

## Effect of Intensive Endurance Training Program on Blood Hecpidin Levels: Relation to Iron Deficiency Anaemia in Long Distance Runners.

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### *Abstract*

*Athletes trained for regular intensive endurance exercise often have sports anaemia characterized by low blood iron and haemoglobin levels with decreased aerobic capacity, increases heart rate and elongates the recovery time after exercise. The antimicrobial hepcidin peptide secreted from liver found also to play an important role in iron metabolism leading to iron deficiency. Hecpidin secretion is induced by IL6 elevation after exercise in a case resembles acute phase. This study was proposed to investigate effect of hepcidin on blood iron due to chronic regular intensive program. Eight female athletes from the physical education field games team constituted subjects of this study. Their blood levels of hepcidin, IL6 and iron were measured pre, post and after 6 hours of exercise before and after the program. They were subjected to run on a treadmill at 75% of their VO<sub>2</sub>max for duration of 60 minutes/day for 5days/week for four weeks. Results revealed both acute and chronic low iron levels but only acute hepcidin and IL6. Theses results conclude that regular intensive training exercise leads to decreased blood iron causing sports anaemia. Also, iron is highly recommended to be supplemented during such periods in any sports program.*

### **Introduction**

Previous studies revealed that long-term regular exercises make positive contributions into human organism including physical, physiological, psychological and motor features (Auersperger et al., 2012). The most important effect of regular exercise is on blood hematology (Koç et al., 2010). More recent studies concerned the effect of regular exercise on hematology stated that these differences in results depend on the severity, duration and frequency of exercise as well as physical and physiological conditions of subjects (Büyükyazı and Turgay, 2000). Furthermore, the severity, duration and frequency of exercise should be well-organized to have similar positive

influence on blood (Baltacı et al., 1998; Shephard and Shek, 1994). Athletes trained for regular intensive endurance exercise often have low blood haemoglobin levels. It may even be below the normal range (13 g/dl in men and 12 g/dl in women) and associated with low haematocrit values giving rise to the concept of 'sports anaemia' with decreased aerobic capacity, increases heart rate and elongates the recovery time after exercise (Chatard et al., 1999).

Iron performs many important roles that are directly relevant to an athlete's performance. It plays a key role in a number of cellular processes such as DNA synthesis and electron transport. Furthermore, it is an essential component of haemoglobin, the oxygen carrying

protein in the blood (Schumacher et al., 2002). Therefore iron depletion can affect physical performance.<sup>5 6</sup> It is not surprising that a significant loss of this metal commonly occurs with exercise. Early previous studies explained the mechanisms involved in exercise-induced iron loss are mainly gastrointestinal bleeding, haematuria, sweating and haemolysis<sup>5-7</sup>, with inflammation and hormone activity also being relevant, according to the more recent research in this field<sup>8</sup>. There is no evidence that iron supplementation increases athletic performance, except in individuals in whom iron deficiency is established (Ottomano and Franchini, 2012).

Hepcidin peptide secreted from liver was known to be an acute phase protein with antibacterial and anti fungal properties (Hunter et al., 2002). Recently, it is discovered that hepcidin play an important role in iron metabolism, was suggested by the observation that mice with disruption of the gene encoding the transcription factor failed to produce hepcidin mRNA and developed spontaneous visceral iron overload with consequent severe iron deficiency anaemia (Nicolas et al., 2002 A).

Hepcidin protein is a small cysteine-rich peptide hormone produced in the liver, circulated in blood in two forms designated as 20 and 25 amino acids peptides. Only hepcidin 25 is the active human form (Young et al., 2009). Hepcidin contains very high percentage of cysteines (eight cysteines in both the 20- and 25-residue peptide). This is an unusually high amount of Cys residues may compromise for its antibacterial activities. Hepcidin is believed to affect iron metabolism by decreasing iron absorption in the gut, iron

recycling from macrophages, and control hepatic iron storage (Nemeth and Ganz , 2006). In the enterocyte, hepcidin binds to the basolateral iron export protein, ferroportin, initiating its internalization and degradation, effectively blocking iron flux from the cell and reducing iron absorption (Nemeth et al., 2004).

It appears that the interleukin-6 (IL-6-hepcidin axis is critically important for sports anaemia developed by regular intensive endurance exercise. In humans, the hepcidin increase elicited by IL-6 infusion (which resembles that induced by intensive endurance exercise) is accompanied by a 30% decrease in serum iron and in transferrin saturation (Nemeth et al., 2004). The induction of hepcidin by IL-6 results in limitation of iron supply to the bone marrow and hence is considered a major contributor to the pathogenesis of sports anaemia. Because most of the iron in the transferrin compartment is destined for the bone marrow, hypoferraemia resulting from excess hepcidin diminishes the amount of iron available for hemoglobin synthesis and erythrocyte production (Ganz and Nemeth, 2006).

Although hepcidin expression was shown to be inversely associated with iron absorption and expression of iron transport proteins, to date a significant relation between hepcidin expression and iron absorption in humans has not been established. Also there is no previous studies investigated the chronic intensive exercise effects on developing iron deficiency anaemia. The purpose of this study was to investigate whether intensive endurance exercise program for field game players for one month will result in sports anaemia. Serum interleukin-6, hepcidin levels and iron status in field game players

will be assayed and investigate if they can be used as predictors for ongoing sports anaemia.

## Material and Methods

### Subjects

8 female subjects of the faculty of physical education field games players were recruited for this study. Subjects were not currently taking any prescription medications or anti-inflammatory medications that can affect results. They were signing a written, informed consent form for participation.

### Program Design

Subjects were required to run on a treadmill at 75% of their VO<sub>2</sub>max for duration of 60 minutes/day for 5days/week for four weeks. Their blood samples were acquired at rest (pre-exercise), after exercise (post-exercise) and after 6 hours of recovery.

### Blood analysis

Blood samples were collected into Vacutainer tubes using standard procedures via venipuncture of the antecubital vein. Serum was used for the measurement of serum iron, hepcidin and IL-6. Whole blood collected in a

Vacutainer serum tube was allowed to clot, and then centrifuged for 15 minutes at 3200 rpm. Serum were separated and then stored at -20°C for later analyses. Commercially available ELISA kits were used for the measurement of hepcidin and IL-6 (Bio-source, USA). Serum iron was measured using the commercially available reagents (Serum Iron: Stanbio Laboratory, USA).

### Statistical analysis

A computer statistical program SPSS version 17 was used to analyze obtained data. Descriptive statistics, including means, standard deviations and ranges, were calculated for all measures. T test analysis was used to determine significant findings, with significance accepted at  $p \leq 0.05$ . A one-way ANOVA with repeated measures was performed for all variables in which significant main effects were observed.

### Results

Anthropometric data that may affect the results of the research as (age- weight, height and training age) are presented in table (1) and all values of the skewness lies between ( $\pm 3$ ) which achieved the equivalence of the subjects.

**Table (1)**  
**Anthropometric Data for subjects volunteered for this study**

Variable	Unit	Mean $\pm$ SD	K	S
Age	Year	21 $\pm$ 0.67	0.223	-0.341
Weight	Kg	60.88 $\pm$ 2.53	-0.492	-0.448
Height	Cm	166 $\pm$ 3	-0.611	0.263
T. age	Year	4 $\pm$ 0.76	0.319	0.782

**K:** Kurtosis

**S:** skewness

**T.age:** Training age

Obtained data revealed significant variations in the Hepcidin and IL6 levels before program but not in iron levels before the program in the three collected

blood samples. After the program, all the three parameters revealed high significance ( $p < 0.01$ ) in the three investigated samples (Table 2).

(Table 2)  
Comparison of concentrations (Means  $\pm$  SD) Pre, post and after 6 hours for Hecpidin, IL6 and iron by ANOVA

Before program						
	Pre-exercise	Post-exercise	6 hrs. recovery	F	p	Sig.
Hepcidin (nmol/l)	1.03 $\pm$ 0.23	5.52 $\pm$ 0.63	3.12 $\pm$ 0.53	166.45	0.00	S
IL6 (ng/ml)	1.54 $\pm$ 0.47	5.23 $\pm$ 0.84	1.70 $\pm$ 0.55	85.29	0.00	S
Iron ( $\mu$ g/l)	83.37 $\pm$ 6.99	86.5 $\pm$ 10.62	78.62 $\pm$ 5.63	1.95	0.17	NS
After program						
	Pre-exercise	Post-exercise	6 hrs. recovery	F	p	Sig.
Hepcidin	0.99 $\pm$ 0.25	5.67 $\pm$ 0.97	3.51 $\pm$ 0.51	104.27	0.00	S
IL6	1.68 $\pm$ 0.82	5.01 $\pm$ 0.34	2.03 $\pm$ 0.6	69.84	0.00	S
Iron	74.37 $\pm$ 4.5	76.37 $\pm$ 4.1	70.12 $\pm$ 3.14	5.21	0.01	S

There was decreased blood iron levels after the program compared to that obtained before program in all samples ( $p < 0.05$ ) while no significant variation could be detected in hepcidin and IL6 levels (Table 3).

(Table 3)  
Paired Samples T Test difference significance for Hecpidin, IL6 and iron parameters in pre-program compared to post-program

pre-exercise			
	t	p	significance
Hepcidin	0.33	0.75	NS
IL6	-0.51	0.63	NS
Iron	3.04	0.02	S
Post-exercise			
	t	p	significance
Hepcidin	-0.34	0.75	NS
IL6	0.56	0.59	NS
Iron	2.71	0.03	S
6 hours after recovery			
	t	p	significance
Hepcidin	-1.41	0.20	NS
IL6	-1.18	0.27	NS
Iron	3.61	0.01	S

## Discussion

The present study was proposed to study assays for blood hepcidin, IL6 and iron to

determine the effects of intensive endurance program for four weeks undergone through for the following reasons; it is long time proved that such

programs lead to what is commonly defined sports anaemia. Also, iron deficiency resulted after the heavy periods of intensive sports programs lead to improper training fate with unexpected low adaptations for athletes in general (Koçi et al., 2012).

The significant increase in post-exercise hepcidin that lasts for more 6 hours after the exercise suggests that hepcidin cause long time effect of iron unavailability although IL6 returns to become –more or less- around its basal levels during this periods of times. Also, there is no chronic effect of the program on either hepcidin or IL6 but that is true for iron levels.

Regulation of hepcidin synthesis follows two major mechanisms; either by iron and oxygen availability or by inflammatory response (Ganz and Nemeth, 2006). Exercise induces acute phase inflammation like case, which lead to increased circulating IL6 levels in blood. IL-6 but not IL-1 $\alpha$  or TNF- $\alpha$  induce hepcidin synthesis in human hepatocytes while anti-IL-6 anti-inflammatory markers inhibit the induction of hepcidin mRNA in human primary hepatocytes (Nemeth et al., 2004; Nemeth et al., 2003; Nicolas et al., 2002 B & Pigeon et al., 2001). This may explain why hepcidin is acutely elevated after the exercise session.

Mechanism of action of hepcidin on iron follows three major pathways; reduces iron to its ferrous (Fe<sup>2+</sup>) form (absorbable form of iron) can be carried on the iron transporter, divalent metal transporter 1 (DMT1), which moves iron across the cell membrane. Secondly, hepcidin directly reduce the expression of ferroprotein on cell membranes, and at last, it binds cell membrane ferroprotein, causes it to be internalized and degraded with the loss of

ferroprotein from the cell membrane ablates cellular iron export (Frazer and Anderson, 2003; Frazer et al., 2002; McKie et al., 2000 & Nemeth et al., 2004). Regular repeating exercise sessions as designed by the program cause regular elevation of hepcidin which lasts for about 6 hours and hence, lead to decreased bioavailability of iron with iron deficiency manifestation comprising subsequent sports anaemia.

### **Conclusion**

Regular, intense physical activity is often associated with chronic low iron levels associated with temporally increased hepcidin levels with subsequent sports anaemia. Administration of iron supplements is highly recommended to avoid this iron anaemia. Also, post exercise hepcidin levels can be used as a novel predictor for subsequent anaemia may arise before competition and during high intensity periods of sports training.

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