

Anxiety associated with parachute jumping as the cause of blood red-ox balance impairment

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Summary

Aim. The aim of the study was to assess the effect of anxiety associated with parachute jumps on selected redox balance parameters in regular soldiers from airborne forces. The study allows estimating whether the paratroopers exposed to high level of mental stress are simultaneously under severe oxidative stress.

Methods. The investigations were carried out on 46 professional soldiers from airborne forces divided into groups depending on the number of performed parachute jumps. Peripheral venous blood samples were obtained under fasting conditions three times for the determination of selected parameters of red-ox balance: on an ordinary working day, on the day when the jump was performed and on the day after the jump. The time of the performed determinations was to reflect the initial balance of the organism, the state at the moment of stress and its effect on the organism.

Results. Our investigations showed lack of differences in characteristics of the activity of antioxidant enzymes (CAT and SOD) in response to mental stress depending on the experience of the investigated group in parachuting. Decrease in GSH-Px activity was demonstrated in response to mental stress in all the investigated groups. The TBARS level was higher in more experienced parachutists.

Conclusions. The analysis of changes in selected redox balance parameters may be useful for monitoring anxiety associated with parachute jumps.

Key words: anxiety, oxidative stress, parachute jumps

Introduction

Recent years have been the period of intensive research studies on the metabolic role of oxygen. Life-giving oxygen has been long known to be also harmful [1]. Scientific reports have produced a detailed explanation of the mechanisms of its toxicity consisting in oxidation-reduction imbalance in the body by inducing the so-called oxidative stress [2]. The oxidation-reduction balance may vary, among other things, under the influence of environmental factors [3] and diseases [4, 5].

People exposed to high levels of stress often suffer from cardiovascular diseases [6], which include the presence of oxidative stress [4, 7]. It raises the question of whether high levels of psychological stress may change the oxidation-reduction balance causing, for example, oxidative stress. In our previous studies [8], we have shown that for professional soldiers from airborne forces a parachute jump is an important anxiety stimulus, and that this stimulus affects the ACTH and cortisol concentrations, depending on the experience of the study group in skydiving [9]. If it turned out that anxiety associated with parachuting causes oxidative stress, one might recommend taking antioxidants before parachuting to prevent changes in the oxidation-reduction balance. This type of behavior can give measurable health-related benefits, since oxidative stress is related to, among others, infertility [10, 11], and the occurrence of many diseases including neurodegenerative disorders [7, 12], and cancer [13, 14].

Aim

The purpose of this study was to assess the parachute jumping-induced anxiety on selected parameters of red-ox balance in regular soldiers from airborne forces. The study allows to estimate whether the paratroopers exposed, due to specificity of the military service, to high level of mental stress are simultaneously under severe oxidative stress.

Material and methods

The investigation was carried out on 46 regular soldiers from 16 Airborne Battalion, patients of Outpatient Clinic – SPZOZ JW. 4495, healthy males, aged from 20 to 45 years – admitted to perform parachute jumping in airborne forces.

Five groups were distinguished:

- Group 1 – soldiers performing their first jump (7 subjects);
- Group 2 – soldiers during their first season of jumping, performing for the first time a standard of at least 5 jumps per year (14 subjects);
- Group 3 – professional soldiers with the parachutist title, performing at least 5 parachute jumps per year (13 subjects);

- Group 4 – parachute instructors, performing at least 20 parachute jumps per year (7 subjects);
- Group 5 – the members of a parachute sport group performing competitive parachuting (5 subjects).

The groups corresponded to the knowledge and experience in performing parachute jumps and thus they reflect respectively the levels of stress associated with parachuting. Additionally, to emphasize the differences in experience, the total sample was divided into two groups depending on the number of parachute jumps already performed:

- Group A – parachute jumpers having already performed up to 20 jumps;
- Group B – parachute jumpers having already performed more than 20 jumps.

During the experiment, fasting peripheral venous blood samples were collected three times in order to determine the selected parameters of red-ox balance:

- 1) on an ordinary working day (Control);
- 2) on the day a parachute jump was performed (Jump), measurement was taken before the jump;
- 3) a day after the parachute jump (24 h after the jump).

The dates of performing the determinations were to reflect the initial balance of the organism, the state at the time of being exposed of stressor and its effects on the body system.

Blood collection was performed in the Outpatient Clinic – SPZOZ JW. 4495, using disposable equipment, in aseptic conditions, by qualified staff in compliance with safety and hygiene principles.

In erythrocyte hemolysate, the following parameters of oxidation-reduction balance were determined:

- the concentration of compounds reacting with thiobarbituric acid (TBARS) acc. to Placer et al. [15];
- superoxide dismutase activity (SOD) acc. to Misra and Fridovich [16];
- catalase activity (CAT) acc. to Beers and Sizer [17];
- glutathione peroxidase activity (GSH-Px) acc. to Little and O'Brien [18].

Considering the impact of a number of diseases on the oxidation-reduction balance, paratroopers with irregularities in blood tests were excluded from participation in the experiment, i.e., those with abnormal peripheral blood cell parameters (RBC, WBC, PLT, HG, HCT); abnormal levels of acute-phase proteins (CRP) and incorrect basic biochemistry of blood (glucose, urea, creatinine, AST, ALT, total bilirubin). Soldiers experiencing a different kind of stress from the one associated with jumps were also excluded – the verification was carried out during the interview.

In order to estimate the level of emotional tension and psychological stress among the participants, personal questionnaire and the State-Trait Anxiety Inventory (STAI) were performed at each stage of the study [19].

The determinations of red-ox balance parameters from the collected blood samples were performed in the Laboratory of the Chair of Basic and Pre-Clinical Sciences, Medical University of Lodz. Blood cell parameters, the level of acute-phase proteins and basic biochemical parameters were determined in the Medical Laboratory (Diagnostics, LLC) in Krakow. The State-Trait Anxiety Inventory (STAI), developed by C. D. Spielberger, R. L. Gorsuch, R. E. Lushene in the adaptation by K. Wrześniewski et al. [19] were performed in cooperation with the person responsible for psychoprophylaxis in the military unit.

Statistical analysis of the results was made in the Laboratory of Bioinformatics and Biostatistics – BioInforStats – in Krakow using statistical package STATISTICA for Windows. The significance of the difference of distributions between the groups was analyzed using the Kruskal-Wallis test and the significance of differences between individual assays in time was analyzed with the Friedman test. The significance of the difference in distribution of the results depending on the group as regards the number of jumps was analyzed using the Mann-Whitney U test and the significance of differences between individual assays in time with the Friedman test. The test probability at the level of $p \leq 0.05$ was considered as significant and at $p \leq 0.01$ as highly significant.

The project of the presented medical experiment was approved by the Bioethics Committee of the Medical University in Lodz (Resolution No. RNN/430/07/KB, dated 24.07.2007) and by the Bioethics Committee of the Military Medical Chamber (Resolution No. 61/08, dated 14.03.2008).

Results

Results – STAI

The results of the STAI – form X-1 (state anxiety) and X-2 (trait anxiety) – have been published by us in *Polski Markuriusz Lekarski* in 2012 [8]. It has been observed that there is a relationship between experience in performing jumps and the number of jumps and the level of the results of the STAI form X-1 in the individual measurements. The measurements made on the day a parachute jump was performed revealed changes in the STAI form X-1 results, depending on the groups – from the highest value in the least experienced jumpers to the lowest in the group with the most experience. At the same time in groups with less experience in performing parachute jumps the highest level of the STAI form X-1 results was observed in measurements performed on the day a parachute jump was performed, lower in measurements 24 h after the jump and the lowest on an ordinary working day. On the other hand, analyzing the data from the STAI form X-2, there was no association between the experience in performing

jumps and the number of jumps and the level of the STAI form X-2 results in individual measurements. In all groups, the highest level of the STAI form of X-2 results was observed in the measurements performed on a jump.

Results of the measurement of oxidation-reduction balance biomarkers

Figure 1 shows the level of thiobarbituric acid reactive substances – TBARS in hemolysate of red blood cells depending on the group and the time of measurement (Figure 1).

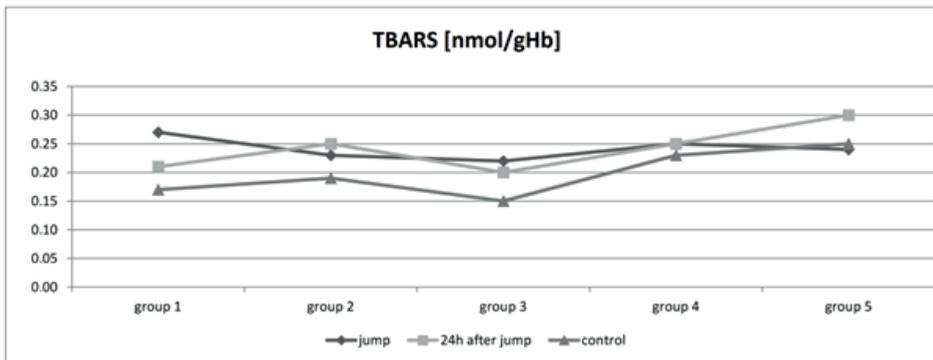


Figure 1. The level of in hemolysate of TBARS in red blood cells depending on the group and time of measurement

There were statistically significant changes in the level of TBARS:

- in measurements performed 24 h after the parachute jump, depending on the group (the lowest value for the group of professional soldiers with the parachutist title and paratroopers performing the first jump and the highest for the parachute sport group; $p = 0.0034$);
- in the control measurement, depending on the group (the lowest values for the group of professional soldiers with the parachutist title and a group of paratroopers performing the first jump, and the highest for the group of instructors and parachute sport group; $p = 0.0104$);
- in the group performing the first jump (the lowest value in the control measurement, higher 24 h after the jump, and the highest on the day of the jump; $p = 0.0388$);
- in the group during the first season of jumping (the lowest value in the control measurement and the highest on the day of the jump and 24 h after the jump; $p = 0.0445$);

- in the group of professional soldiers with the parachutist title (the lowest values in the control measurement and the highest 24 h after the jump and on the day of the jump; $p = 0.0021$);
- in the parachute sport group (the lowest values in the control measurement and on the day of the jump, and the highest 24 h after the jump; $p = 0.0150$).

Figure 2 shows the level of TBARS in hemolysate of red blood cells in each measurement depending on the number of jumps.

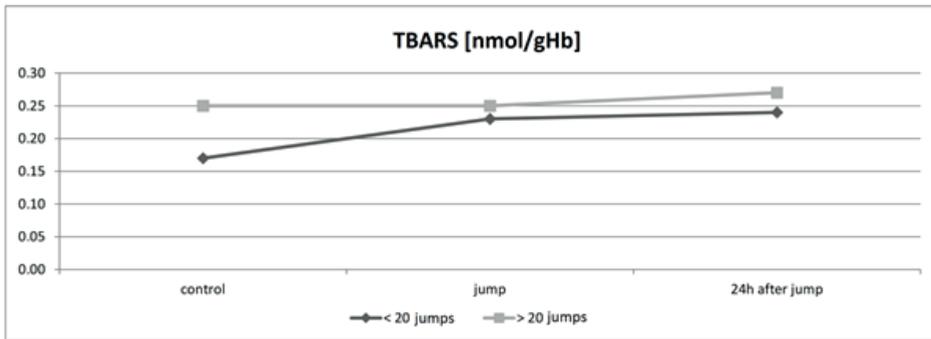


Figure 2. The level of TBARS in hemolysate of red blood cells in each measurement depending on the number of jumps

There were statistically significant changes in the level of TBARS:

- in the measurement 24 h after the jump, depending on the number of jumps (lower values for the group of < 20 jumps and higher for the group of > 20 jumps; $p = 0.0051$);
- in the control measurement, depending on the number of jumps (lower values for the group of < 20 jumps and higher for the group of > 20 jumps; $p = 0.0033$);
- in the group of > 20 jumps (the lowest values on the day of the jump and the control measurement, and the highest 24 h after the jump; $p = 0.0389$);
- in the group of < 20 jumps (the lowest values in the control measurement, the highest 24 h after the jump and on the day of the jump; $p < 0.0001$).

Statistically significant conclusions

There is a relationship between the experience in performing jumps, the number of jumps and the levels of TBARS in the individual measurements.

Higher levels of TBARS were observed in the groups with more experience in performing parachute jumps than in the less experienced groups in the measurements performed 24 h after the jump and during the control period.

In groups with less experience in performing jumps, an increase in TBARS levels, in relation to the control group, in the measurement on the day of the jump and 24 h after the jump was observed.

At the same time in groups with more experience in performing jumps the highest levels of TBARS were found in measurements performed 24 h after the jump compared to the measurements on the day of the jump and during the control period.

Figure 3 shows results of superoxide dismutase (SOD) activity in hemolysate of red blood cells depending on the group and time of measurement.

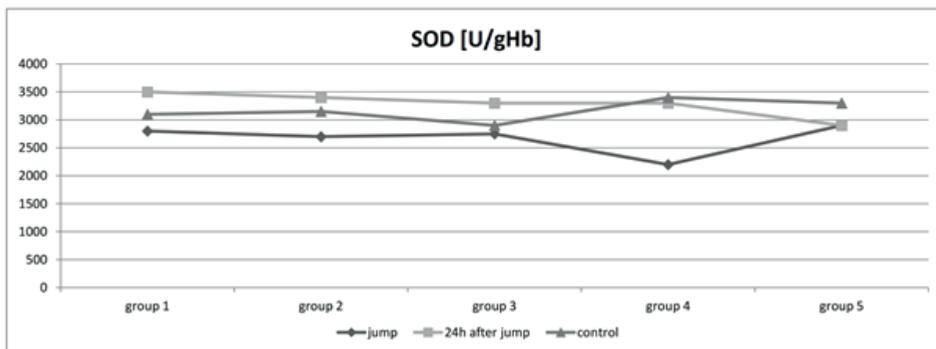


Figure 3. Results of superoxide dismutase (SOD) activity in hemolysate of red blood cells depending on the group and time of measurement

Statistically significant changes in SOD activity were found:

- 24 h after the parachute jump, depending on the group (the lowest values for the group of parachute instructor and the members of a parachute sport group, and the highest for two groups: performing their first jump and during their first season of jumping; $p = 0.00324$);
- in the control measurement, depending on the group (the lowest values for professional soldiers with the parachutist title, and the highest for the group of parachute instructors and the members of a parachute sport group; $p = 0.0221$);
- in professional soldiers with the parachutist title (the lowest on the day of the jump and in the control measurement and the highest 24 h after the jump; $p = 0.0347$);
- in the group of parachute instructors (the lowest on the day of the jump and the highest 24 h after the jump and in the control measurement; $p = 0.0111$);

- in a parachute sport group (the lowest on the day of the jump and 24 h after the jump and the highest in the control measurement; $p = 0.0150$).

Figure 4 shows results of superoxide dismutase activity (SOD) in hemolysate of red blood cells in each measurement depending on the number of jumps.

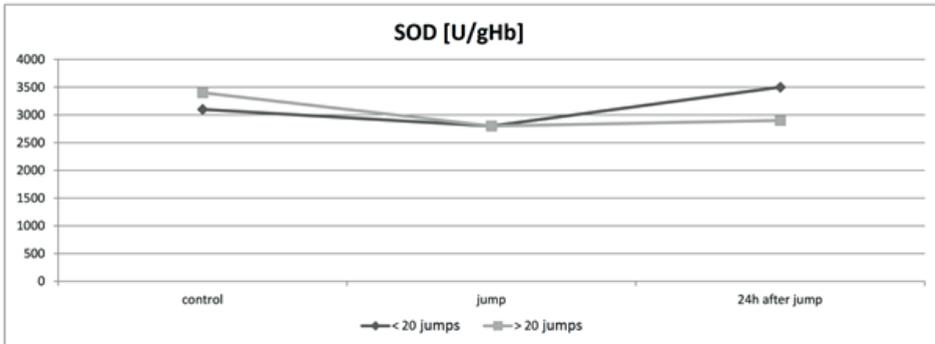


Figure 4. Results of superoxide dismutase activity (SOD) in hemolysate of red blood cells in each measurement depending on the number of jumps

Statistically significant changes in SOD activity were observed:

- 24 h after the jump, depending on the number of jumps (lower values for the group of > 20 jumps and higher for the group of < 20 jumps; $p = 0.0267$);
- in the control measurement, depending on the number of jumps (lower values for the group of < 20 jumps and higher for the group of > 20 jumps; $p = 0.0090$);
- in the group of > 20 jumps (the lowest on the day of the jump and 24 h after the jump and the highest in the control measurement; $p = 0.005$);
- in the group of < 20 jumps (the lowest on the day of the jump, highest in the control measurement and the highest 24 h after the jump; $p < 0.0001$).

Statistical conclusions

The correlation between the experience in performing jumps, the number of jumps and SOD activity was found in the individual measurements.

In measurements performed 24 h after the jump, higher levels of SOD activity were observed in groups with less experience in performing jumps in comparison to the groups of more experienced parachute jumpers.

In measurements performed on an ordinary working day, higher levels of SOD activity were observed in groups with more experience in performing jumps in comparison to the groups of less experienced parachute jumpers.

The lowest values of examined parameter in all groups were found on the day a parachute jump was performed. However, in groups with less experience in performing jumps, the highest SOD activity was found 24 h after the jump, and in groups with more experience – in the control measurement.

Figure 5 shows results of catalase (CAT) activity in hemolysate of red blood cells depending on the group and time of measurement.

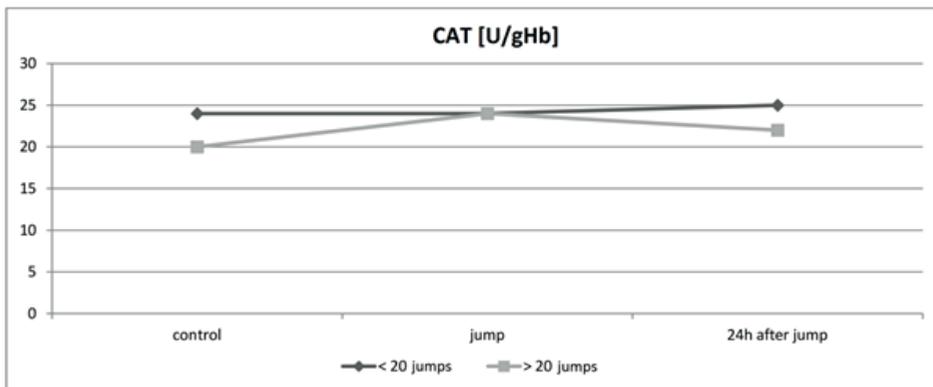


Figure 5. Results of catalase (CAT) activity in hemolysate of red blood cells depending on the group and time of measurement

Statistically significant changes in CAT activity were observed:

- on the day of the jump depending on the group (the lowest in the group of professional soldiers with the parachutist title and the highest in the group during the first season of jumping; $p = 0.0005$);
- 24 h after the jump, depending on the group (the lowest for the group of instructors and the highest in the group performing the first jump; $p = 0.0124$);
- in the control measurement, depending on the group (the lowest in the members of a parachute sport group and the highest in a group of paratroopers performing the first jump; $p = 0.0078$);
- in the group of professional soldiers with the parachutist title (the lowest on the day of the parachute jump and the highest in the control measurement and 24 h after the jump; $p = 0.0211$);
- the group of parachute instructors (the lowest in the control measurement, highest 24 h after the jump and the highest on the day of the parachute jump; $p = 0.0062$);

- in a parachute sport group (the lowest in the control measurement, the highest on the day of the parachute jump and 24 h after the jump; $p = 0.0067$).

Figure 6 shows results of catalase (CAT) activity in hemolysate of red blood cells in each measurement and depending on the number of jumps.

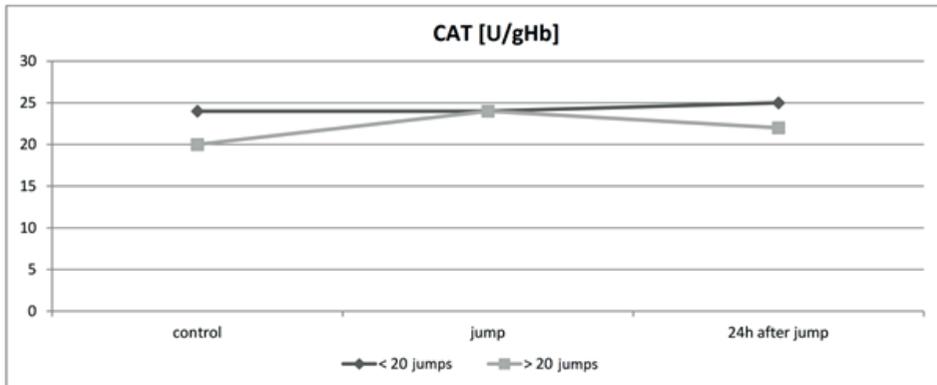


Figure 6. Results of catalase (CAT) activity in hemolysate of red blood cells in each measurement and depending on the number of jumps

Statistically significant changes in CAT activity were found:

- 24 h after the jump, depending on the number of jumps (lower values for the group of > 20 jumps and higher for the group of < 20 jumps; $p = 0.0083$);
- in the control measurement, depending on the number of jumps (lower values for the group of > 20 jumps and higher for the group of < 20 jumps; $p = 0.0015$);
- the group of > 20 jumps (the lowest in the control measurement, higher 24 h after the jump and the highest on the day of the parachute jump; $p = 0.0003$).

Statistical conclusions

The correlation between the experience in performing jumps and the number of jumps and CAT activity was found in the individual measurements.

In measurements performed 24 h after the jump and in the control measurement, higher levels of CAT activity were observed in groups with less experience in performing jumps in comparison to the groups of more experienced parachute jumpers.

At the same time, in groups with more experience in performing jumps, CAT activity was elevated in the control measurement as compared to the day of the parachute jump and 24 h after the jump.

Figure 7 shows results of glutathione peroxidase (GSH-Px) activity in hemolysate of red blood cells depending on the group and time of measurement.

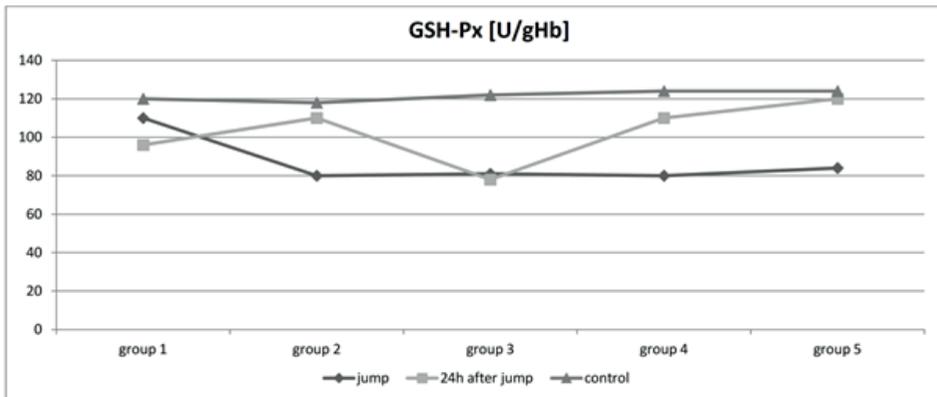


Figure 7. Results of glutathione peroxidase (GSH-Px) activity in hemolysate of red blood cells depending on the group and time of measurement

Statistically significant changes in GSH-Px activity were found:

- in parachute jumpers during their first season of jumping (the lowest on the day of the parachute jump, the highest 24 h after the jump and in the control measurement; $p = 0.0302$);
- in professional soldiers with the parachutist title (the lowest on the day of the parachute jump and 24 h after the jump and the highest in the control measurement; $p = 0.0562$);
- in the group of parachute instructors (the lowest on the day of the parachute jump, higher 24 h after the jump and the highest in the control measurement; $p = 0.0155$).

Figure 8 shows results of glutathione peroxidase (GSH-Px) activity in hemolysate of red blood cells in each measurement and depending on the number of jumps.

Statistically significant changes in GSH-Px activity were found:

- in the group of > 20 jumps (the lowest on the day of the jump, higher 24 h after the jump and the highest in the control measurement; $p = 0.0023$);
- in the group of < 20 jumps (the lowest on the day of the jump, higher 24 h after the jump and the highest in the control measurement; $p < 0.0021$).

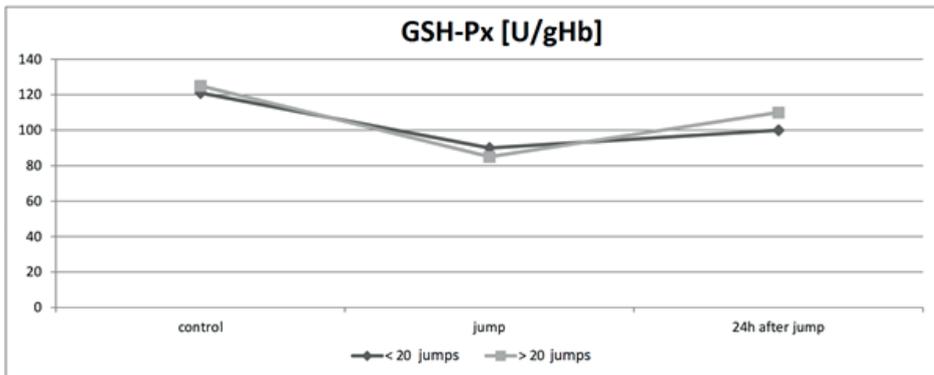


Figure 8. Results of glutathione peroxidase (GSH-Px) in hemolysate of red blood cells activity in each measurement and depending on the number of jumps

Statistical conclusions

No correlation between experience in parachute jumping, the number of jumps and GSH-Px activity in the individual measurements was found.

In all investigated groups, the lowest values of examined parameter were observed on the day of the jump and the highest in the control measurement.

Discussion

Many researchers evaluating the oxidation-reduction balance of the body examine the so-called biomarkers of oxidative stress, among others, the content of lipid peroxidation products, the content of carbonyl groups or thiol groups of proteins and protein activity of antioxidant enzymes [20, 21]. There are no studies on the effects of psychological stress on biomarkers of oxidative stress in healthy people. The most commonly stress was induced in animals, e.g., mice. Romana-Souza et al. [22] reported increased lipid peroxidation and the increase in the content of carbonyl groups of proteins in mice undergoing a 28-day mental stress.

Experimental studies, conducted by other researchers, on mice demonstrated a correlation between the level of anxious behavior and intracellular level of reactive oxygen species in peripheral blood cells [2, 23]. In humans a correlation was observed between the level of anxiety and the quantity of reactive oxygen species in monocytes of patients with arterial hypertension [24]. An increase of oxidative stress parameters was observed in mental illnesses associated with anxiety disorders [25, 26]. These reports pointed to the association between mental stress and anxiety disorders and they suggested the correlation between the level of stress response and red-ox balance parameters.

In our earlier studies we found out that a parachute jump is an important stress stimulus especially for soldiers less experienced in performing parachute jumps. The level of anxiety (stress) in these groups on the day of the jump was higher in relation to the initial results as well as in relation to the results of more experienced parachutists [8]. Taking into account the effect of many factors on the red-ox balance in the soldiers examined by us, a number of determinations of peripheral blood cell parameters, biochemical parameters and the level of acute-phase protein were performed to exclude basic diseases, first of all infections which could have been associated with oxidative stress. Subjects with abnormal results of the above mentioned tests were excluded from further studies. No deviations from laboratory standard were observed in the group of soldiers qualified for the study and thus all of them were exposed to the testing of red-ox balance parameters.

In lipid peroxidation assessment the most frequently applied method is the one based on the reaction of malondialdehyde (MDA) with thiobarbituric acid [27]. Then a colored adduct is generated in the reaction between thiobarbituric acid and some products of lipid peroxidation in acidic conditions, at elevated temperature. This reaction is sensitive but non-specific; apart from malondialdehyde a number of other compounds react with thiobarbituric acid, e.g., bilirubin, sialic acid and glucose degradation products. In our study the TBARS absolute value was not of interest but the possible dynamics of its changes under the effect of anxiety associated with parachute jumps. The analysis of TBARS levels points to the association between the experience in parachute jumping, the number of performed jumps and the level of TBARS in the individual measurements. In the groups less experienced in parachute jumping, a relatively low TBARS level was observed in comparison to more experienced groups. TBARS level was found to be higher in both groups when determined 24 h after the jump.

Furthermore, in our study we also demonstrated that experience in parachute jumping as well as the number of performed jumps affect not only TBARS level but also CAT and SOD activity. Higher levels of CAT and SOD activity were detected in groups with less experience in parachute jumping in relation to the more experienced groups in the measurements performed 24 h after the jump and during the control period. Different profiles of changes in red-ox balance in parachutists can be associated with hormonal changes occurring in them. Among experienced parachutists we observed low initial levels of ACTH and cortisol and a significant mobilization of these hormones in response to stress. In the groups less experienced in parachuting, a slight decrease in the level of ACTH was observed in the determinations performed 24 h after the jump and small cortisol level fluctuations. Perhaps the high reactivity of HPA axis among experienced parachutists is a mechanism developed in response to chronic, repetitive stress stimuli [9].

However, these correlations can be more complex and perhaps further research will bring the explanation of the changes observed by us.

Conclusions

In this research aiming at assessing the effect of anxiety associated with parachute jumps on selected parameters of red-ox balance in regular soldiers:

- there was demonstrated different characteristics of the activity of antioxidant enzymes (CAT and SOD) in response to mental stress depending on the experience of the investigated group in parachuting;
- decrease in GSH–Px activity was demonstrated in response to mental stress in all the investigated groups;
- the effect of stress stimulus, which is parachute jump, was found on TBARS level in all the investigated groups but this level was higher in more experienced parachutists.

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