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## **Special Features of the State of Functional Activity of Neutrophil Granulocytes in Blood During the High Level of Physical Exercises**

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*In recent years researchers in different countries pay much attention to changes in immune system, which appear as a result of intensified physical exercises. The main reason for such attention lays in the necessity of finding the mechanisms of adaptation to physical loading and a task of corrections of widely spread clinical expressions of immunodeficiency of highly qualified sportsmen, in particular, a high level of susceptibility to infectious diseases.*

*Our work presents results of our comparative research of the functional activity of neutrophil, expressed through a calculated index of activation, for representatives of different sports and the control group, consisting mostly of healthy people, who do not expose to high physical loading. We detected a reliable decrease of the activation index of neutrophils of sportsmen in more than 2 times in comparison to the control group. These results provide grounds for asserting that a high level of physical loading, which is typical of highly qualified sportsmen, has a substantial suppression impact on the immune system, in particular, on the functional activity of neutrophils.*

*Keywords: sport, immune system, neutrophil, spontaneous chemiluminescence, induced chemiluminescence.*

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### **Introduction**

The examination of reaction of human body to a high level of physical loading, typical of highly qualified sportsmen, has a considerable interest from both – the fundamental and practical point of view. Clear understanding of cellular and molecular mechanisms of adaptation for an intensive motional activity is a key to the development of optimal systems of sport selection, individualization of training and correction of a functional state. The results presented in the

paper form a part of comprehensive research undertaken by Siberian Federal University, Research Institute of Medical Problems of the North (Siberian Branch of the Russian Academy of Medical Sciences), Krasnoyarsk Subdivision of the Hematological Center (Siberian Branch of the Russian Academy of Medical Sciences).

One of important indices reflecting significant changes in human body is a state of immune system. Foreign literature contains a considerable amount of research works on the

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impact of physical loading on a state of the immune system, most of which allow us to believe that it results in immune suppression (Murakami, 2009; Levada-Pires, 2008; Moreira, 2006). Thus, in one of works (Kakanis, 2010) we see a decrease of ingestion rate of neutrophiles combined with the general increase of their number in 2 hours after the end of the physical loading and lasting for at least 24 hours. Another work (Horn, 2010) gives results of a 10-year study, proving a considerable decrease of leukocytes in blood and neutrophiles in particular for sportsmen in types of sports requiring stamina. The maximum decrease was detected for cyclists and triathletes. The study (Levada-Pires, 2010) pays much attention to changes in a level of production of active forms of oxygen by neutrophiles of sportsmen, participants of poly-triathlon competitions Ecomotion Pró. There was discovered that the production of active forms of oxygen by neutrophiles was decreased in 2.2 times after the end of the competition in comparison to a state of relaxation. It confirmed the increase in the intensity of apoptosis of lymphocytes after the competitions. Another study (Yaegaki, 2008) displays a substantial decrease of the production of active forms of oxygen by neutrophiles in blood of judo sportsmen after the end of a training period in comparison to the initial state. There are few works in Russian devoted to the reaction of immune system to a high level of physical loading (Kolupaev, 2010). In general this subject remains to be topical and needs additional examination.

Neutrophiles form the first line of human body protection from outer pathogens, such as bacteria, and amount to 40-60 % of the whole population of leukocytes. The comprehension of a neutrophile's role has substantially changed for the last several years. Activated neutrophiles are able to perform the majority (almost all) functions of macrophages, to produce a number of cytokines, take part in the activation of T- lymphocytes.

The disturbance of activation of neutrophiles is a pathogen chain for a number of diseases. In some of the stated cases neutrophiles produce compounds, which can cause tissue damage, such as protease, or molecules, inducing inflammatory processes – chemoattractants (eicosanoids and chemokines) or cytokines (Helen, 2009).

In an adult blood flow neutrophiles circulate in a non-activated state, which prevents them from the release of toxic intercellular contents, which are able to cause damage to their own tissues. In vivo neutrophiles become activated in two stages. The primary activation takes place under the influence of components of bacteria and cytokines or chemokines, such as TNF- $\alpha$ , GM-CSF, IL-8 and IFN- $\gamma$ . The secondary activation happens during the mobilization of neutrophiles to a nidus of infection, where they receive activation signals to destroy bacteria (Hallett, 1995). The principal “weapon” of neutrophiles are proteases and active forms of oxygen (AFO). The functional state of phagocytes can be characterized by the level of “respiratory explosion”, which is followed by a drastic increase of the level of oxygen consumption through its transformation into active forms. The ability of neutrophil granulocytes to develop a necessary amount of active forms of oxygen (AFO) can be regarded as a predictive sign for the assessment of the further inflammatory process, and the level of response to the inductor of “respiratory explosion” can characterize the efficacy of protection abilities of the body.

There are different ways of assessment of formation of AFO. One of the most sensory methods is chemiluminescence, based on the activators of chemiluminescence, which widely use luminol. Thus, having reacted to luminol, a new appeared hydroxyl radical emits bright light, and the detection of its intensity forms the method of chemiluminescence. Neutrophiles stimulation in vitro can be done by the adding of

a suspension of bacteria, latex balls, zymosan, quartz crystals and other compounds. A cell stimulated by chemiluminescence with luminal is an informative index of the functional state of blood phagocytes and tissues, their ability to produce AFO, i.e. to perform its protective function. This ability reveals itself after the appearance in human body some inflammatory nidi (during acute disease. When there is a lack of oxygen for a considerable amount of time combined with the general weakening of our body (during chronic illnesses), phagocytes activity and chemiluminescent responses experience a decrease (Vladimirov, 2001; Vladimirov, 1989).

The main aim of our research is to examine changes of the functional activity of neutrophiles under the influence of high physical loading for highly qualified sportsmen.

### **Materials and methods**

In order to accomplish the current task we used samples of venous blood, taken from the representatives of different sports, during different phases of one-year macro-cycle. The venous blood (2ml) from the median cubital vein was gathered to spinner flasks, and was mixed with 80 units of heparin and 1 ml of polyglycine. In order to settle erythrocytes we incubated this mixture for 25 minutes at 37° C and for 30 at room temperature. After the final incubation we put leukocytal supernatant into clean spinner flasks and three times washed it in Hanks solution without phenol red for 5 minutes at 400 g. When the third centrifugation was over the supernatant was removed and cells that left were mixed with 2 ml of Hanks solution. The calculation of previously mixed by 10 times cells (10 % acetic acid solution and 0,25 % solution of trypan blue) was done in a Goryaev chamber.

The evaluation of purity of attained leukocytes (to do so the cells were covered with a Romanovsky-Gimza dye) detected lymphocytes

and erythrocytes. However, since the presence of non-adhesive cells have not had any substantial impact on the character of development of chemiluminescent response, the lysis of contamination granulocytes cells is not done. After the calculation the cells were mixed with Hanks solution till the concentration reached 2 mln/mole.

The reactive solution for a chemiluminescent reaction consisted of 40 micro liters of donor serum AB (IV), 100 micro liters of luminal in the concentration of  $10^{-5}$  M, 50 micro liters of an inducing component (for the detection via induced chemiluminesce), 610 micro liters of Hanks solution without staining agent and 250 micro liters of leukocytal suspension (2 mln/mole) for the detection of spontaneous chemiluminesce or 685 micro liters of Hanks solution and 125 micro liters of leukocytal suspension – for the induced chemiluminesce.

The evaluation of spontaneous and induced chemiluminesce was given for 90 minutes at a 36-channeled chemiluminescent analyzing device «CL3604» (Special Designing And Technological Bureau “Nauka”, Krasnoyarsk). The registration of results and control over the analyzing device was done through a computer. The following characteristics were defined: time of reaching the maximum (T Max), the maximum value (I Max) and the area of the curve (S) .

Oposonized zymosan («Sigma», USA) was used as an inductor of the respiratory “explosion”. Suspension of the oposonized zymosan was prepared in the following way: a zymosan sample was thoroughly mixed with donor serum AB (IV) Rh(-) in the concentration of 2 milligrams of zymosan for 1 milliliter of serum. Incubation for 30 minutes at 37° C. After the incubation the mixture was centrifuged for 10 minutes at 800 g, supernatant was removed and the settled zymosan slurred into 10 milliliters of physiological solution and three times for 10 minutes each washed it

Table 1. Chemiluminescent activity of neutrophil granulocytes for sportsmen in different periods of a training cycle (Me, C<sub>25</sub>-C<sub>75</sub>)

Indices	Control n=62 1		Recovery n=20 2		Fatigue n=9 3	
Spontaneous chemiluminescence						
T max, (sec.)	1351,5	798,0-1983,0	1026,5	605,0-1261,0	631,0	324,0-1082
			p <sub>i</sub> =0,024		p <sub>i</sub> =0,013	
I max, (o.e.)	8580,0	4005,0-14250,0	6886,0	3369,5-18936,0	5663,0	2777,0-6027
S, (o.e.× sec. × 10 <sup>5</sup> )	2,69	1,22-5,76	3,36	1,92-10,03	2,71	1,78-6,47
Zymosan-induced chemiluminescence						
T max, (sec.)	1221,0	984,0-1526,0	980,0	682,5-1231,0	1365,0	445,0-1954
			p <sub>i</sub> =0,019			
I max, (o.e.)	16806,1	5943,0-26458,0	15188,5	6589,0-45184,0	8787,0	3482,0-10274
S, (o.e.× sec. × 10 <sup>5</sup> )	5,06	1,70-8,64	8,85	3,16-15,35	4,62	2,89-12,60
S ind./ S spon.	1,67	1,33-2,39	0,61	0,40-0,79	0,57	0,45-0,66
			p <sub>i</sub> <0,001		p <sub>i</sub> =0,002	

at 800 g. The received opsonized zymosan was dissolved in Hanks solution without phenol red to the concentration of 2 milligram / milliliters.

The enhancement of the chemiluminescence induced by zymosan in comparison with the spontaneous one was evaluated by the ration S zymosan / spontaneous, which was defined as activation index.

The description of the selection was done with the help of the median calculation (Me) and cross-quarterly range of 25 and 75 percentile (C<sub>25</sub> and C<sub>75</sub>). The reliability of differences between the indices of independent selections was assessed by a non-parametrical criterion of Mann-Whitney. The statistical analysis was done in batches by the application Statistica 7.0 (StatSoft Inc., 2004).

### Results and its discussion

The comparative study of the functional activity of neutrophiles has been undertaken. The neutrophiles' activity is expressed by a calculated index of activation for the representatives of

different sports and the control group, which consists of relatively healthy people, who are not exposed to high physical loading. The number of people in the group of sportsmen is 29, 9 of whom feel fatigue after the end of the competition period, 20 of whom feel recovered after a period of rest and 62 people belong to the control group. The both groups have similar characteristics in sex and age. Types of sports: biathlon, ski racing, track and field athletics, orienteering. The given types of sports are characterized by a cycled aerobic physical load. The results of the comparative analysis of the parameters of the functional activity of neutrophiles in correlation with the phase of one-year sport macro-cycle are presented in the Table 1. "Fatigue" means a state after the end of a competition period, "Recovery" – a state after a period of rest.

The given data describes the state of hyperactivation of neutrophiles – the time of attaining the maximum of intensity (T Max) for spontaneous chemiluminescence, especially

when feeling fatigue, is substantially low than in the control group. Meanwhile the level of maximum activity (I Max), displayed by neutrophiles is lower than in the control group. A special interest is found in the reaction of neutrophiles to the activation by zymosane in the "fatigue" group: the table shows us that there is a considerable increase (T Max) in comparison with the spontaneous activity. This peculiarities are revealed in the credible differences of the activation index: 1,67 in the control group; 0,61 and 0,57 in the groups «recovery» and «fatigue» correspondingly.

Thus, bearing in mind the analyzed date we can conclude that activation reserves are exhausted, and neutrophiles respond to

stimulation by a decrease in their activity. A period of recovery gives a slight change in functional activity of neutrophiles, but it is not enough for full restoration of their normal reaction.

### Conclusion

We have detected a decrease of neutrophiles activity in the sportsmen group in more than 2 times in comparison to the control group. These results correspond to the data received by other researchers, and give substantial grounds for asserting that a high level of physical loading, typical of highly qualified sportsmen, has a considerable suppression impact on the state of immune system, in particular – on the functional activity of neutrophiles.

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## **Особенности состояния функциональной активности нейтрофильных гранулоцитов крови при высоком уровне физических нагрузок**

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*В последние годы внимание исследователей в различных странах привлекают изменения в иммунной системе, возникающие под влиянием высоких физических нагрузок. Причиной такого интереса является как потребность выявления механизмов адаптации к физической нагрузке, так и задача коррекции достаточно распространенных клинических проявлений иммунодефицита у высококвалифицированных спортсменов, в частности, высокой восприимчивости к инфекционным заболеваниям.*

*В нашей работе проведено сравнительное исследование функциональной активности нейтрофилов, выраженной через расчетный индекс активации, у представителей различных видов спорта и контрольной группы, состоящей из практически здоровых лиц, не испытывающих значительных физических нагрузок. Установлено достоверное снижение индекса активации нейтрофилов в группе спортсменов более чем в 2 раза по отношению к контрольной группе. Эти результаты позволяют обоснованно говорить о том, что высокий уровень физических нагрузок, характерный для спорта высших достижений, оказывает существенное супрессивное влияние на состояние иммунной системы, в частности на функциональную активность нейтрофилов.*

*Ключевые слова: спорт, иммунная система, нейтрофилы, спонтанная хемилюминесценция, индуцированная хемилюминесценция.*

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