70.20±5.33
Sports years	11.08±4.75	6.02±3.78

The age of the basketballers and cyclist were 21.45±1.63, 20.00±1.49 years, the heights of the basketballers and cyclist were 195.09±5.72, 178.60±4.88cm, the body weight of the basketballers and cyclist were 97.36±8.88, 70.20±5.33kg, the sports years of the basketballers and cyclist were 11.08±4.75, 6.02±3.78 respectively.

Table 2. The Comparison of Erythrocyte, Hemoglobin and Hematocrit values at Basketballers and Cyclists

Variables	Basketballers (n=11)	Cyclists (n=10)	z	p
Erythrocyte($x10^6$)	4,73±0,31	4,55±0,27	-1.444	0.14
Hemoglobin	13,34±0,92	12,27±0,59	-2.289	0.02
Hematocrit(%)	39,86±2,46	37,32±2,15	-2.078	0.03
MCV	84,52±3,39	82,16±2,99	-1.056	0.29
MCH	28,20±1,53	26,98±1,56	-1.831	0.67
MCHC	33,42±1,75	32,87±1,36	-0.775	0.43

Hemoglobin and hematocrit values were significantly higher in professional basketball players. Although erythrocyte count, MCV, MCH and MCHC values were also high in basketball players, no statistically significance was observed.

Table 3. The Comparison of Leukocyte count and Leukocyte Formula values at Basketballers and Cyclists

Variables	Basketballers (n=11)	Cyclists (n=10)	z	p
Leukocyte($x10^3$)	7,27x±1,52	5,91±1,27	-2,253	0,02
Lymphocyte (%)	33,81±4,09	45,90±6,95	-3,591	0,00
Monocyte(%)	4,19±0,72	5,37±0,93	-2,325	0,02
Granulocyte(%)	62,00±3,85	48,73±6,78	-3,521	0,00

Total leukocyte count and granulocyte percentage were significantly higher in basketball players, whereas lymphocyte and monocyte percentages were significantly higher in cyclists.

Table 4. The Comparison of Cortisol and Growth Hormone values at Basketballers and Cyclists

Variables	Basketballers (n=11)	Cyclists (n=10)	z	p
Cortisol (µg/dl) Reference values Morning 6.0-30.0 Evening 3.0-16.0	9,50±1,81	12,77±1,97	-3,206	0,001
Growth Hormone(ng/ml) Reference values 0-3ng/ml	1,20±0,14	3,02±0,95	-3,883	0,00

Cortisol and Growth hormone levels were significantly higher in cyclists.

Table 5. The Comparison of Lymphocyte Subpopulation values at Basketballers and Cyclists

Variables	Basketballers (n=11)	Cyclists (n=10)	z	p
$CD3^+$(%)	68,01±6,06	66,89±6,66	-0,141	0,88
$CD4^+$(%)	38,49±1,38	32,73±2,17	-3,874	0,00
$CD19^+$(%)	8,40±1,50	16,24±8,32	-2,078	0,03
$CD45^+$(%)	97,58±1,63	95,49±2,43	-1,022	0,30

While number of $CD4^+$ T helper/inducer cells was significantly higher in basketball players and the number of $CD19^+$B cells is significantly higher in cyclists, no significant difference was found in terms of number of total $CD3^+$ T cell count.

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Table 6. The correlation between stress hormone level and $CD3^+$, $CD4^+$, $CD19^+$ cells.

Variables	$CD3^+$	$CD4^+$	$CD19^+$
Cortisol	-0,245 0,28	-0,743 0,00	0,430 0,05
Growth Hormone	-0,153 0,50	-0,688 0,001	0,439 0,04

The negative correlation between cortisol level and $CD4^+$ cell count, growth hormone level and $CD4^+$cell count were found statistically significant. Also a positive correlation was found between growth hormone level and $CD19^+$ cell.

Table 7. The correlation between stress hormone level and leukocyte count, granulocyte, lymphocyte and monocyte percentage.

Variables	Leukocyte	Granulocyte	Lymphocyte	Monocyte
Cortisol	-0,502 0,02	-0,405 0,06	0,461 0,03	0,553 0,009
Growth Hormone	-0,593 0,005	-0,683 0,001	0,642 0,002	0,429 0,05

A positive correlation was found between lymphocyte count and growth hormone level, also a negative correlation were found between granulocyte count and growth hormone level, and between cortisol and leukocyte number.

Discussion

Moderately intensive endurance training causes no change in lymphocyte and NK cell count, total T cell ($CD3^+$) count, T-helper ($CD3^+$ $CD4^+$)/ T-suppressor ($CD3^+$ $CD8^+$) ratio, mitogen-stimulated lymphocyte proliferation, serum immunoglobulin levels and in vitro immunoglobulin production (Gannon, 1997; Green, 2003). Unlike moderately intensive training, long-term and intensive training causes physiological stress and suppression in immune system. This enhances the risk of upper respiratory tract infection (Nieman, 1999; Shephard 1994).

While stress hormones such as cortisol, growth hormone, glucagon and adrenalin are increased in intensive training, insulin concentration is decreased. It has been suggested that mobilization of lymphocyte subgroups is correlated with hormone levels during exercise (Singh, 1996).

Rhind et al. (1999) found negative correlation between cortisol level and $CD14^+$ monocyte, $CD19^+$ B cell and $CD4^+$T cells, but a positive correlation with granulocytes. In the present study, a positive correlation was found between lymphocyte count and growth hormone level (r= 0.642, P=0.002), but a negative correlation between granulocyte count and growth hormone level (r=-0.683, P=0.001). The negative correlation between cortisol level and $CD4^+$ cell (r= -0.743 P=0.000), growth hormone level and $CD4^+$cell (r=-0.688 P=0.001) was found statistically significant. Also a positive correlation was found between growth hormone level and $CD19^+$ cell (r=0.439 P=0.047).

In the recent years, the relation between growth hormone and biological aging has been intensively studied. It has been suggested that growth hormone therapy increases plasma IGF-1 levels in old animals and human, causes and increase in skeletal muscle and lean body mass and decrease in adipose tissue, enhances immune functions, improves learning and memory, and enhances cardiovascular functions (Khan, 2002).

Stokes et al. (2002) demonstrated that growth hormone level is high in cycling people and is increased in line with the load. The present study found that cortisol and growth hormone levels are significantly higher in cyclists versus basketball players.

Viru et al. (2001) reported that insulin is decreased, growth hormone concentration is increased, and cortisol and testosterone responses showed variations after 2-hour jogging.

Van der Pompe et al. (2001) showed that ACTH, cortisol, growth hormone, prolactin release, and $CD3^+$, $CD4^+$, $CD16/56^+$ and $CD8^+$ cell counts are increased after utilizing cycling training in middle-aged postmenopausal women.

Shek et al. (1995) demonstrated an increase in peripheral T cell ($CD3^+$), helper ($CD4^+$), and suppressor ($CD8^+$) T cell count after utilizing endurance training. Since increase in $CD4^+$ count was less than increase in $CD8^+$ cell count, they reported that $CD4^+$ /$CD8^+$ ratio is depressed in the training period. It has been reported that, T cells progressively decrease during training, they are decreased by 60% two hours after training versus before training, but $CD19^+$B cells are not much influenced by training.

Khanfer et al. (2011) reported that cortisol/DHEAS ratio was increased in the subjects aged over 65 years, who had lost one of the relatives, due to emotional stress and that neutrophil superoxide production was suppressed.

Cordova et al. (2010) conducted a study in professional volleyball players during competition and reported that T and B lymphocyte count was remarkably increased after maximum cycling training and remained high during recovery, cortisol level was remarkably increased just after the training and decreased to the basal level during recovery.

In conclusion, the present study found that cortisol and growth hormone concentrations and $CD3^+$, $CD4^+$, and $CD19^+$ (T and B cell) counts are within the normal reference range in cyclists and basketball players of training period. Besides, stress hormone levels were found to be significantly lower, $CD4^+$ T cell count was significantly higher and $CD19^+$B cell count was significantly lower in the basketball players versus cyclists.

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