

## Use of integral hematological indices for diagnostics of athletes' adaptive processes

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### Abstract:

The article investigates the changes in blood indices of athletes-runners of various qualifications at a state of rest, in conditions of physical maximum load and during the restoration period. On the basis of leukograms, integrated hematological indices were calculated for substantiation of the adaptation processes to physical load. A total of 35 male runners at short distances were examined. Two groups were formed: the first group included athletes of low qualification (II-III grade) (n = 20) and the second group included athletes of high qualification (I grade - Masters of Sports) (n = 15). On the basis of lesser changes in the leukogram indices, as well as lower changes in the values of integral hematological indices observed in high-skilled runners, it was concluded that athletes of high qualification have more perfect regulation mechanisms of adaptive-compensatory states of organism under conditions of physical load "to refusal". The obtained data confirm that integral hematological indices can be functional state markers for assessing athletes' adaptive reactions to vigorous physical activity.

**Key words:** leukogram, hematological indices, marker, adaptation, runners, extreme physical load

### Introduction

Adaptation of the human body to the action of extreme environmental factors, including vigorous physical activity (PA), is a fundamental problem of physiology and medicine [3, 4]. The problem of adaptation to exhausting, including submaximal physical load, has been sufficiently covered in scientific publications [4-9]. It is known that the complex of reactions that occur in the body under the influence of strong stimuli was first described by G. Selye as a "general adaptive syndrome", which includes, as a compulsory component, changes in the blood system. Blood, directly involved in any physiological or pathological process, reflects all changes in the body's functional state [3, 7, 9].

Characteristics of the morphological and functional state of the peripheral part of the blood system, in particular leukocytes, the ratio of which in most depend on the influence of neuro-humoral factors responsible for the adaptation of the organism, can serve as a universal index of the body's homeostasis disorders. At the same time, the response to the physical maximum load of the organism's resistance system, represented by a heterogeneous population of leukocytes, without which it is difficult to assess objectively the degree of the organism's adaptation to PA [5, 7] is not sufficiently studied today.

The relevance of our study is to find blood markers that can reflect the athletes' adaptive processes influenced by physical activity, and can become the criteria for overtraining.

Therefore, the purpose of this study was to determine the markers of leukograms for the diagnosis of athletes' adaptive processes in conditions of vigorous physical activity.

### Material and methods

Two randomized groups of male athletes-runners at short distances aged 18-20 were examined: I group – low-skilled runners (LS) - II-III grade (n = 20) and II group - high-skilled runners (HS) - I grade – Master of Sports (MS) (n = 15). The study was conducted at three states: 1) in rest, 2) after physical activity - bicycle ergometric test of increasing intensity to individual maximum ("to refusal") and 3) after 30 minute restoration. Every examined athlete signed a voluntary consent form for participation in the experiment. The indices of leukograms were investigated with the help of a non-invasive diagnostic method for determining blood formulae and biochemical regulatory indices of metabolism and circulation according to Malihin-Pulawski (device and method of measurement that have several patents and is on sale in Ukraine and many other countries). In rest conditions, leukogram indices and integral hematological values were also studied in the control group. The control group consisted of fit volunteers of the corresponding age and gender who were not involved in sports (n = 12).

The leukogram indices were analyzed in detail and in addition to the relative and absolute number of lymphocytes, monocytes, eosinophils, basophils, neutrophils, the value of the adaptation index (AI) was calculated, according to which the types of adaptation reactions were characterized by L. Harkavy et al., [3]. According to the findings of these researchers, some adaptive reactions (ARs) develop in the human body in response to various physiological and pathological stimuli. According to the value, AIs are identified as the following types of AR: stress (0.3 and <); orientation (0.31 - 0.5); quiet adaptation (0.51 - 0.7); re-activation (0.71-0.9) and increased activation (0.9 and >).

The orientation reaction and the reaction of quiet adaptation with the transition to the orientation reaction represent a physiological response to the various magnitude and power stimuli. They are accompanied by an increase in nonspecific resistance and adaptive potential of the human body. The response to stress and the related ones have pathological nature. They are accompanied by a decrease in non-specific resistance of the organism and its adaptive potential. Complex neuro-endocrine changes that characterize each of the adaptation reactions are reflected in the morphological composition of the leukocyte formula [5, 7, 9].

In addition, the leukocyte index of intoxication according to Kalf-Kalif [14] and other integral hematological indices [1, 2, 11] were calculated:

1. Leukocyte index of intoxication (LII) of Kalf-Kalif, which characterizes the degree of inflammation, destruction, intoxication:

$LII = (2B + S) / (L + M) \cdot (E + 1)$ , where P and C are band and segmental neutrophils; L - lymphocytes; M - monocytes; E - eosinophils.

2. Leukocyte index (LI), which reflects the relationship between humoral and cellular immunity:  $LI = L/S$ .

3. The index of the ratio of neutrophils and lymphocytes (IRNL), which provides information on the ratio of cells of specific and non-specific immune defense:  $IRNL = (B + S) / L$ .

4. The index of the ratio of neutrophils and monocytes (IRNM), changes can indicate to the ratio of components of the microphage-macrophage system:  $IRNM = (P + C) / M$ .

5. The index of the ratio of lymphocytes and monocytes (IRLM), which characterizes the relationship between the effector and afferent chains of the immunological response:  $IRLM = L/M$ .

6. Index of the ratio of lymphocytes and eosinophils (IRLE), which characterizes the processes of immediate and delayed hypersensitivity:  $IRLE = L/E$ .

The results were statistically processed using the Microsoft Excel 2010 program and the SPSS 11 statistical software package. The Wilcoxon criterion used to determine the probability of related samples and the nonparametric Mann-Whitney U-criterion for unrelated samples and the Spearman rank correlation coefficient. The result was considered statistically significant if  $p < 0.05$ .

## Results

In the initial state, according to certain leukogram indices, there were some differences in the control group, which obviously represented signs of incomplete restoration after physical activity. In particular, having examined high-skilled runners we noted a tendency to increase the number of leukocytes ( $11.05 \pm 3.3 \times 10^9 / l$ ). These athletes showed a decrease in the absolute amount of monocytes ( $0.21 \pm 0.04 \times 10^9 / l$ ), which was reflected in the hematological indices: IRNM and IRLM (Table 1). The values of these indices in the control group were significantly different in low-skilled athletes and high-skilled ( $P < 0.01$ ). The values of these indices significantly go beyond reference values (IRNM - 0-21 and IRLM - 0-14) [12], which is a sign of violation of the effector and afferent chains of the immune response [2, 11].

Table 1. Features of integrated hematological indices both in runners of various skills and in the control group at rest ( $M \pm m$ )

Indices	Examined groups			P between GII and GI
	CG (n = 12)	GI (n = 20)	GII (n = 15)	
LI	$0,54 \pm 0,03$	$0,61 \pm 0,02^*$	$0,55 \pm 0,01$	$P > 0,05$
LII	$2,22 \pm 0,43$	$2,18 \pm 0,19$	$3,01 \pm 0,32$	$P < 0,05$
IRNL	$2,16 \pm 0,11$	$1,97 \pm 0,13$	$2,02 \pm 0,08$	$P > 0,05$
IRNM	$11,11 \pm 1,73$	$6,31 \pm 0,36^*$	$33,12 \pm 6,83^{**}$	$P < 0,01$
IRLM	$5,52 \pm 1,07$	$3,33 \pm 0,29^*$	$16,59 \pm 3,57^{**}$	$P < 0,01$
IRLE	$16,99 \pm 2,9$	$6,58 \pm 0,45^{**}$	$6,18 \pm 0,19^{**}$	$P > 0,05$

Note: \* -  $P < 0,05$

\*\* -  $P < 0,01$  – probability between GI, GII and CG

Athletes of high qualification confirmed the state of overtraining. They observed an imbalance in the peripheral and central phases of the immune response. This was confirmed by a significantly increased index of

IIR ( $19.33 \pm 4.15$ ) ( $P < 0.01$ ), compared with the control group and the group of low-skilled athletes. The increased index testified to the presence of phenomena of inflammation, destruction, and intoxication [10, 13].

Athletes of low qualification also showed signs of influence of training loads, but less than high-skilled athletes. They showed a tendency to shift the formula to the left (increase of bNT -  $7.9 \pm 1.19\%$  and reduction of the absolute amount of sNT -  $2.9 \pm 0.23 \times 10^9 / l$ ).

In a state of rest, the type of adaptive response to the AI value for all athletes was defined as "quiet adaptation" that is characterized by high resistance and high adaptive potential of athletes. In the initial state, there were also no features of leukogram in athletes of various qualifications.

After physical activity "to refusal", the values of the indices characterizing the morphological and functional status of leukocytes, especially in low-skilled athletes, changed significantly. There the number of leukocytes increased twofold ( $P < 0.01$ ), alongside with significant changes in leukogram. The number of lymphocytes decreased by 5.5% ( $P < 0.05$ ), and the number of eosinophils decreased almost to zero ( $P < 0.01$ ). At the same time there was a tendency to monocytes and neutrophils increase ( $P > 0.05$ ).

After 30 minute recovery, the number of leukocytes continued to increase ( $P < 0.05$ ) with a pronounced shift of the leucogram to the left, as the number of band neutrophils (bNT,  $P < 0.05$ ) sharply increased, while all other populations of leukocytes were restored to the initial level, except for lymphocytes, the number of which continued to be reduced ( $P < 0.05$ ).

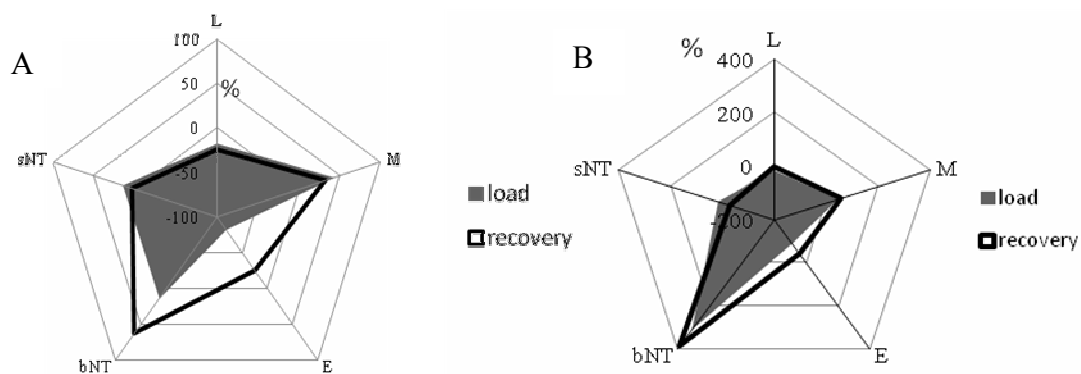


Fig.1 Dynamics of morphological changes in blood of low-skilled (A) and high-skilled (B) runners after a physical load "to refusal" (% out of initial level)

Thus, immediately after exercising, low-skilled athletes had an almost classical second phase of leukocytosis - the first neutrophil phase. This phase occurs in the body due to the effect of significant physical activity and is characterized by an increase in the amount of leukocytes twice ( $5.95 \pm 1.47 \times 10^9 / l$  - before physical activity and  $12.42 \pm 3.21 \times 10^9 / l$  - after physical activity,  $p < 0.05$ ), lymphocytopenia ( $p < 0.05$ ), but with significant monocytosis; decrease in the number of eosinophils, practically to zero ( $0.27 \pm 0.03 \times 10^9 / l$  - before physical activity and up to  $0.09 \pm 0.04 \times 10^9 / l$  - after physical activity,  $p < 0.01$ ), as well with an increase in the number of segmental neutrophils with the shift of the blood formula to the left, which is characterized by a significant increase in bNT ( $P < 0.05$ ). The second phase of leukocytosis is clearly observed in the presentation of changes in the leucogram indices in percentage (Fig. 1A).

Changes in the percentage of leukogram were even more pronounced in high-skilled athletes, as they showed signs of non-recovery from previous loads at the initial level (Fig. 1B).

After exercising, athletes of high qualification experienced a pronounced second phase of leukocytosis with the transition to the third phase (the second neutrophilic phase). This stage is characterized by further leukocytosis ( $P < 0.05$ ), lymphocytopenia and significant monocytosis ( $7.44 \pm 1.04\%$  ( $0.55 \pm 0.06 \times 10^9 / l$ ) - after exercise and  $11.74 \pm 2.76\%$  ( $1.93 \pm 0.58 \times 10^9 / l$ ) - after 30 minute recovery) ( $P < 0.05$ ), reduction of the number of eosinophils practically to zero ( $4.86 \pm 0.03\%$  ( $0.54 \pm 0.18 \times 10^9 / l$ ) - before physical activity and  $0.94 \pm 0.1\%$  ( $0.08 \pm 0.02 \times 10^9 / l$ ) - after exercising) ( $P < 0.05$ ), neutrophils and changes in the formula blood to the left (a significant increase in the number of bNT) ( $17.58 \pm 1.93\%$  ( $1.88 \pm 0.52 \times 10^9 / l$ ) - after physical activity and  $20.24 \pm 3.88\%$  ( $1.65 \pm 0.18 \times 10^9 / l$ ) - after 30 minute recover) ( $P < 0.05$ ).

As it is shown in Fig. 1A and 1B, after 30 minute recovery the shift of the blood formula to left increased in both low-skilled athletes and high-skilled athletes. It has been established that hematological indices significantly change in high-skilled athletes after physical activity (Fig. 2).

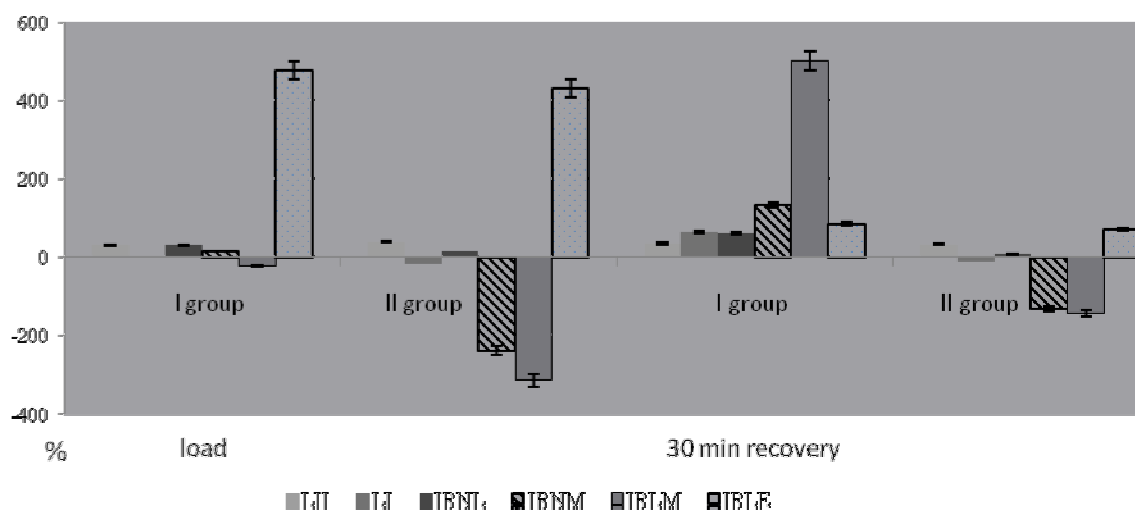


Fig. 2. Dynamics of hematological indices in athletes-runners (% of the initial level)

However, immediately after physical activity, only the IRLE index ( $P < 0.01$ ), which characterizes the balance between lymphocytes and eosinophils, and reflects the ratio of processes of hypersensitivity of the immediate and delayed type has changed. At the same time, changes in the indexes of IRNM and IRSM were marked in high-skilled athletes in a state of rest. After 30 minute recovery, more significant changes in indices were observed in low-skilled athletes. They experienced particularly large changes in the IRNM and IRLM indices, which characterize the ratio of components in the microphage-macrophage system and the effector and afferent chains of the immune response. At the same time, high-skilled athletes displayed normalization of the hematological indices values during the recovery process.

## Discussion

The largest changes were observed in the IRLE and LII indexes, which include the number of eosinophils. It is known that eosinophils are a source of a number of cytokines, some of which are involved in maintaining homeostasis, while others carry out a proinflammatory function and emit proinflammatory mediators (a factor that activates platelets, proinflammatory prostaglandins) - these are cells that are involved in damaging tissue. Eosinophils secrete transforming growth factor; participate in the development of reactions caused by T-lymphocytes. Eosinophils can modulate immediate hypersensitivity reactions by inactivating mast cell release (histamine, leukotrienes, lysophospholipids and heparin) [15]. In conditions of vigorous physical activity, due to a sharp decrease (almost to zero) of the number of eosinophils, respectively, there is a higher increase in the indexes of hematological indices IRLE and LII in low-skilled athletes than in high-skilled athletes ( $P < 0.01$ ). In addition, high-skilled athletes have better restitution of indices.

Consequently, the tolerance of high-skilled athletes to extreme physical activity was higher. This was confirmed by little changes in the values of the hematological indices that characterize the ratio between the individual subunits of the leukocytes, as well as the constant type of adaptive response and the unchanged value of the L. H. Harkvy adaptation index during physical activity and recovery period.

The reaction of low-skilled runners to physical activity "to refusal" indicated less complete mechanisms for regulating the blood system of these athletes, since the misbalance in the ratio of individual indices of leukogram was deeper, indicating less adequate adaptation to maximal physical load.

## Conclusions

It was established that the violation of the balance between the subpopulations of leukocytes, in particular neutrophils and monocytes, and between lymphocytes and monocytes, expressed in the dynamics of the values of the IRNM and IRLM indices, can serve as a criterion for the level of overtraining, since these indices were the most variable in low-skilled athletes engaged in physical activity and increased at restoration as a result of excessive physical stress.

A lot of information was found in the following indices: the Harkavy Adaptation Index (AI), as well as LI that is related to endotoxicity, and the indices associated with the absence of anti-inflammatory mediators released by eosinophils - IRLE and LII.

It was established that high-skilled athletes showed better tolerance to physical activity (reduction of LII, unchanged AI, practically identical changes of leukograms, better restitution of indices).

Consequently, changes in the leukogram indices and integral hematological indices can be used in further studies as a marker for the adequacy of physical activity and monitoring of the athletes' functional state.

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