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*Original article*

## A STUDY ON THE INFLUENCE OF TRAINING AT ALTITUDE (2000m) ON THE BLOOD HEMOGLOBIN AND ERYTHROIETIN VALUES IN ATHLETICS (AEROBIC RESISTANCE)

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### Abstract

*Aim.* Training at altitude as factor of progress in the preparation of the endurance athletes has become a concern for researches, once with the Olympic Games in Mexico from 1968 at an altitude of 2200m. The research aims to achieve a significant increase in blood hemoglobin and erythropoietin (EPO) values in the athletes' body, after introducing in the training plan a preparatory stage of 21 days at altitude.

*Methods.* The research has been conducted on a group of 10 competitive athletes during a training stage of 21 days in the 'Piatra Arsă' National Sports Complex of the Bucegi Mountains, situated at an altitude of 2000 m. In order to carry out this research we have used the experimental method and the laboratory analysis method, the static-mathematical method and the graphical representation method.

*Results.* The data analysis has shown an increase in the hemoglobin values, by an average of 0,64g/dl namely with 4,4%, and of the erythropoietin with 1,37UI/l namely with 26,5% in terms of performing the altitude training stage.

*Conclusions.* Performing one or more altitude training stages, significantly improves the performance of the runners by triggering some physiological, hematologic changes in the athletes' body favorable to performance increase.

*Keywords:* altitude, hemoglobin, erythropoietin, hypoxia

### Introduction

Exposure to high altitudes has long been considered beneficial for athletes during training. The higher the altitude, the lower is the amount of oxygen, which means that the body is working hard to increase the level of oxygen. It is believed that this process can lead to better athletic performances, but until now it was unclear at what altitude is most beneficial. The athletics competitions in the Olympic Games from 1968 held on the stadium of Ciudad de Mexico at an altitude of 2240m have marked a reference point in the study of the effects of training at altitude (over 2000m). The results achieved in this competition have been remarkable, unprecedented in the history of the modern Olympic Games. There have been 14 world records (established and/or equaled) and at the same time Olympic ones, 14 more Olympic records. Among these we mention; 100m/9"9 – Jim Hines, respectively 11"0 – Wzomia Tyus, 200m/19"8 – Tommie Smith, respectively 22"5 - Irena Kirszenstein Szewinska, 400m.p./43,8 – Lee Evans, 800m/1'44"3 Ralph Doubell, 400m.g./48,1 – David Hemery, long jump/8,90m - Robert Beamon, respectively 6,82m / Viorica Viscopoleanu, triple jump/17,39m - Viktor

Saneev, 4x100m/38"2 – S.U.A., respectively 42"8 / S.U.A. 4x400m/2'51"1 etc. As it can be seen, most of these were performed in the sprint, jump and throw tests. World records and/or Olympic records were also recorded in the 800m run tests, – women and men and 1500m men. At that time, the women's 800m run test was the longest in the Olympic program. We can't say that there have been results of the same level in male endurance tests: 5000m, 10000m, marathon, 3000m obstacles, 20km mars and 50km mars. The erythropoietin (EPO), a glycoprotein hormone encoded by a gene located on the long arm of chromosome 7 and secreted primarily in the kidneys, intervenes in the control of erythropoiesis. Normally, the erythropoietin levels vary in inverse proportion with the value of the hematocrit. The hypoxia stimulates the release of EPO, which in turn induces the production of erythrocytes at the level of the bone marrow. The increased values of the number of erythrocytes, hematocrit and hemoglobin suppress the release of EPO.

The erythropoiesis is among the extensively described (and researched) benefits of prolonged exposure to altitude, due to the direct effect on the

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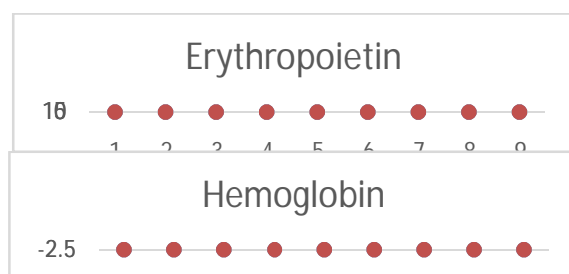
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transport capacity of  $O_2$ ; this may induce an improving of the  $VO_2$  max. and of the aerobic performance. In the case of hypoxia, a biphasic evolution of the blood concentration is observed in the EPO ([EPO]): after only a few hours a rapid increase is found, followed by a gradual decline until the values return to their initial level of the sea level. The first phase occurs in the next 2 – 4 hours after installing at altitude, and the amplitude of this increase depends basically on the level of altitude: e.g. between 150% – 200% of the value at sea level to an altitude between 2000 m – 3000 m (Wilber, 2004). The next phase, of gradual reduction, occurs after approximately a week at moderate altitude, and is due to the increase in mass of the red blood cells (RBC), and therefore, the oxygenation of tissues, including the kidney (Berglund, 1992).

Klausen and others (1991), as well as Fridman and others (1999) noted that during the first 5 days at

altitude, the [EPO] is higher. Ge and others (2002) postulate that this increase of the [EPO] depends on the „hypoxic dose”. In other words, it seems that the threshold altitude that allows an increase in the [EPO] lies to 2000 m for most of the subjects. However, the concept of „hypoxic dose” also takes into account the duration of exposure (Levine, 2002; Levine and Stray-Gundersen, 2006; Wilber and others, 2007). Therefore, as a result of exposure of very short duration, many studies (Ashenden and others, 2000; Berglund, 1992; Vallier and others, 1996) do not notice any increase in the [EPO] after following the protocols of simulated altitude training, nor any increases too low to induce an increase in mass of Hb. Thus, Ashenden and others (1999), in a study on athletes from different disciplines (triathlon, cycling, cross-country skiing) have not found increases in RBC after 23 nights at 3000 m, with 8 – 10 hours/ 24 hours of hypoxic stimulation.



**Figure no. 1** Change of A. [EPO]; percentage of reticulocytes; C. reticulocyte hemoglobin (retHb) that quantifies the circulating contents of Hb in reticulocytes; D. average corpuscular concentration in hemoglobin (CHCMr), of the runners who stay overnight at an altitude of 2650 m. (High, ■) or at sea level (Control 600 m, ○). The horizontal bars correspond to the 3 bits of 5 nights at simulated altitude, separated by 3 nights at sea level; °P < 0.05. (Ashenden and others, 2000).

The control group is installed and trained at 600 m altitude. The same team (Ashenden and others, 2000) has tested then the effects of the 3 sessions of 5 nights at 2650 m altitude (8 – 11 hours/ day in hypoxia) separated by 3 nights in normoxia, on 6 well trained runners, the 5 control-runners being installed and trained at 600 m altitude. They have not revealed either, in this study, increases in reticulocytes despite a strong stimulation of the EPO production (+42%) in the first bit of 5 days. The next 2 bits indicate a lower and insignificant increase in relation to the basic values (between –4 and +26%). Interestingly, in this study, although there has been an increase of [EPO], it was insufficient to stimulate the production of reticulocytes and thus to allow the increase of the  $O_2$  transport capacity (**Figure no. 1**) (Ashenden and others, 2000). One of the most important effects of training at altitude regards the increase in red blood cells mass and in

blood hemoglobin concentration. As previously described, the increase of [EPO] stimulates the erythropoiesis in the bone marrow (Berglund, 1992) and as a consequence there is an increase in [Hb], which – logically – improves the  $O_2$  transport capacity and, therefore, the performance of submaximal and maximal exercise. Klausen and others (1999) have noticed a number of reticulocytes (ret) higher during the first 5 days of altitude training. The fact is confirmed by Mairbaurl and others (1986), who report an increase in red blood cells mass in the athletes trained in hypoxia. Increases of 10% of the total mass in the red blood cells are reported, in time, after a training camp. Levine and Stray-Gundersen (1997). Conversely, after acclimatization, the Hb affinity compared with  $O_2$  decreases in parallel with the increase of mass in Hb (Hbm) Calbet and others, 2003).Hypotheses: Including an altitude training stage



of 21 days that triggers in the runners' body physiological and biochemical effects favourable to support effort capacity. In this context the erythropoietin and the blood hemoglobin values increase.

**Methods**

We note that the research protocol was in accordance with the Helsinki Declaration, the Amsterdam Protocol and the Directive 86/609/CEE and approved by the Ethics Commission of the Physical Education and Sports Department of the Babeş-Bolyai University of Cluj-Napoca, with regard to the research on human subjects. The research procedures were fully explained to the participants in the study and their written agreements have been obtained prior to the commencement of research. Period and place of research: The studies have been carried out in the period of 01.08.2014-22.08.2014 in the 'Piatra Arsă' National Sports Complex of the Bucegi Mountains and the Stadium School Sports Club of Blaj

- 'Avram Iancu' Park no. 2, Blaj. Subjects: 20 competitive athletes specialized in the sports branch/mountain race. The athletes were divided into two groups of 10 and they have done for 21 days the same workout program, they had the same diet and have received the same effort-sustaining medication. The first group of 10 athletes has done the training stage in Blaj, at an altitude of 600m above sea level, and the other group of 10 athletes in 'Piatra Arsă' at an altitude of 2000m above sea level. Tests applied: There were performed laboratory tests through which the initial and final value of the erythropoietin and the hemoglobin has been determined (after performing the training stage).

**Results**

As a result of the laboratory tests recorded before and after the training stage of 21 days, for the two groups: experiment - control, the following results have been recorded:

**HEMOGLOBIN**

**Experiment group**

**Table no.1** Hemoglobin values, of the subjects from the experiment group, before and after the altitude training stage

No. crt.	Name and first name	Hemoglobin	
		Before the training stage	After the training stage
1.	B.N.	15,80g/dl	16,40g/dl
2.	B.G.	14,90g/dl	15,40g/dl
3.	C.D.	13,90g/dl	14,70g/dl
4.	G.N.	12,60g/dl	13,10g/dl
5.	M.V.	13,9g/dl	14,4g/dl
6.	P.A.	15,30g/dl	15,40g/dl
7.	P.R.	14,10g/dl	15,80g/dl
8.	Z.I.	15,20g/dl	15,80g/dl
9.	G.S.	14,80g/dl	15,40g/dl
10.	T.D.	14,60g/dl	15,10g/dl

**Table no.2** Hemoglobin statistical analysis before and after the training stage experiment group

TRAINING STAGE	Average	Average difference	Median	Standard deviation	Minimum	Maximum	Amplitude	Coef. of variation
Before	14.51	0.64	14.70	0.92	12.60	15.80	3.20	6.3%
After	15.15	4.4%	15.40	0.92	13.10	16.40	3.30	6.1%

The average hemoglobin value has increased in average with 0.64 g/dl (4.4%), from 14.51 g/dl before the training stage, at 15.15 g/dl after the stage. The hemoglobin varies between 12.60 and 15.80 g/dl

before the training stage and between 13.10 and 16.40 g/dl at the end of the stage. At both trials the data dispersion around the average is homogeneous.



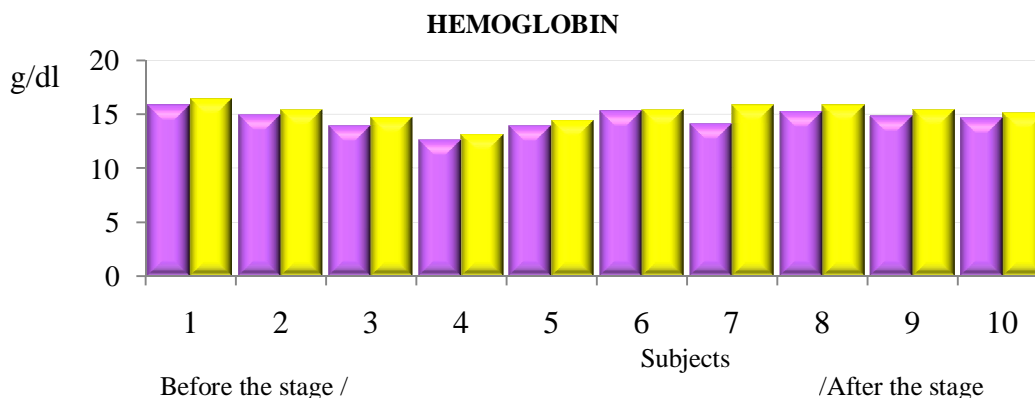
### THE WILCOXON TEST

**Table no.3** The Wilcoxon test analysis hemoglobin experiment group

Ranks difference tests	N	Average ranks	Sum of ranks	Test parameters	Result
Negative	0	0.00	0.00	Z	-2.829
Positive	10	5.50	55.00	P (2-tailed)	0.005
Equal	0	0.00	0.00	Effect size	0.63

The nonparametric Wilcoxon test demonstrates that the difference of the results on the two moments of the training has reached the threshold of statistical significance,  $z = -2.829$ ,  $p = 0.005 < 0.05$ . The effect size (0.63) indicates a very large difference between the two tests. The null hypothesis is rejected and it is accepted the research hypothesis according to

which the average increase of the hemoglobin is significant at the end of the training stage. The graphical representation of the results corresponding to those two checks from the experimental group is shown in **chart no. 1**.



**Chart no. 1** Hemoglobin values before and after the altitude training stage, experiment group

### HEMOGLOBIN- Control group

**Table no.4** The hemoglobin values on subjects of the control group, before and after the training stage

No. crt.	Name and first name	Hemoglobin	
		Before the training stage	After the training stage
1.	B.A.	15,9g/dl	15,9g/dl
2.	D.S.	15,9g/dl	15,8g/dl
3.	D.R.	14,3g/dl	14,5g/dl
4.	R.A.	14,6g/dl	14,7g/dl
5.	S.M.	14,9g/dl	14,9g/dl
6.	S.R.	16,2g/dl	16,2g/dl
7.	V.A.	14,9g/dl	14,8g/dl
8.	B.M.	14,6g/dl	14,8g/dl
9.	S.I.	14,1g/dl	14,2g/dl
10.	L.D.	13,6g/dl	13,8g/dl

**Table no.5** Hemoglobin statistical analysis before and after the training stage control group

Training stage	Average	Average difference	Median	SD	Minimum	Maximum	Amplitude	CV%
<b>Before</b>	14.90	0.06	14.75	0.85	13.60	16.20	2.60	5.7%
<b>After</b>	14.96	0.40%	14.80	0.77	13.80	16.20	2.40	5.2%

We notice an increase of hemoglobin in an average of 0.06 g/dl (0.4%), from 14.90 g/dl before the training stage to 14.96 g/dl after the stage. The hemoglobin varies between 13.60 and 16.20 g/dl before the training

stage and between 13.80 and 16.20 g/dl at the end of the stage. At both trials the data dispersion around the average is homogeneous.

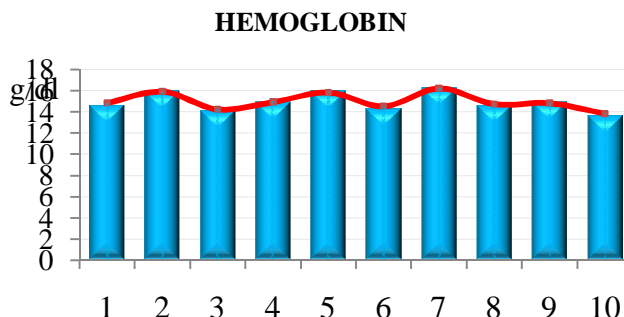
### THE WILCOXON TEST

**Table no 6.** The Wilcoxon test analysis hemoglobin, control group

Ranks difference tests	N	Average ranks	Sum of ranks	Test parameters	Result
Negative	2	2.50	5.00	Z	-1.561
Positive	5	4.60	23.00	P (2-tailed)	0.119
Equal	3	0.00	0.00	Effect size	0.35

The nonparametric Wilcoxon test demonstrates that the difference of the results on the two moments of the training has not reached the threshold of statistical significance,  $z = -1.561$ ,  $p = 0.119 > 0.05$ . The Effect size (0.35) shows a middle difference between the two

tests. It is accepted the null hypothesis according to which the average increase of hemoglobin is not significant. The graphical representation of the results corresponding to those two checks from the control group is shown in **chart no. 2**.



**Graficul nr. 2** Valorile hemoglobinei înainte și după stagiul de pregătire, grupa de control.subjects, / Subjects / After the stage

### EXPERIMENT Vs CONTROL/AFTER THE TRAINING STAGE.HEMOGLOBIN

**Table no.7** Comparative statistical analysis experiment vs control group – hemoglobin

GROUP	Average	Average difference	Median	Standard deviation	Minimum	Maximum	Amplitude	Coefficient of variation
Control	14.96	0.19	14.80	0.77	13.80	16.20	2.40	5.2%
Experiment	15.15	1.27%	15.40	0.92	13.10	16.40	3.30	6.1%

On average, the hemoglobin in the group experiment is higher than in the control group with 0.19 g/dl (1.27%), the averages being equal 15.15 in the experiment group respectively 14.96 g/dl in the control group. The hemoglobin varies between 13.80

and 16.20 g/dl in the control group and between 13.10 and 16.40 g/dl in the experiment one. At both trials the data dispersion around the average is homogeneous.

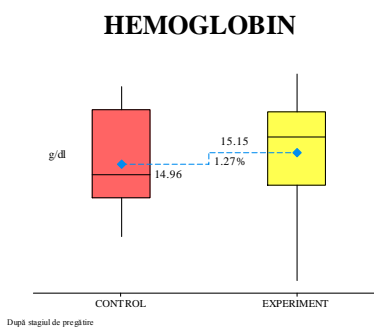
### THE MANN-WHITNEY U TEST

**Table no.8** Comparative analysis - the Mann-Whitney U test- hemoglobin

	GROUP	N	Average ranks	Sum of rank	Test parameters	Result
<b>HEMOGLOBIN</b>	Control	20	9.65	96.50	Mann-Whitney U	41.50
	Experiment	20	11.35	113.50	Z	-0.645
	Total	40			P (2-tailed)	0.519
					Effect size	0.14

The nonparametric Mann-Whitney U test results applied for the two groups at the end of the training stage show that, that there are no statistically significant differences,  $z = -0.645$ ,  $p=0.519 > 0.05$ . The effect size (0.14) indicates from little to

medium difference between the two groups. It is accepted the null hypothesis according to which the average increase of hemoglobin is not significant. The graphical representation of the results corresponding to the two groups is shown in figure no. 2.



**Figure no. 2** The graphical representation of the results corresponding to the two groups - hemoglobin

### ERYTHROPOIETIN

**Table no.9** Erythropoietin statistical analysis before and after the training stage – experiment group

Training stage	Average	Average difference	Median	Standard deviation	Minim	Maxim	Amplitude	Coef. Of variation
Before	5.18	1.37	4.60	2.08	2.50	10.20	7.70	40.2%
After	6.55	26.5%	5.90	2.71	3.40	13.30	9.90	41.4%

We notice an increase of erythropoietin in an average of 1.37 ui/l (26.5%), from 5.18 ui/l before the training stage to 6.55 ui/l after the stage. The erythropoietin varies between 2.50 and 10.20 ui/l before the training

stage and between 3.40 and 13.30 ui/l at the end of the stage. At both trials the data dispersion around the average is inhomogeneous.

### THE WILCOXON TEST

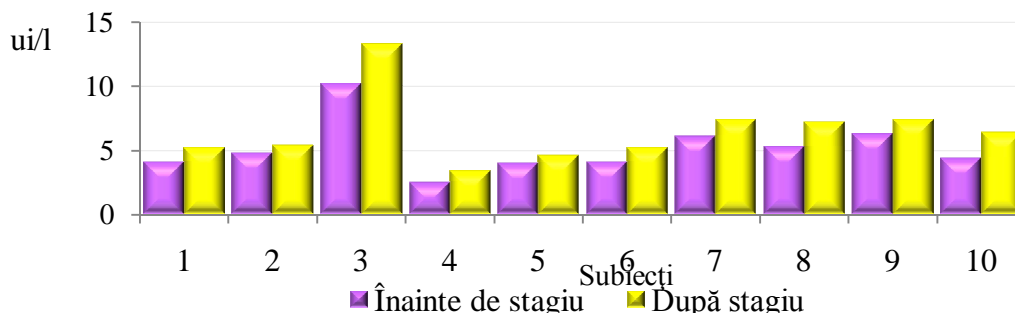
**Table no.10** The Wilcoxon test analysis EPO experiment group

Ranks difference tests	N	Average ranks	Sum of ranks	Test parameters	Result
Negative	0	0.00	0.00	Z	-2.812
Positive	10	5.50	55.00	P (2-tailed)	0.005
Equal	0	0.00	0.00	Effect size	0.63

The difference of the results on the two moments of the training has reached the threshold of statistical significance, conform to the nonparametric Wilcoxon test,  $z = -2.812$ ,  $p = 0.005 < 0.05$ . The effect size (0.63) shows that there is a very large difference between the two tests. The null hypothesis is rejected and it is accepted the research hypothesis according to

which the average increase of the erythropoietin is significant at the end of the training stage. The graphical representation of the results corresponding to those two checks from the experimental group is shown in **chart no. 3**.

**Chart no. 3.** The EPO values before and after the altitude training stage, experiment group



**ERITROPOIETIN (EPO)- control group**

**Table no.11** The EPO values, on subjects from the control group, before and after the training stage

No. crt.	Name and first name	EPO	
		Before the training stage	After the training stage
1.	B.A.	4,6UI/L	4,6UI/L
2.	D.S.	7,2UI/L	7,2UI/L
3.	D.R.	8,1UI/L	8,1UI/L
4.	R.A.	6,5UI/L	6,55UI/L
5.	S.M.	6,9UI/L	5,9UI/L
6.	S.R.	6,1UI/L	6,1UI/L
7.	V.A.	6,0UI/L	6,1UI/L
8.	B.M.	2,5UI/L	2,5UI/L
9.	S.I.	5,3UI/L	5,3UI/L
10.	L.D.	4,5UI/L	4,4UI/L

**Table no.12** The EPO statistical analysis, before and after the training stage control group

TRAINING STAGE	Average	Average difference	Median	Standard deviation	Minim	Maxim	Amplitude	Coef. of variation
Before	5.680	0.005	6.00	1.56	2.50	8.10	5.60	27.5%
After	5.685	0.09%	6.00	1.59	2.50	8.10	5.60	27.9%

We notice an increase of erythropoietin in an average of 0.005 ui/l (0.09%), from 5.680 ui/l before the training stage to 5.685 ui/l after the stage. The erythropoietin varies

between 2.50 and 8.10 ui/l both before and after the training stage. At both trials the data dispersion around the average is relatively homogeneous.

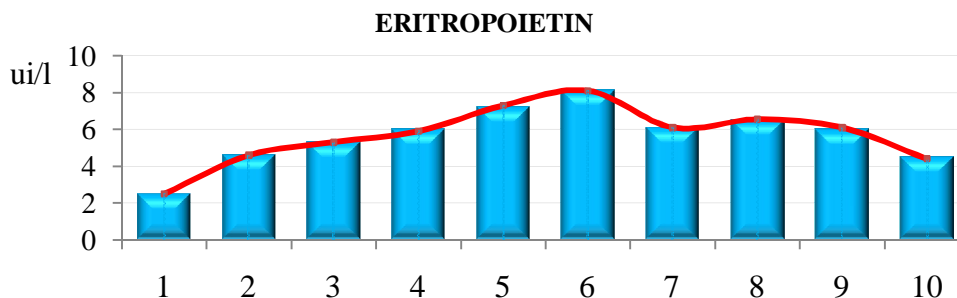
### THE WILCOXON TEST

**Table no.13** The Wilcoxon test analysis EPO control group

Ranks difference tests	N	Average ranks	Sum of ranks	Test parameters	Result
Negative	2	3.50	7.00	Z	-0.141
Positive	3	2.67	8.00	P (2-tailed)	0.888
Equal	5	0.00	0.00	Effect size	0.03

According to the nonparametric Wilcoxon test the difference of the results on the two moments of the training has not reached the threshold of statistical significance,  $z = -0.141$ ,  $p = 0.888 > 0.05$ . The effect size (0.03) shows a very little difference between the

two tests. It is accepted the null hypothesis according to which the average increase of erythropoietin is not significant. The graphical representation of the results corresponding to those two checks from the control group is shown in **chart no.4**.



**Chart no. 4** The EPO values, before and after the training stage, control group

### EXPERIMENT vs CONTROL AFTER THE TRAINING STAGE ERITROPOIETIN

**Table no.14** Comparative statistical analysis experiment vs control group - EPO

GROUP	Average	Average difference	Median	Standard deviation	Minim	Maxim	Amplitude	Coefficient of variation
Control	5.69	0.87	6.00	1.59	2.50	8.10	5.60	27.9%
Experiment	6.55	15.22%	5.90	2.71	3.40	13.30	9.90	41.4%

The average erythropoietin in the experiment group is higher than in the control group with 0.87 UI/L (15.22%), the averages being equal 6.55 UI/L in the experiment group respectively 5.69 UI/L in the control group. The erythropoietin varies between 2.50 and 8.10

UI/L in the control group and between 3.40 and 13.30 UI/L in the experiment one. At both trials the data dispersion around the average is relatively homogeneous in the control group and inhomogeneous in the experiment one.

### THE MANN-WHITNEY U TEST

**Table no.15** Comparative analysis - the Mann-Whitney U test - EPO

	GROUP	N	Average ranks	Sum of ranks	Test parameter	Result
ERITROPOIETIN	Control	20	9.85	98.50	Mann-Whitney U	43.50
	Experiment	20	11.15	111.50	Z	-0.492
	Total	40			P (2-tailed)	0.623



Effect size 0.11

Between the two groups, at the end of the training stage, conform to the nonparametric Mann-Whitney U test, there are no statistically significant differences,  $z = -0.492$ ,  $p = 0.623 > 0.05$ . The effect size (0.11) shows a small difference to medium between the two groups. It is accepted the null

hypothesis according to which the average increase of erythropoietin is not significant. The graphical representation of the results corresponding to those two checks from the two groups is shown in fig. no. 3.

### ERITROPOIETIN

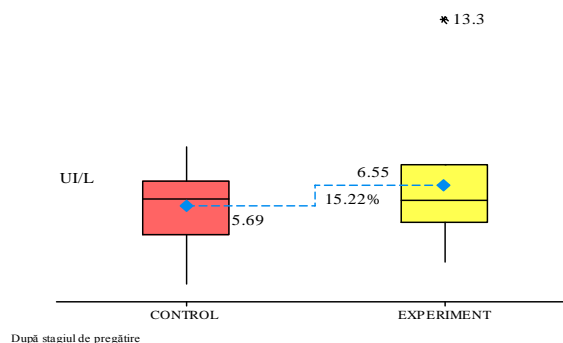


Figure no. 3 The graphical representation of the results corresponding to the two groups – EPO

### Discussions

Wehrlin and Marti have shown, on 2 world-class runners in races of 5000 m and marathon, developments of 3.9 and 7.6% of the hemoglobin mass and of 5.8 and 6.3% in erythrocytes after a LHTL-type training camp of 28 days, 18 hours/day at 2456 m with trainings performed at 1800 m altitude, and others (Wehrlin et al., 2006). This result is interesting because it indicates a positive effect on very high level athletes, on whom the improvement of the physiological capacities is particularly difficult, since they are already highly developed through training. In another study, the same authors (Wehrlin and others, 2006), with well trained triathletes and control group, they have found, also with the LHTL method for the altitude of 2500m, 18 hours/day and workouts at 1800m and 1000m., an increase of the hemoglobin mass of 5.3% and of the red blood cells of 5.0%. However, the results concerning a systematic increase of [Hb] in hypoxia are highly controversial (Bailey and others, 1998; Faulkner and others, 1967; Friedmann and others, 1999; Gore and others, 1998; Hahn and others, 1992; Ingjer & Myhre, 1992; Klausen and others, 1991; Laitinen et al., 1995; Levine and Stray-Gundersen, 1997; Svedenhag and others, 1997),

although – inversely – Ashenden et al., 2000; 1999; 1999) they report minimal increases.

Clark and others (Clark and others, 2004), in a study on 29 athletes (cyclists and triathletes) divided into a control group and two LHTL groups (the first of these, permanent; the second spending 2 days in normoxia 4 nights, at an altitude of 2650 m, 9 – 10 hours/ 24 hours in normoxia), have not manifested any increase in the concentration of Hb despite a significant reduction of ferritin after 20 days of LHTL.

### Conclusions

In relation to the biochemical composition of the blood, the largest changes have been recorded in the group experiment: the EPO has increased after the altitude training stage with 26,5% from an average of 5,18 ui/l to an average of 6,55 ui/l in comparison with the control group in which the increase is insignificant 0,09% , from an average of 5.680 ui/l before the training stage to 5.685 ui/l after the stage.

The hemoglobin increased by 4,4% from an average of 14,51 g/dl before to 15,15 g/dl after the training experiment for the experiment group. We notice an increase of hemoglobin for the control group, in average of 0.06 g/dl (0.4%), from 14.90 g/dl before the training stage to 14.96 g/dl after the stage. The hemoglobin varies between 13.60 and 16.20 g/dl before



the training stage and between 13.80 and 16.20 g/dl at the end of the stage. Finally, there can be concluded a significant increase in performance capacity, due to favorable physiological and biochemical changes, produced as a result of exposure to hypoxia. As a result of theoretical and experimental research, allow us to make the following recommendations:

1. Using the preparatory training stage at altitude – as a means of improving aerobic performance capacity;
2. The individualization of the training necessary for maximizing the athlete's capacity;
3. The performance of altitude trainings to be made under strict medical supervision; the determination of biochemical indicators which determine the biological reactivity of the athletes to be made before, during and after performing the training stage.

#### Acknowledgments

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