Survival Training Effects on Oxidative Stress and Muscle Damage Biomarkers of Naval Cadets

Andrzej Tomczak; Ewa Jówko; Paweł Różański

INTRODUCTION: It is important for military commanders to know the extent of fatigue experienced by soldiers undergoing a long-term military training. This knowledge can enable them to determine the level of physical capabilities of soldiers. The present study aimed to evaluate changes in the level of chosen biochemical parameters in blood during the survival training of Polish Naval Academy cadets.

METHODS: Participating voluntarily in the research study were 14 cadets. All subjects were men, ages 23.1 $\pm$ 2.0 yr. During the 36-h survival training, the subjects were deprived of sleep. The following biochemical parameters were assessed in venous blood collected from the cadets: creatine kinase (CK) activity, concentration of lipid hydroperoxides (LOOHs), superoxide dismutase activity (SOD), and glutathione peroxidase activity (GPx).

RESULTS: After 36 h of training a significant increase was observed in CK (from 183.1 up to 530.2 $U \cdot L^{-1}$), LOOHs (from 1.72 up to 3.74 $\mu mol \cdot L^{-1}$), and GPx (from 27.4 up to 36.4 $U \cdot gHb^{-1}$). After 12 h of rest, the level of LOOHs returned to the initial level, GPx activity did not change significantly, and CK activity was significantly higher than those at baseline (422.3 $U \cdot L^{-1}$).

DISCUSSION: The 36-h survival training increased oxidative stress, which contributed to the damage to muscle cells in the group of cadets of the Polish Naval Academy. The intensity of postexercise changes in the level of oxidative damage indicators is dependent on the initial level of enzymatic antioxidant defense. The 12-h recovery proved to be too short to regenerate the damaged muscle tissue.

KEYWORDS: lipid peroxidation, antioxidant capacity, creatine kinase activity, SERE, soldiers.

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The most frequently chosen method to evaluate readiness of soldiers to perform military operations is assessing the level of their overall physical fitness. Each country has its own physical fitness tests and all of them consist of simple trials to evaluate physical fitness (e.g., sit-ups, push-ups, zig-zag run, shuttle run 10 $\times$ 10 m, 3000-m run, and 2-mi run). Tests (trials) can be performed in a sports hall or on a sports fields, in sports clothing, or without any specialized equipment.[10,24,25]

Several investigators have conducted tests (trials) that show how the level of physical fitness changes in the conditions of real soldier training, that is, during field training or long-term military exercises.[3,6,18] The most interesting research studies with a substantial practical implication were those conducted during Survival, Evasion, Resistance, Escape (SERE) trainings or survival trainings. SERE practical trainings are characterized by their long period of time and performing military operations under the influence of stress, emotions, time pressure, and considerable physical efforts. During such trainings, soldiers can find themselves in the role of a captive, an interrogated person, or a fugitive who fled from the place of detention. It is generally known that SERE trainings are targeted at soldiers of land forces and military pilots.[2,15,23] It is related to essential tasks assigned to soldiers, which are conducted on the land, whereas for military pilots, it pertains to a situation after the aircraft is shot

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down and emergency landing occurs in the enemy territory or within occupied boundaries. Nevertheless, incidents regarding armed conflicts can be unpredictable to such an extent that seamen can find it useful to know how to survive on the land according to the SERE procedures. Hence, it is fully justified to conduct the training in the Navy.

Literature on this subject revealed that research studies conducted on the course of SERE training were concerned with changes in psychological, physiological, fitness, and efficiency-related aspects.[11,14,27] The novelty of this research on changes in fitness and efficiency during military operations specifically related to school of survival or SERE is tests linked to coordination motor abilities. These tests were conducted in various groups of soldiers and civilians.[23,24,26] The studies demonstrated varying degrees of disturbance of coordination motor abilities due to a long-term moderate physical effort combined with sleep deprivation or some limitation of sleep. A certain regularity probably exists that encompasses a lower level of performing tasks when larger groups of muscles are involved (run motor adaptation; maximum strength of forearm muscles, and rotational test). On the other hand, while performing tasks that require precision of action, those tasks are deteriorated to a lesser degree or at times tend to increase the analyzed values. Situations of this kind were observed in psychomotor tests, such as the multiple choice reaction time test (MCRT), as these tests generally lack any impact of long-term moderate physical efforts on the response time change.[9,23,26]

Biochemical tests are another method of evaluating the influence of military training on soldiers and are very scarce. Tests conducted in the army are modeled on observations made in athletes (the research procedure). There are many similarities between preparing athletes to undertake physical efforts and preparing soldiers for certain tasks.[5,8] During an intense military training, the disturbance of the physiological balance between the production of reactive oxygen forms and their inactivation is noted. An increased production of free radicals leads to damage of basic cellular structures (lipids, proteins, and DNA).[28] Consequently, it causes development of inflammatory processes and dysfunctions of various organs.[7,17,21]

Biomarkers related to oxidative stress have been used to assess the influence of some physical efforts on an organism. The blood concentration of lipid hydroperoxides was assessed as an indicator of lipid peroxidation, along with the activity of superoxide dismutase and glutathione peroxidase as representatives of the enzymatic antioxidant system in the blood, while the activity of creatine kinase in the blood was used as a marker of muscle cell damage.[4]

In other studies, the following biochemical markers were assessed: cortisol, DHEA-s, neuropeptide Y (NPY), testosterone,[11] glycerol, glucose, free fatty acids, growth hormone, insulin growth factor 1 (IGF-1), and IGF-binding protein 3.[9] During the military survival training, Szivak et al. assessed the biochemical markers epinephrine, norepinephrine, dopamine, cortisol, testosterone, and NPY, and they showed statistically significant changes in these parameters after some physical effort (excluding NPY).[19] Lieberman et al. assessed the markers cortisol, testosterone, NPY, and DHEA-s during SERE training and showed a substantial increase in the level of these biomarkers.[11]

As mentioned above, SERE training or survival training should involve all soldiers regardless of their military specialty. Thus, the present study aimed to evaluate the level of chosen biochemical parameters in the blood of Polish Naval Academy cadets subjected to survival training. During this training, the examined cadets were deprived of rest in the form of sleep.

METHODS

Subjects

Participating voluntarily in the research study were 14 cadets of the Polish Naval Academy. All the subjects were men, ages 23.1 $\pm$ 2.0 (20–27) yr, and with a military service period of 2.1 $\pm$ 1.5 (1–5) yr. The average body height of the surveyed soldiers was 179.9 $\pm$ 4.6 (174–190) cm, with an average body mass of 79.3 $\pm$ 6.3 (71–94) kg and BMI of 24.7 $kg \cdot m^{-2}$. The research protocol was approved by the Senate Scientific Research Committee of the Józef Piłsudski University of Physical Education in Warsaw (Approval No.: SKE 01-16/2014). The surveyed subjects provided written consent to participate in the research.

Design

During the 36-h survival training, the subjects were deprived of sleep. In total, during the training and research, the cadets covered 30 km on foot (18 km on the first day and 12 km on the second day) and simultaneously performed tasks: first aid in the battlefield, building makeshift military camps, water crossing to the enemy base, topography, marching to the azimuth, operations in the recon team, and conducting observations. The average air temperature during the training ranged from 1°C to 4°C, with continuous but moderate rain.

Throughout the experimental period, all participants received the same meals and had unlimited access to mineral water. The main meals were consumed at 07:00, 13:00, and 19:00. The daily energy intake amounted to 3800 kcal, with protein, fat, and carbohydrates contributing to 14%, 30%, and 56% of dietary energy intake, respectively.

Procedure

Blood samples from an ulnar vein were collected into EDTA tubes four times from every solider: prior to the participation in training (M1), at 24 h of training (M2), after completing 36 h of training (M3), and after 12 h of recovery (M4). Blood collection before training, at 24 h of training, and after 12 h of recovery was performed in the morning: from 06:00 to 06:30, whereas blood collection after 36 h of training was performed in the evening: from 18:00 to 18:30. Blood collection before training and after 12 h of recovery was performed on an empty stomach (after rest at night and after at least 10 h from the last meal), whereas blood collection after 24 and 36 h of training was performed after at least 5–6 h from the last meal because during the training soldiers were provided with light meals, which

were the same for everyone (they also had constant access to mineral water in unlimited quantities).

Venous blood samples were drawn into heparinized test tubes and then centrifuged (for 10 min at 3000 × g at 4°C) to separate erythrocytes and plasma. Subsequently, the erythrocytes were washed three times with a cold isotonic saline solution. Erythrocytes, plasma, and whole blood were frozen and stored at −80°C.

The following markers were assessed in venous blood collected from soldiers:

- in plasma: creatine kinase (CK) activity—the index of muscle cell damage—and concentration of lipid hydroperoxides (LOOH)—the lipid peroxidation index;
- in red blood cells (after the centrifugation of whole blood and washing of erythrocytes with NaCl solution): superoxide dismutase activity (SOD); and
- in whole blood: glutathione peroxidase activity (GPx).

The CK activity in plasma was assessed with a kinetic method using a kit designed by the Alpha Diagnostics company (Warsaw, Poland). Diagnostic kits from Randox (Crumlin, United Kingdom) were used to measure the parameters of enzymatic antioxidants in the blood, i.e., the activity of SOD and GPx, which were expressed as units per gram hemoglobin ($U \cdot gHb^{-1}$). The concentration of hemoglobin was also measured using the Randox Diagnostic kit. The concentration of LOOHs in plasma was assessed using a kit provided by Oxis Research (Portland, OR, USA).

Statistical Analysis

The obtained results were analyzed with a univariate analysis of variance and the post hoc NIR test (using the Statistica 10.0 program). The level of statistical significance was set at $P < 0.05$.

RESULTS

The results of the study are presented in **Table I**. A significant increase in the activity of CK in plasma was noted after 24 h (as compared to baseline) and it remained at a considerably higher level after 36 h of survival training (595.8 $U \cdot L^{-1}$ and 530.2 $U \cdot L^{-1}$, respectively; $P < 0.00001$). After 12 h of recovery, CK activity decreased significantly (422.3 $U \cdot L^{-1}$) as compared to that at 24 and 36 h of training. Nonetheless, despite this decrease after 12 h of recovery, the CK level remained significantly higher than that before training (422.3 vs. 181.1 $U \cdot L^{-1}$, respectively; $P < 0.00001$).

Similar to CK activity, the training caused a substantial increase in the LOOHs level in plasma. The level after 24 and 36 h of training was considerably higher than that before training (3.45 $\mu mol \cdot L^{-1}$ and 3.74 $\mu mol \cdot L^{-1}$ vs. 1.72 $\mu mol \cdot L^{-1}$, respectively; $P < 0.0001$). During the 12-h recovery period, the LOOHs level returned to the pretraining level.

An increase in SOD activity which was significant compared to the initial value (prior to the training) was noted after the 12-h recovery period. GPx activity significantly changed due to training. An increase in this parameter was observed after 36 h of training as compared to the initial value (36.4 $U \cdot gHb^{-1}$ vs. 27.4 $U \cdot gHb^{-1}$, respectively; $P < 0.005$).

DISCUSSION

The cadets of the Polish Naval Academy constitute a group of soldiers who were selected to serve at sea after the multistage medical and physical efficiency examination. As their education in the Polish Naval Academy proceeded, they had to participate in obligatory physical education classes at least 6 h a week. These classes offered various physical activities, which included team games, field athletics, gymnastics, swimming, and close combat. Thus it can be established that cadets of the Polish Naval Academy are healthier and more physically fit than most members of the general public.

On the basis of the results of the present study, it was concluded that the survival training made an immense impact on the physical load of the cadets from the Polish Naval Academy. The results of creatine kinase indicate damage to muscle cells. During the initial test, the CK level was normal, whereas it gradually increased during training. Even the 12-h recovery period did not cause the marker to return to its initial values that are recognized as a standard value for men. In our previous study,[4] which was also conducted during the survival training for civilians that lasted for 36 h, the increase in plasma CK activity was also noted after 24 and 36 h of training. However,

Table I. Changes in the Concentration of Chosen Biochemical Indicators Under the Influence of 36 h of Survival Training of Polish Naval Academy Cadets ($N = 14$).

	MARKED INDICATORS			
PARAMETER	**CK [$U \cdot L^{-1}$] [df = 52]**	**LOOHs [$\mu mol \cdot L^{-1}$] [df = 52]**	**SOD [$U \cdot gHb^{-1}$] [df = 52]**	**GPx [$U \cdot gHb^{-1}$] [df = 52]**
Before (M1)	183.1 ± 61.2^{a}	1.72 ± 0.67^{d}	1158.1 ± 140.7^{f}	27.4 ± 7.9^{h}
After 24 h (M2)	595.8 ± 121.9^{b}	3.45 ± 1.17^{e}	1238.3 ± 330.8^{f,g}	31.6 ± 8.1^{h,i}
After 36 h (M3)	530.2 ± 110.9^{b}	3.74 ± 1.08^{e}	1281.0 ± 147.7^{f,g}	36.4 ± 15.7^{i}
After 12 h of recovery (M4)	422.3 ± 144.9^{c}	2.08 ± 0.50^{d}	1366.4 ± 101.5^{g}	34.4 ± 11.1^{h,j}
Main time effect	$P < 0.00001$	$P < 0.00001$	$P < 0.05$	$P < 0.05$

The table shows mean values ± SD.

CK: creatine kinase; LOOHS: lipid hydroperoxides; SOD: superoxide dismutase activity; GPx: glutathione peroxidase activity.

$^{a–i}$ Differences in values of the analyzed parameter in the group (one-way ANOVA). Values (at different time points) in a given parameter which do not have a mutual letter differ significantly ($P < 0.05$).

^{a,b,c} Significant differences at $P < 0.0001$; ^{d,e} significant differences at $P < 0.0001$; ^{f,g} significant differences at $P < 0.01$; ^{h,j} significant differences at $P < 0.05$.

after the 12-h recovery period, the CK level dropped even below the initial level.[4] This difference in the behavior of CK activity can be explained by the different characteristics of load during exercises linked to performing survival tasks and by varying atmospheric conditions. During the training of the Polish Naval Academy cadets, it was cold and there was a constant rain; these conditions could reduce the tolerance of a body to physical stress and disturb biochemical parameters.

Another marker measured in the current study was LOOHs. It indicates the level of free radical damage to lipids and, consequently, it shows the degree of oxidative stress. Statistically significant differences were observed between the first and the subsequent measurements during the training; that is, a substantial increase in the level of this parameter in plasma proves that free radical damage became more severe during the survival training. The 12-h recovery period caused a considerable decrease in the LOOHs value, which exceeded the initial level only by a small degree. This indicates that the 12-h recovery is sufficient to reduce the high level of oxidative stress which was caused by the 36-h survival training with moderate physical load.

Comparison of the results obtained for the group of the Polish Naval Academy cadets in the current study and those obtained for civilians in our previous study[4] clarifies the difference in the test result for SOD. The cadets had a markedly lower initial level of this biomarker than civilians and, while SOD decreased under the influence of training in civilians,[4] it increased in the cadets. The increase in SOD activity in this group in the current study proves that there was an enhanced production of oxygen free radicals, particularly superoxide anion radicals, considering that SOD is the first line of defense against the harmful effect of free radicals and catalyzes the reaction of dismutation of this free radical form to hydrogen peroxide.[7] A similar observation was noted in the course of GPx changes when the cadets were compared to the group of civilians. The cadet group showed increased GPx activity after 36 h of training. Conceivably, this change might result from the low initial level of this enzyme (which was at the lowest limit of the normal); this change was indication of a low level of adaptation to effort and low protection against free radicals. The increase in the postexercise GPx activity in the cadet group indicates an increased production of hydrogen peroxide, because this reactive oxygen form is the substrate in a reaction catalyzed by GPx. This fact explains that under the influence of the survival training, the cadet group showed higher increases in markers of free radical damage (LOOHs) and muscle damage (CK) than the civilian group.[4]

The results of biochemical markers for the cadets can be compared to those of athletes practicing aerobic sports. For example, long-lasting physical exertion in an ultra-marathon is, to some extent, comparable to the fatigue experienced in survival training. This exertion is particularly related to changes in the lipid hydroperoxide marker (LOOHs). Mastaloudis et al.[12] reported a 43% increase in F2-isoprostanes (another marker of lipid peroxidation) after a 50-km ultramarathon. But these levels returned to the baseline by 24 h of recovery.

Oxidative stress biomarkers were also studied while playing judo, CrossFit, and a handball game and after 24–48 h of recovery.[12,20,22] From a military point of view, it is interesting to investigate these biomarkers because in close contact combat, which may occur while the soldier is in isolation, these military activities can have a similar energy structure. The authors stated that after the game and during 24 h of recovery, the concentration of all oxidative stress indices changed significantly, which indicates increased oxidative stress in the blood and erythrocytes.[12] Muscle damage indices also increased significantly after the exercise.

A review of articles on this subject revealed biochemical indicators that can be possibly used to monitor training in soldiers. It is worth mentioning the studies which investigated reliable indicators of changes that may occur during survival training. During military survival training that lasted for 2 wk, Szivak et al. conducted physical fitness and biochemical tests.[19] They measured the maximum strength of the hand, the explosive force (the leap), and the blood levels of epinephrine, norepinephrine, dopamine, cortisol, testosterone, and NPY markers.

Epinephrine is defined as a hormone whose level increases with emotions associated with "fight or flight" situations.[1,16] The increased secretion of norepinephrine occurs in stressful and dangerous situations. Dopamine secretion is related to the body's motor control for alertness and attention, whereas cortisol is a stress hormone and is related to adrenaline levels. The impact of the 24-h recovery on changes in the levels of the abovementioned indicators was also determined.[1,16,19]

In the abovementioned studies, the survival school training in men resulted in a significant increase in plasma epinephrine, norepinephrine, and dopamine levels as well as in serum cortisol concentration. No significant changes in plasma NPY level were observed after survival training; however, the NPY level significantly reduced after the recovery period. Generally, the results of these studies show that a long-term survival training causes bodily stress reactions as evidenced by the release of stress hormones.[11] Chicharro et al. discussed the free testosterone/cortisol ratio (FTCR), which might be used to monitor exercise training in military units in order to prevent overtraining.[3] Moreover, considering the finding of our study, it seems that it would be beneficial for the army to determine a group of biomarkers that to the greatest extent could reflect the level of oxidative stress related to the SERE/survival training. Additionally, similar to the research conducted on athletes by Nishimaki et al.,[16] further research on military soldiers should be conducted to understand the effect of weight loss, dehydration, oxidative stress, and sleep deprivation on their physical performance.

The current study did not include tests performed on a control group. However, the authors have previously conducted studies with a control group with a similar course of training and physical load (duration, sleep deprivation, type of classes), which were carried out in a group of civilians. In these studies performed in the control group, no significant differences were observed in the course of changes in oxidative stress and muscle damage biomarkers.[4]

The 36-h survival training enhanced oxidative stress, which contributed to the damage to muscle cells in the group of cadets of the Polish Naval Academy. The intensity of post-exercise changes in the level of oxidative damage indicators is dependent on the initial level of enzymatic antioxidant defense.

The low resting level of GPx and SOD in the cadet group indicates the low adaptation of muscle tissue to physical efforts as well as low defense against the detrimental effects of oxygen free radicals. This finding can serve as an explanation for a considerable increase in the indicators of oxidative stress (LOOHs), enzymatic antioxidant defense (SOD and GPx), and muscle damage (CK), which were observed in this group under the influence of the training. Therefore the 12-h recovery proved to be too short to regenerate damaged muscle tissue.

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