

THE EFFECT OF MAXIMAL PHYSICAL EFFORT (THE REFUSAL TEST) ON ERYTHROCYTIC SYSTEM PARAMETERS, HEMOPROTEINS AND ERYTHROPOIETIN CONCENTRATIONS IN BLOOD OF JUNIOR ICE HOCKEY TEAM

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Abstract. We tested the influence of maximal physical effort on selected blood parameters. This exercise was performed by twenty-two junior ice hockey players during the work on a cycle ergometer with the increasing load i.e. the refusal test. In blood taken before and just after exercise red blood cells (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin concentration (HGB), myoglobin concentration (Mb) and erythropoietin concentration (EPO) were determined. It was found, that the maximal physical effort caused statistically significant increase of following parameters: mean red blood cells, hematocrit value and average hemoglobin concentration. Statistically significant increase of the average myoglobin concentration in sportsmen with the initial myoglobin concentration which was at the normal level (subgroup A, n=11) was found. On the other hand, mean corpuscular hemoglobin concentration underwent statistically significant decrease. It was proved, that after the refusal test mean corpuscular volume, mean corpuscular hemoglobin, average myoglobin concentration in athletes with the initial relatively high myoglobin concentrations (subgroup B, n=11) and average erythropoietin concentration did not show any statistically significant changes. Physical effort causes the plasma volume changes, as a result of water migration between extravascular and intravascular spaces. It was calculated that the plasma volume decreased on average about $9.055 \pm 4.293\%$. In the group of examined athletes, the statistically significance decreases of blood plasma volume caused the increase of plasma components. Ascertained changes of myoglobin concentration in subgroup A after maximal work are big enough that they do not result only from decrease of the plasma volume.

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Introduction

The work of skeletal muscles causes a number of functional immediate nature changes in an organism. The nature of these changes is heterogeneous, which means that they proceed faster or more slowly. The factors such as the kind of training stimulus or individual genetic conditioning to physical work decide on the nature of the changes. The range of changes in the plasma volume and blood composition depends on both intensification and the length of effort. The intensification of changes is conditioned by the form. The more trained the person is the smaller intensifications of changes are. Oxygenic efficient delivery in organism determine possibility to do physical work. Oxygenic delivery depends on cardiac output and oxygen contents in blood. While, oxygen contents in blood depends on oxygen saturation degree of blood and oxygenic capacity which are conditioned by transporting oxygenic proteins concentration (mainly hemoglobin) and by their ability to binding oxygen [10,14,16].

During the exercise water migrates between extravascular and intravascular space. The direction of this migration depends on the length of effort. When the exercise is short-lived (<30 min) the water moves from vessels to extravascular space. However, when the exercise is long-lasting (30-60 min) at the beginning the direction is the same like during short-lived exercise then, as the exercise is continued, it submits to change and at a stated point it turns away. It is the result from: blood concentration, increase of the plasma osmotic and oncotic pressure and increase of intratissue pressure [14,16,20,21]. You can calculate the plasma volume change ($\Delta PV\%$) as follows [10]:

$$\Delta PV\% = \left(\frac{100}{100 - HCT_{pre}} \right) \times \frac{100(HCT_{pre} - HCT_{post})}{HCT_{post}} \quad (1)$$

or by means of formula which makes allowance for changes of MCV under the influence of plasma osmolarity increase



$$\Delta PV\% = 100 \frac{Hb_{pre}}{Hb_{post}} \times \frac{1 - HCT_{post} \times 10^{-2}}{1 - HCT_{pre} \times 10^{-2}} - 100 \quad (2)$$

where

$\Delta PV\%$ – the percentage change in the plasma volume,

Hb_{pre} – hemoglobin concentration before an exercise (change PV),

Hb_{post} – hemoglobin concentration after an exercise (change PV),

HCT_{pre} – haematocrit value before an exercise [%],

HCT_{post} – haematocrit value after an exercise [%].

Physical effort is the basic element of sport practice. The form arises through the organism adaptation to physical work. The organism adaptation is got by tot up, for the longer period of time, the aforementioned functional changes.

Ice hockey players' effort during the game has changeable nature. When a sportsman is skating he is doing cyclical work, however, during the fight with an opponent he is doing no cyclical work. During the game the hockey player covers the 5-8 km long distance, he loses 3-4 kg weight and 2930.760-4186.8 J (700-1000 kcal.) energy. A wing and a center forward sportsman do effort on the similar intensity range. Effort of the defenders is smaller in a third on the maximum intensity range [6,14].

Exercise test, whose parameters are the most similar to ice hockey players' effort is the refusal test. That test is carried out with the increasing load on a cycle ergometer [6].

Materials and Methods

The research was performed by twenty-two junior ice hockey players from the national team, aged from 16 to 17-years, weighed from 73 to 88 kg (average 77.41 kg) and from 173 to 191 cm. tall (average 180.9 cm.) at the end of the calendar year. Twelve sportsmen from this group were the forwards, nine of them were the defenders and one was a goalkeeper. The test was accomplished in Department of Individual Sports on Academy of Physical Education in Katowice.

The evaluation of: erythrocytic parameters, haemoproteins and EPO concentration quantitative change was conducted under the influence of maximal physical effort performed during the work on the cycle ergometer. Athletes carried out physical effort with the increasing load i.e. the refusal test. For each sportsman the location of a handlebar and the height of a saddle (according to the height), and also the initial load (according to the body mass: $0.5W \cdot kg^{-1}$ body mass) were



selected individually. During the test the load was increased every 3 min by $0.5\text{W}\cdot\text{kg}^{-1}$ body mass until the complete exhaustion (the average time of performing the test was 26.16 ± 3.26 min). After the refusal sportsmen were doing another 5 min work on the cycle ergometer without the load i.e. the restitution time [3]. During the exercise test the automatic registration of parameters such as: a total work, a time of work, a pulse (taken just after the test) as well as VO_2max and pVO_2max , were being conducted by the computer connected to the cycle ergometer.

Blood from an elbow vein was taken of subjects just before and right away after the test. Of the $5\ \mu\text{m}^3$ (5 ml) venous blood of each sportsmen, $2\ \mu\text{m}^3$ (2 ml) were injected to prepared standard tubes which included dried anticoagulant (dipotassium versenate, K_2EDTA) of a firm Medlab. This blood was used to morphologic determinations. The remaining $3\ \mu\text{m}^3$ (3 ml) were left to coagulation in order to get serum.

Morphologic parameters such as red blood cells (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and hemoglobin concentration (HGB) were calculated by means of eighteen-parameter haematologic analyzer MICROS 60 OT 18M, of a firm AVL – ABX Diagnostics Systems. Coagulated blood in the tubes was removed by centrifugation (10 min, $760 \times g$) and serum was drawn off. In these serums erythropoietin (EPO) and myoglobin (Mb) concentration were determined by radioimmunological methods. Erythropoietin concentration was determined by means of radioimmunoassay EPO-TracTM ^{125}I RIA of a firm DiaSorin. The lower range of the determinable of EPO was $4.4\ \text{mU}\cdot\mu\text{m}^{-3}$ ($4.4\ \text{mU}\cdot\text{ml}^{-1}$). A control sample, which the declared range of concentration was from $51.5\ \text{mU}\cdot\mu\text{m}^{-3}$ to $79.1\ \text{mU}\cdot\mu\text{m}^{-3}$ ($51.5\text{-}79.1\ \text{mU}\cdot\text{ml}^{-1}$), was added by the producer to a kit. The concentration of the control sample measured during the determination, was $73.6\ \text{mU}\cdot\mu\text{m}^{-3}$ ($73.6\ \text{mU}\cdot\text{ml}^{-1}$), which means that the EPO concentrations in the samples are credible [5]. Myoglobin concentration was determined by means of the immunoradiometric kit made by Immunotech International (Prague), and which the determinable lower range was $5\ \text{ng}\cdot\text{ml}^{-1}$ [8]. Two controlled samples were added to the kit. Their the declared range of concentration was: $223\text{-}335\ \text{mU}\cdot\mu\text{m}^{-3}$ and $359\text{-}539\ \text{mU}\cdot\mu\text{m}^{-3}$ ($223\text{-}335\ \text{ng}\cdot\text{ml}^{-1}$ and $359\text{-}539\ \text{ng}\cdot\text{ml}^{-1}$). Concentrations of these samples were measured during the determination and the result was adequately: 296 and $478\ \text{mU}\cdot\mu\text{m}^{-3}$ (296 and $478\ \text{ng}\cdot\text{ml}^{-1}$). This proves that the determinations of myoglobin concentration are credible. In all the tubes activity was counted with the help of an automatic gamma counter –Wallac-Wizard-1470.



The results of the statistical study were subjected by means of computer Microsoft®Excel 2000 and Statgraphics Plus software. To assess if the results have normal distribution, the calculation of skewness and kurtosis was made. To the purpose of this paper was assumed that the values of skewness and kurtosis above 2 means that the range of values do not have normal distribution. In those cases geometric mean and quartile deviation were calculated and nonparametric Kolomogorov-Smirnoff test was applied. In order to define the significance of the influence of physical effort on erythrocytic system parameters change in ice hockey players, function TEST.T was applied and the probability connected with Student's t-test was calculated. The t-test coupled with unilateral distribution was applied. The $p \leq 0.05$ level of significance was taken as the lowest one.

Results

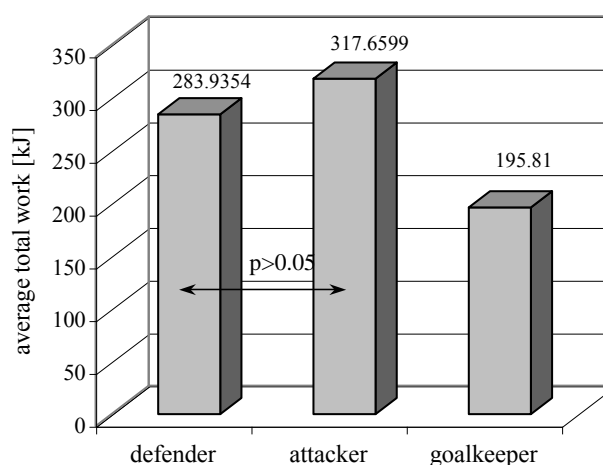


Fig. 1

The average total work value in the groups of: attackers (n=12), defenders (n=9) and goalkeeper (n=1) during the refusal test

Ice hockey sportsmen during the game do physical effort with different intensity which depends on the position they occupy [6], that is why during the refusal test the average total work in the groups of attackers and defenders was calculated (Fig. 1). Although the attackers carry out one-third bigger effort than the others during the hockey games [6], any significant differences between the average total



work value in the refusal test in these groups were found. The obtained dependence allows to treat the group of studied ice hockey players in the further assessments as a homogenous group.

Table 1

Average values of red blood cells (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), erythropoietin (EPO), before and after physical effort

	$\bar{x} \pm s$		p
	before effort	after effort	
RBC [$\times 10^{12}/l$]	5.131 \pm 0.292	5.404 \pm 0.277	<0.05
HCT [%]	44.74 \pm 2.22	47.28 \pm 2.12	<0.05
HGB [g/dl]	15.61 \pm 0.69	16.42 \pm 0.66	<0.05
MCV [fl]	87.4 \pm 2.7	87.5 \pm 2.8	NI
MCHC [g/dl]	34.92 \pm 0.51	34.74 \pm 0.55	<0.05
MCH [pg]	30.38* \pm 0.66**	36.63* \pm 0.75**	>0.086*** NI
EPO [mU/ml]	19.33* \pm 5.39**	30.48* \pm 8.78**	>0.39*** NI

*geometric mean; **quartile deviation; ***in Kolmogorov-Smirnoff test

Mean red blood cells, average hematocrit volume, average hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration as well as average erythropoietin concentration are presented in Table 1.

Due to the big dispersion of the initial myoglobin concentrations, which range from $3.3 \cdot 10^{-5} \text{ kg} \cdot \text{m}^{-3}$ (33 ng·ml⁻¹) to $37.8 \cdot 10^{-5} \text{ kg} \cdot \text{m}^{-3}$ (378 ng·ml⁻¹), the studied group was divided into two subgroups: subgroup A – with the initial myoglobin concentration below $8.5 \cdot 10^{-5} \text{ kg} \cdot \text{m}^{-3}$ (<85 ng·ml⁻¹) and subgroup B – with relatively high myoglobin concentration ranging from $26.9 \cdot 10^{-5} \text{ kg} \cdot \text{m}^{-3}$ (269 ng·ml⁻¹) to $37.8 \cdot 10^{-5} \text{ kg} \cdot \text{m}^{-3}$ (378 ng·ml⁻¹). The obtained results are shown in Table 2.

It has been found, that the maximal physical effort of the ice hockey players causes the statistically significant increase of red blood cells, hematocrit and average hemoglobin concentration.

Because during the exercise water migrates between extravascular and intravascular spaces, it was important to determine changes in plasma volume for athletes after refusal test. It was calculated that the plasma volume decreased on average about $9.055 \pm 4.293\%$. Because the plasma volume change might have

influenced the studied parameters value of blood, it was possible to count how much circulating blood volume decreased in the group of studied athletes. For each ice hockey player circulating blood volume on the basis of body mass was calculated. According to references [4] circulating blood volume in convent to 1 kg body mass amount to 60-75 μm^3 (60-75 ml). Average circulating blood volume before exercise was $5225.11 \pm 361.2 \mu\text{m}^3$ (5225.11 ± 361.2 ml), while after exercise was $4765.34 \pm 539.46 \mu\text{m}^3$ (4765.34 ± 539.46 ml) ($p < 0.05$). In the group of studied athletes statistically significance decrease of the plasma volume concentrated blood thus submit increase of components included in plasma.

Table 2

Average myoglobin concentrations in subgroup A (n=11) and B (n=11) before and after physical effort

myoglobin concentration [ng/ml]	$\bar{x} \pm s$		p
	subgroup A	subgroup B	
before effort	46.95 \pm 13.39	305.18 \pm 35.45	<0.05
after effort	108.12 \pm 98.55	275.05 \pm 98.45	= 0.064
corrected myoglobin concentration after effort	103.59	-	-

Increase of red blood cells and decrease of the plasma volume caused increase of haematocrit value, which was proved in many researches. Average hemoglobin concentration in blood increased and because this increase was proportional to the amount of red blood cells mean corpuscular hemoglobin was not change. However, increase of hemoglobin content in blood was incommensurable to increase of haematocrit value so that mean corpuscular hemoglobin concentration value decreased. Change of mean corpuscular volume was not found, what is not equivalent to change of mean corpuscular volume in particular sportsmen group. Therefore, for each sportsman in whom MCV was changed, the percentage plasma volume change according to equation (2) was calculated. Estimating the results in Table 2 in sportsmen belong to subgroup A increase of average myoglobin concentration and in sportsmen of subgroup B – decrease was noticed.



After exercise made by ice hockey players increase of average erythropoietin concentration and decrease of myoglobin concentration in subgroup B in serum were not statistically significant. Because it was proved that after maximal physical effort blood volume in the group of studied sportsmen submitted decreased at about $9.055 \pm 4.293\%$, it was decided to correct the received myoglobin values of subgroup A (Table 2). Ascertained myoglobin concentration changes in this subgroup after done maximal work are big enough that they do not follow only from decrease of the plasma volume.

The average total work and the time of work in subgroups A and B was compared. Sportsmen from subgroup A statistically significant were doing the refusal test longer and they did bigger the average total work in comparison to sportsmen from subgroup B. These differences at Fig. 2 and Fig. 3 were illustrated.

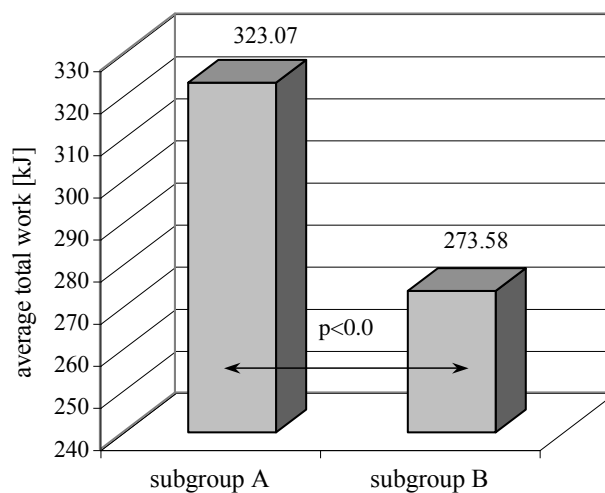
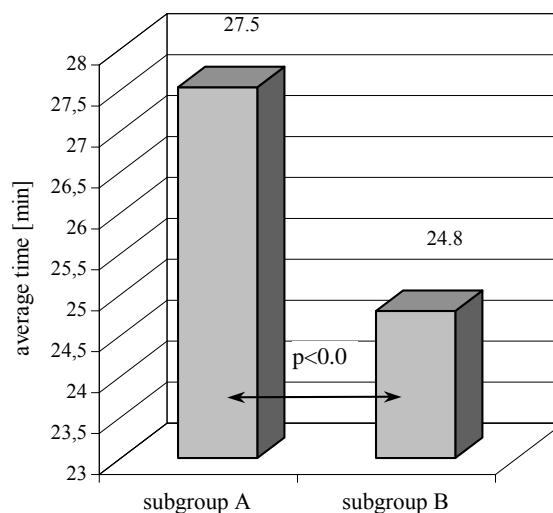


Fig. 2

The average total work performed by sportsmen from subgroup A (n=11) and B (n=11) during the refusal test

**Fig. 3**

The average time of work performed by sportsmen from subgroup A (n=11) and B (n=11) during the refusal test

Discussion

Researches over the influence of physical effort on red blood cells count, haematocrit and hemoglobin concentration can be found in many references [3,10,16-21]. Earlier investigations show, that the decrease in the plasma volume at about $9.055 \pm 4.293\%$ caused by the 26.16 ± 3.26 min lasting effort, gives an increase of RBC, HCT and HGB values of about 5%. Kozłowski and Nazar [10] explain the observed increase of RBC in blood unit volume and increase of HCT during short-lived high intensity efforts, exclusively by decrease of the plasma volume. Szyguła [16] explains also increase of RBC, HGB and HCT values at about 3-10% just after lasting 30-60 min effort by blood concentration as the result of the plasma volume decrease at about 5-16%. According to Watts [23], small (1-2%) initial increase of above-mentioned parameters after short-lived effort, might be due to the circulation blood of with the big hematocrit count, which “lingered” in capillary vessels on inactive earlier muscles. It was found by Laub *et al.* [12] on



the basis of isotopic researches proved that red blood cells from spleen, which contract during intensive physical effort, might be responsible for increase of hematocrit in quarter at least.

References to researches about the influence of physical effort on erythrocytic indexes were not found. Because these parameters depend on aforementioned RBC, HGB and HCT changes, similarly one can explain the influence of physical effort on erythrocytic indexes change. Decrease of MCHC value was most probably caused by decrease of blood circulation volume.

Since during the games attackers' effort is bigger than defenders', one should suppose that the attackers should be better trained than the defenders. So, erythrocytic system parameters changes of the defenders ought to be the biggest, but dependence between work done value and RBC, HGB and HCT changes were not found. Only mean corpuscular hemoglobin change was the smallest in the groups of attackers. The lack of correlation between erythrocytic system parameters and position occupied, could be explained by missing of oriented training for sportsmen on individual positions.

Ice hockey players with correct initial myoglobin concentrations (subgroup A) represent higher ability to realize effort and higher effort tolerance in comparison with the group of ice hockey players with the high initial myoglobin concentrations (subgroup B). The high initial myoglobin concentrations for subgroup B may indicate that sportsmen were overtrained. Hemoglobin concentration changes under the influence of physical effort bring practical information, which allow to modify training program and might prevent sportsmen overtraining.

References on the influence of physical effort on myoglobin concentration comprise another athlete's groups than in our paper. However, authors of those papers obtained the similar results to the our results for the subgroup A. Bogacz *et al.* [3] similar results regarding myoglobin junior footballers after maximal physical effort (the refusal test) were found. Statistically significant increase of average Mb concentration just after and 1.5 h after the test was observed by them. For these sportsmen, statistically significant ($p < 0.05$) linear dependence ($r = 0.5$) between Mb concentration (1.5 h after exercise) and work done value (kJ) was also proved. Kyrolainen *et al.* [11] observed an increase of Mb concentration in athletes directly after effort (400 jumps). Goodman *et al.* [7] measured myoglobin concentration in twenty male runners after 21 km run, which was done as fast as time possible. They use this parameter as indicators of muscle damage. They obtained a material just prior to, immediately after, and 24 h after the exercise. They corrected the results for the percentage plasma volume change. Mb concentration revealed statistically significant increase directly after run. Another



researching group analysed the influence of an 8-day training camp on myoglobin concentration in ten well trained long distance runners. Athletes each day run average 30 ± 3 km. Myoglobin concentration in analysed runners showed statistically significant increase after the camp [13]. Vuorimaa *et al.* [22] selected two groups in random ten well-trained middle-distance runner. Each of groups performed 28-min effort on moving track. The first one performed 14 bouts of 60-s runs with 60 s of rest between each run. The second one performed 7 bouts of 120-s runs with 120 s of rest between each run. In these sportsmen before the exercise, 2 h after and 1, 2 and 3 days after the exercise myoglobin concentrations were measured. In both groups myoglobin concentration increased statistically significant 2 h after the exercise. Immediately after the exercise they did not find statistically significant changes of myoglobin concentration, the same was found in our paper for subgroup B. Probably if myoglobin concentration after the maximal exercise was still monitored, it would be found increase of this protein concentration in blood.

Statistically significant change of erythropoietin concentration was not found, although, decrease of the plasma volume may cause increase of EPO concentration and in this case it should be explain as RBC, HGB and HCT change. Similar results on the influence of physical effort on erythropoietin concentration in serum blood were found in many papers [1,2,9,15]. In those papers erythropoietin concentration under the influence of physical effort did not show any significant changes, as it was found in our earlier study. Kozłowski and Nazar [10] suppose, that the reason of anaemia in athletes is (beside loss iron with sweat) erythropoiesis disorder caused by hormonal factors. In their opinion increase of 2,3 diphosphoglycerate in red blood cells causes shift of hemoglobin dissociation curve to the right and increase oxygen leave in kidneys, what leads to the inhibition of the erythropoietin synthesis. They mention also about the influence of testosterone concentration decrease on decrease of erythropoiesis in athletes. Szyguła [16] enumerates many factors which may cause erythropoiesis disorders in athletes. He shows also, that after application of more precision methods for erythropoietin diagnosis (radiocompetitive methods), higher concentrations of EPO immediately after intensive efforts have been not found.

In conclusion, under the influence of maximal physical effort, in the group of studied ice hockey players, statistically significant increase of mean red blood cells, hematocrit value, average hemoglobin concentration and average myoglobin concentration in sportsmen with the initial myoglobin concentration at the normal level, were found. On the other hand, mean corpuscular hemoglobin concentration underwent statistically significant decrease.



It was found that after the refusal test, mean corpuscular volume, mean corpuscular hemoglobin, average myoglobin concentration in athletes with the initial relatively high myoglobin concentrations and average erythropoietin concentration did not show any statistically significant changes.

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