Effect of exercise on Special Aviation Gymnastics Instruments on blood serum levels of selected biochemical indices in cadets

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Abstract

Introduction. Aim of this study was the training effect evaluation on the Special Aviation Gymnastics Instruments (SAGI) on blood metallothionein (MT), zinc (Zn), copper (Cu), protein, neuron-specific enolase (NSE), and physical fitness in the examined cadets.

Material and methods. The study comprised 55 cadets, aged 20, divided into two groups: examined group A (N=41) and control group B (N=14). In both groups, blood material was collected twice, i.e. before (baseline) and after training (series I), during (series II), and after completion of training on the SAGI (Series III). Blood serum MT, Zn, Cu, protein, and NSE were assayed with commercially available kits). Physical fitness was assessed with commonly used fitness tests.

Results. A significant decrease in serum MT was noted in both groups in all three series of assays after training, except group B in series II. NSE significantly increased in group A in series II after training. NSE activity increased significantly in group B in series I and III. In both groups, a significant decrease in blood serum Zn was noted after training in series I and II. Serum Cu significantly decreased in group A in all three series of assays. Blood serum protein significantly decreased in group A in series III. In series II, blood serum protein increased significantly in both groups. The remaining values were not changed significantly.

Conclusions. Training intensity on SAGI lowered serum MT levels after training in comparison with the control group. This might be associated with Zn, Cu, and protein metabolism.

Key words

Metallothionein, zinc, copper, physical fitness, Special Aviation Gymnastic Instruments, neuron-specific enolase

INTRODUCTION

In Polish military aviation, Special Aviation Gymnastics Instruments (SAGI) consist of the following devices: looping, aerial wheel, and gyroscope. Training on SAGI involves movements in all body axes, resulting in the work of the defined muscle groups, circulatory-respiratory system, and CNS load. SAGI is moved with the aid of specific muscles. Isometric exercises predominate during the training on SAGI. This training aims at preparing the future pilots to extreme conditions during flight. It was found that training on SAGI improves psychomotor capability [1]. Physical exercise produces some changes in biochemical blood parameters, which depend on both exercise duration and intensity. Physical exercise, stress, and negative environmental factors may produce a so-called acute phase, manifested by an increase in blood protein level. In the available literature, there are not reports on the changes of the blood serum biochemical indices produced by training on SAGI. Therefore, metallothionein, (MT), zinc (Zn), copper (Cu), protein, and neuron-specific enolase (NSE) were used to assess body load produced by the training on SAGI.

Metallothionein is a low-molecular weight protein (molecule weight 7 to 10 kDa). It has been shown that MT has a high affinity to the following metals: Cd, Hg, Cu, and Zn, and may play a role in both metabolism and detoxication. Mammalian MT molecule consists of a polypeptide cysteine-rich chain and clusters of bivalent ions [2]. Studies have shown that metallothionein is a stress-induced protein and belongs to the acute-phase proteins [3]. Metallothionein is considered an effective free radicals scavenger, several times more effective than glutathione [4]. It plays a valuable diagnostic role in the monitoring of physical exercise and disorders of both pro- and antioxidative balance [5,6]. Zinc and copper play a marked role in the physical exercise as antioxidative agents. Blood protein level usually increases during physical exercise and is considered an effective antioxidant [7]. NSE is one of three enolase isoenzymes in the neuroendocrine cells cytoplasm. An increase in beta-enolase is seen in marathon runners [8].

An attempt at determining sensitive biochemical indices which could be used to assess body adaptation, exercise-induced load and related stress, was undertaken because of the specificity of training on SAGI. Therefore, the authors used these indices, which change significantly during a physical exercise [5,6,7,8]. The authors noted such changes after the training on SAGI within aerobic metabolism – the subject of the study.

OBJECTIVES

The presented study aimed at: 1) Assessing the effect of training on SAGI on blood MT, Zn, Cu, protein, and NSE
levels in the examined cadets, in comparison with a control group. 2) Assessing the effect of training on SAGI on the general physical fitness in the examined cadets, using physical fitness tests, in comparison with a control group.

**MATERIAL AND METHODS**

**Subjects.** Fifty-five cadets (male) participated in the study, mean age: 20 years, body weight: between 67.59 kg – 71.03 kg, height: between 173.62 cm – 176.64 cm, BMI: between 22.49 kg/m$^2$ – 22.82 kg/m$^2$ (Tab. 1). Cadets were divided into group A (examined; N=41), trained on SAGI special programme [9], and Group B (control; N=14), undertaking a standard programme of physical education. In both groups, blood served as the assayed material. Samples were collected twice: prior to (BT) and after training (AT) at the beginning (series I), during (series II), and after (series III) completion the training programme on SAGI. Samples were collected at 09:00 (i.e. two hours after breakfast) and 11:00 (i.e. immediately after training) in each series of assays. In both groups, the programme of physical education included 20 two-hour training units for 70 days (Fig. 1).

![Figure 1. Scheme of the study](image)

Consent for the study was obtained from the Ethical Commission on Biomedical Research Studies at the Military Institute of Aviation Medicine in Warsaw (Decision No. 03A/2009 of 08.07.2009).

**Measures.** The following biochemical indices were assayed in blood serum: MT with immunoenzymatic technique ELISA [10,11] with antibodies DacoCytomation, standards isolated from the human liver, containing isoforms MT-I and MT-II and color reaction produced by the commercially available horseradish peroxidase. Zn and Cu were assayed with atomic absorption spectro-photometry (AAS) technique. Serum protein was assayed with burette test using Roche reagents and Integra 400/800. NSE with enhanced chemiluminescence immunoassay (ECLIA) test with Roche reagents using Elecsys NSE analyzer.

POLAR TEAM-2 PRO system was used for the monitoring training intensity and HR registering. It included a straps with HR placed on the cadets’ chest, which recorded HR during training and base station interpreting the results.

**Procedure and design.** Training stimulus of a similar intensity and the same set of exercises were used in the Group A in all three series. Training was carried out with interval method in all three series. In every series, the training unit consisted of the following consecutive exercise:

- **Looping**: two series of turns on the fixed looping. Each series consisted of 10 turns forward and 10 turns backward (Fig. 2). Interval between series was 20 seconds. Pause before the next exercise on gyroscope was 3 minutes.
- **Gyroscope**: two series of a 60-second dynamic turns with 20-second intervals (Fig. 2). Pause before the next exercise on the aerial wheel was 3 minutes.
- **Aerial wheel.** Two series of turns. Each series consisted of 3 turns sideways to the right and 3 turns sideways to the left. Each series was repeated 4 times (Fig. 2). Interval between series was 20 seconds. Pause before the next exercise on free looping was 3 minutes. The above listed exercises were repeated trice.

Training intensity in group B was similar in all 3 series. General fitness training was applied with special reference to all motor features. This programme included team sports, light athletics, gymnastics, and field running games. Training was quite intensive to prepare the body for higher loads during the further education cycle. Training was carried out with task, repetition, and interval methods.

Cadets’ physical fitness was assed before (series I) and after study completion (series II) with physical fitness tests, e.g. Aero-Synthetic Efficiency Test (ASET) [12], shuttle sprint 10×10 m, pull up on the bar, sprint for 16.5 m, and bending forward.

Board and lodging was the same for all cadets participating in the study. Cadets received standard diet. Daily ration contained 4,500 kcal on the average, including 150 g of fat (30%), 112.5 g protein (10%), and 675 g (60%) carbohydrates. This daily ration contained 8 – 10 mg of zinc, and 1.5 – 3.0 mg of copper.

**Statistical analysis.** Mean values and standard deviation were calculated for all parameters. Differences before and after training were calculated with the test of differences for dependent and independent groups in both groups of examined cadets. The obtained results were statistically analyzed with computer STATISTICA 9 programme. Differences in the mean values are treated as statistically significant when the calculated $p$ value was lower than 0.05.

**RESULTS**

The obtained results showed statistically insignificant differences between both groups in the following demographic data: age, height, body weight, and BMI (Tab. 1). HR recorded during training in series I, II, and III increased significantly in group B, compared with group A at $p<0.001$ (Tab. 1).

A statistically significant decrease in AT serum MT was seen in group A in series I ($p<0.005$), series II ($p<0.05$), and series...
III (p<0.001), compared with BT values (Tab. 2). In group B, a decrease in AT serum MT was also noted in series I (p<0.05) and series III (p<0.001), compared with BT values (Tab. 3). It should be stressed that significantly higher BT serum MT values were seen in group B than the BT values group A in series I at p<0.001, series III at p<0.001, and AT values in series I at p<0.01, series II at p<0.01, and series III at p<0.001. Only BT serum MT value was insignificant in series II (Tab. 3).

In group A, AT serum Zn values decreased significantly in group A in series I at p<0.001, series II at p<0.005, and insignificantly increased in series III, in comparison with BT values (Tab. 2). In group B, serum Zn values decreased significantly in series I at p<0.01, series II at p<0.05, and insignificantly in series III, in comparison with BT values (Tab. 3). Statistically significantly higher serum Zn values were noted in group A than those in group B, both before and after training in series I (p<0.001 and p<0.001, respectively), in series II (p<0.001 and p<0.001, respectively). In series III, the difference in serum Zn was insignificant (Tab. 3).

A statistically significant decrease in AT serum Cu was seen in group A in series I at p<0.05, series II at p<0.001, and series III at p<0.05, in comparison with the BT values (Tab. 2). AT serum Cu values decreased insignificantly in group B in series I and II, and increased significantly in series III at p<0.01 (Tab. 3). Before training, serum Cu values were statistically significantly higher in group A than those in group B in series II at p<0.05. AT, serum Cu values were significantly higher in group B than that in group A in series III at p<0.01, while the difference was insignificant in series I and II (Tab. 3).

AT serum protein decreased statistically significantly in group A in series II and series III at p<0.001 and p<0.001, respectively, compared with BT values, while this decrease was insignificant in series I (Tab. 2). In group B, AT serum protein levels increased in all 3 series, but it was significant only in series II at p<0.001 (Tab. 3). BT serum protein values were statistically significantly higher in group A in comparison with group B in series I and II at p<0.001 and p<0.01, respectively, and significantly higher at p<0.001 and p<0.001 after training (Tab. 3).

AT serum NSE activity increased significantly in group A in series II at p<0.005. This increase was insignificant in the remaining series of assays (Tab. 2). In group B, AT serum NSE increased in all 3 series, but statistically significantly in series I and III at p<0.05 and p<0.005 (Tab. 3). Both BT and AT statistically significant higher serum NSE values were noted in group A, in comparison with group B in series I at p<0.001 and p<0.001, respectively, (Tab. 3).

Zn/Cu and MT/protein ratios were also calculated. AT Zn/Cu decreased significantly in series I at p<0.001 and increased significantly in series III at p<0.05 in group A, while in series II this decrease was insignificant (Tab. 2). In group B, AT Zn/Cu values decreased in all 3 series, but significantly only in series I and series II at p<0.001 and p<0.05, respectively, (Tab. 3). BT and AT Zn/Cu values were significantly lower in group B, compared with those in group A in series I at p<0.01 and p<0.001, respectively. In series II, these values were significantly higher at p<0.001 and p<0.005, and significantly lower at p<0.005 in series III after training (Tab. 3).

AT MT/protein values was significantly lower in group A in all 3 series, in comparison with BT values at p<0.01, p<0.001, and p<0.005 (Tab. 2). In group B, MT/protein values after training were significantly lower in series I and series III at p<0.01 and p<0.001, respectively, and in series II, MT/protein decrease was insignificant (Tab. 3). Both BT and AT values of this parameter were significantly higher in group B in series I at p<0.01 and p<0.001, compared with that in group A. In series II, AT values were statistically significantly higher at p<0.001 and p<0.001, respectively, while in series III both BT and AT values were significantly higher at p<0.005 and p<0.05, respectively (Tab. 3).

Physical fitness analysis showed a statistically significant improvement in the results of pull-up on the bar, running for 16.5 m, body bending – both forward and backward, and ASET in group A in series II in comparison with the results achieved in series I (Fig. 3). In group B, the results of pull-up on the bar at p<0.005, forward bending, and ASET at p<0.005 improved significantly in comparison with the results in series I. Improvement in the results of running for 16.5 m was insignificant. Insignificant improvement in results of shuttle sprint 10×10 m was noted in both groups in series II, in comparison with series I (Fig. 3).

### Table 1. Demographic data and HR in groups A and B [9]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20.5±3.102</td>
<td>20.2±0.42</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.64±5.78</td>
<td>173.62±5.12</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>71.03±6.94</td>
<td>67.59±6.36</td>
</tr>
<tr>
<td>BMI (kg·m⁻²)</td>
<td>22.82±1.95</td>
<td>22.9±1.87</td>
</tr>
</tbody>
</table>

**Mean ±SD – standard deviation, “+” – statistically significant difference at p<0.001 in relation to the values in group A**

### Table 2. Changes in biochemical indices before and after exercise on SAGI in series I, II and III in group A (N=41)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Series I</th>
<th>Series II</th>
<th>Series III</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT (ng/mL)</td>
<td>4.98</td>
<td>3.72⁺</td>
<td>2.94</td>
</tr>
<tr>
<td>NSE (ng/mL)</td>
<td>±2.48±1.43</td>
<td>±3.55±2.07</td>
<td>±1.06±0.30</td>
</tr>
<tr>
<td>Zn (µmo/L)</td>
<td>12.99</td>
<td>11.72⁺</td>
<td>11.85</td>
</tr>
<tr>
<td>Cu (µmo/L)</td>
<td>±1.55±1.62</td>
<td>±2.09±1.08</td>
<td>±1.45±1.55</td>
</tr>
<tr>
<td>Protein (g/L)</td>
<td>8.79</td>
<td>8.67</td>
<td>6.59⁺</td>
</tr>
<tr>
<td>NSE (µmo/L)</td>
<td>±0.51±0.53</td>
<td>±0.40±0.60</td>
<td>±0.33</td>
</tr>
<tr>
<td>Zn/Cu</td>
<td>±0.92±0.84⁺</td>
<td>±0.75±0.74</td>
<td>0.34±0.23⁺</td>
</tr>
<tr>
<td>MT/protein (ng/g)</td>
<td>±0.56±0.16</td>
<td>±0.28±0.16</td>
<td>±0.04</td>
</tr>
</tbody>
</table>

BT – before training; AT – after training; Mean ±SD – standard deviation;
⁺ – statistically significant difference at p<0.001 in relation to the values BT;
⁻ – statistically significant difference at p<0.001 in relation to the values BT;
⁻⁻ – statistically significant difference at p<0.005 in relation to the values BT;
⁻⁻⁻ – statistically significant difference at p<0.01 in relation to the values BT.
Table 3. Changes in biochemical indices before and after exercise on SAGI in series I, II, III in group B (N=14) and comparison results with group A (N=41)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BT</th>
<th>AT</th>
<th>BT</th>
<th>AT</th>
<th>BT</th>
<th>AT</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT (ng/mL)</td>
<td>±3.10</td>
<td>±1.12</td>
<td>±5.11</td>
<td>±4.29</td>
<td>±1.72</td>
<td>±0.49</td>
</tr>
<tr>
<td>Zn (µmo/L)</td>
<td>11.37+</td>
<td>9.01+</td>
<td>13.88+</td>
<td>12.81+</td>
<td>11.65</td>
<td>11.30</td>
</tr>
<tr>
<td>Cu (µmo/L)</td>
<td>14.89±</td>
<td>15.10</td>
<td>13.86±</td>
<td>14.26</td>
<td>14.38</td>
<td>14.73**</td>
</tr>
<tr>
<td>Protein (g/L)</td>
<td>7.91±</td>
<td>8.81±</td>
<td>7.80**</td>
<td>8.25*</td>
<td>8.01+</td>
<td>8.21+</td>
</tr>
<tr>
<td>NSE (ng/mL)</td>
<td>0.51±</td>
<td>0.42±</td>
<td>0.29±</td>
<td>0.37±</td>
<td>0.46±</td>
<td>0.51±</td>
</tr>
<tr>
<td>Zn/Cu</td>
<td>±0.51</td>
<td>±0.37</td>
<td>±0.60</td>
<td>±0.60</td>
<td>±0.54</td>
<td>±0.56</td>
</tr>
<tr>
<td>MT/protein (ng/g)</td>
<td>±0.51</td>
<td>±0.50</td>
<td>±0.40</td>
<td>±0.47</td>
<td>±0.27</td>
<td>±0.21</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The obtained results show that the physical exercise applied to the cadets of both groups brought about changes in the majority of the biochemical indices AT. Differences between group A and group B resulted from the intensity of physical exercise, as confirmed by the HR recording (Tab. 1). Lower intensity of physical exercise in group A was a result of the shorter duration of training due to the longer intervals during changing SAGI, but also due to the character of the exercise.

It seems that training on SAGI produces a higher body load because of +Gz (direction head-legs) and –Gz (direction legs-head) being present during the training. Training on SAGI is associated with a marked effect on appropriate receptors in the arteries and CNS.

The changes in serum Zn, Cu, and protein values seen in group A in all 3 series of assays AT indicate a markedly increased body requirement for these elements and for protein. This is confirmed by the significant decrease in serum Zn (except in series II, in which an insignificant increase was noted), Cu, and protein (insignificant in series I and significant in series II). In the control group (B), direction of changes in serum Cu level was opposite in all 3 series of assays. Serum Zn decreased significantly in series I and II (insignificant in series III). Serum protein also increased significantly in series II (insignificantly in series I and III). There is evidence that an intensive physical exercise brings about a significant increase in blood Zn levels and decrease in blood Cu [13], as well as an increase in the protein levels [5] in the athletes of both genders. Studies have shown significantly increased serum Zn levels in volleyball players and controls at the beginning of training, using a progressive cycle ergometer test, while urine Zn levels were markedly increased in the volleyball players than that in the control group AT [14].

In the presented study, changes in serum Zn and Cu were different in comparison with reports by other researchers. This might be associated with the character of the physical exercise, simultaneous effect of the positive (+Gz) and negative (-Gz) acceleration, and intensity of training on SAGI. A statistically significant decrease in serum Zn and Cu levels and insignificant urine levels of these elements were noted in the body exposure to +Gz [15]. This suggests that the degree of changes in serum bioelements result from increased transfer into the tissues of the higher physiological work due to the applied acceleration.

In group B, in which serum Cu increased significantly AT, while serum Zn decreased in series I and series II, it might be connected with the different character of physical exercise and also decreased liver and kidney Cd. Physical exercise decreases Cd levels in the internal organs [16]. Cd interactions with Zn and Cu consists of mutual displacement from the complex with metallothionein. Zn displaced from the bound with proteins, so-called free Zn, stimulates MT gene expression and increases its synthesis [17]. Zn generally maintains an activity with enzymes which neutralize free radicals effects resulting from the oxidation stress [18]. Decreased Zn levels may lower the activity of superoxide dismutase and increase hepatocytes sensitivity to the free radicals-induced damage. Cu deficit is associated with superoxide dismutase defect and impaired free radical scavenging. It is assumed that MT plays an important regulatory role [19] and has high affinity to hydroxyl and superoxide radicals [20]. A decrease in serum Cu in group...
A in all 3 series of assays indicates a weaker anti-oxidative defence and MT taking up the function of an antioxidant. This was clearly seen in both groups in series II.

It was shown that a Zn-deficient diet induces increased MT levels during intensive physical exercise which may result from both Zn/Cu and MT levels. A Cu-deficient diet decreased body adaptation to physical exercise which could be confirmed by other researchers who showed an increase in MT levels during intensive physical exercise in groups B than those in group A. In group A, this tendency was reversed, and confirmed by other researchers who showed an increase in MT levels during intensive physical exercise [21].

In analyzing MT levels in the presented study series of assays, attention should be paid to the significant changes of BT and AT values, where a decreasing tendency was observed in both groups. This indicates a decrease in MT synthesis during prolonged training and possibly its reduced function. It seems that it might be associated with the high nutritional value of the diet protein which, however, was lower than the requirement, especially in group A, which had a different character than the group B.

The decrease in Zn/Cu ratio in group A in series II may result from the specificity of training on SAGI acting on the hypothalamic-pituitary-adrenals axis after training, decreasing glucose levels. MT could take the role of a superoxide dismutase regulator. In series III, a return of Zn/Cu to the values seen in series I after training may indicate an important Zn role in the CNS activity. This seems to be confirmed by NSE levels in series III of assays AT, showing a decreasing tendency. NSE levels in group A in comparison with group B showed that the intensity of training did not exceed body compensation capability in series III. NSE increase in group B indicated a demand for oxygen higher than that in group A. This might be associated with the difference in MT, NSE, and protein levels in both groups. A physical exercise-induced lower oxygen intake may stimulate NSE activity. Such a stimulation has been noted in oxygen-deficient patients with pneumonia [28].

Animal studies (in rats) showed that prolonged consumption of a diet deficient in Zn and Cu may weaken superoxide dismutase activity and damage CNS neurons, leading to an increase in NSE activity and MT changes in the Zn and Cu binding with CNS proteins [29].

Summing up, it should be stressed that both groups in the presented study differed in the character and intensity of physical exercise, as confirmed by the difference of MT, Zn, and Cu, NSE, and protein levels between the studied groups. This was a result of the lower intensity and character of the physical exercise in group A in comparison with group B (control). The character of training on SAGI increased the body requirement for protein, Zn and Cu. Lower MT levels in group A compared with group B after training were probably due to the fact that the training applied to the cadets produced a marked load on CNS and the internal organs, producing better results obtained in ASET, which determined movements coordination. In a such situation, MT plays the role of an antioxidant (regulator), combining its role with protein, Zn, and Cu metabolism. Training on SAGI improved cadets’ general physical fitness during exercise tests, but insignificantly in comparison with the results in group B.

CONCLUSIONS

This study shows that:
1. Training on SAGI increased body requirement for Zn, Cu, and protein in the study group in comparison with the control group.
2. Intensity of training on SAGI resulted in statistically lower serum MT levels AT in comparison with the control group. This might be associated with Zn, Cu, and protein metabolism.
3. Training on SAGI increased cadets’ general fitness more than in control group.
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