Original Article

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Antioxidative system capacity after a 10-day-long intensive training course and one-month-long recovery in military cadets

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Abstract

Optimization of training and minimization of injuries are topical for the physical performance of military personnel. Physical and psycho-emotional load, fatigue, sleep deprivation, and dietary limits can lead to the development of oxidative stress (OS) and injuries in specific military training. This study investigated markers of OS and muscle damage in military cadets after a 10-day-long intensive training course and a one-month-long recovery. The sample included 42 cadets (2 females and 40 males) aged from 22 till 34. Myoglobin, catalase activity (CAT), superoxide dismutase activity (SOD), and total antioxidants capacity (TAC) in plasma were measured. OS was assessed by the glutathione index. The results revealed an increasing level of myoglobin, increasing glutathione index, and no changes in CAT, SOD, and TAC during the intensive training course. After the one-month-long recovery, myoglobin was back to normal, the activity of CAT and TAC was higher than before and after the training course, while SOD did not change after the recovery. The glutathione index decreased after the one-month-long recovery, but it was not reached the initial level before the intensive training. In sum, the observed grade of OS positively affected the capacity of the antioxidative system with some sign of a need for a longer rest.

Keywords: recovery, myoglobin, oxidative stress, SOD, CAT, TAC

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INTRODUCTION

Optimization of training adaptations and minimization of training-related injuries and overload are aiming at enhancing the physical performance of military personnel. Based on a review of Kyröläinen et al. [1], training programs must be well planned, and the total training load must increase progressively, including sufficient recovery periods. In addition, some individualization of programs is required to avoid unnecessary injuries and overloading because of the variability of the initial physical fitness of soldiers [1]. Training load, combined with external stress factors, can lead to overreaching and overtraining associated with increasing musculoskeletal injury rates [2]. It forms a topical health problem for military personnel in different countries [3-5]. Specific military surrounding contains the risk factors such as high physical load, psycho-emotional stress, fatigue, sleep deprivation, and dietary limits [6]. Detection of oxidative stress markers in blood provides significant information for the assessment of physical load intensity and successful recovery [7].

During metabolic processes, oxidative reactions occur in each live cell that leads to the formation of free radicals (e.g., reactive oxygen, nitrogen, carbonyl, sulfur), including skeletal muscle during the rest and contractile activity [8]. The antioxidative system includes enzymatic and non-enzymatic proteins, reducing the concentration of oxidants. In accordance with Sies [9], an imbalance between oxidants and antioxidants in favor of the oxidants is termed oxidative stress (OS), which potentially leads to cell damage. OS is a key mechanism in aging processes [10] and a risk factor for various pathologies, including oncological diseases [11]. The negative effect of OS is related to his intensity and duration [12]. Simultaneously, there is evidence that free radicals are not only agents of cell damage, but they are necessary for different processes, as myogenic regeneration after acute muscular injuries [8].

Increasing levels of oxidants associate more frequently with pathophysiological conditions [9], but they can be also detected during physical load [13-17], sleep deprivation [18], and military training [19]. The studies [7,20] show that increasing OS markers associate with changes in antioxidative system activity and correlate with the physical load level during training. Regular and optimal (low- and mild-intensive) physical load favors the increasing concentration of free radicals, stimulating the antioxidative system. This stimulation results in increased stress resistance and diminishes the risk of pathological conditions [15,21-23].

Lushchak [12] divided OS into four ranges: basal, low-intensity, intermediate, and high-intensity OS. In the first phase of low-intensity OS, the activity of antioxidant or associated enzymes up-regulated, but in the second phase, they are normalized to the level of no observable effect. In the intermediate OS, the level of oxidants is rather high, whereas antioxidants are decreasing. Finally, in the high-intensity OS, both functions reach some plateau. Oxidants reach it at their maximum when antioxidants at their minimum [12].

In military training, physical load intensity can be higher and longer than in a standard training course. As a result, the recovery period can be an important factor for minimizing the negative effect of OS and preventing musculoskeletal injuries. For example, a study on the effect of 36-hours of physical activity with sleep deprivation in young physically active men indicated that a 12-hours recovery was sufficient for the normalization of oxidative and muscle damage markers and restoration of blood prooxidant-antioxidant homeostasis [18]. In the case of prolonged activity, stress and fatigue have a cumulative effect on biochemical parameters of blood [24,25]. Our study investigated markers of oxidative stress and muscle damage in military cadets after a 10-day-long intensive training course and a one-month-long recovery.

MATERIALS AND METHODS

The research sample included 42 cadets – 2 females and 40 males – aged from 22 till 34 (mean age 24.7, SD = 2.9 years). They passed a medical examination of health status. All participants were informed about the examination protocol and gave their written consent to participate in the study. The study protocol was approved by the National Defence Academy of Latvia and the Ethical Committee of Rīga Stradiņš University (Latvia).

Combat training course (CTC) with high physical load exercises took place in summer in Latvia when daily outdoor temperatures ranged from +8 ºC to +20 ºC, with an average of + 15 ºC (data from the local weather station). The overall CTC was set according to the study program of the National Defence Academy of Latvia. The CTC schedule for 10 days included various activities: aerobic physical load, marching exercises in full military equipment, overnight field exercises, and tactical exercises. The average intensity of physical activity in the daily program was planned as moderate on the first day, increased gradually during the next days, and slang down till the end of CTC (10^{th} day). Cadets had sleep deprivation spending all CTC time in outdoor activities. As well they had dietary limits for meals (one intake daily from 6 p.m. to 7 p.m). The water intake was not restricted. Before CTC, cadets underwent the six-month-long pre-intervention phase included military tactical preparation training. During CTC, cadets participated in tactical exercises in the military surrounding. The average distance per day was 25 km. Participants had tactical military tasks in full military equipment (20-25 kg). The target of military training was to improve military performance during tactical exercises, training the skills, and getting military knowledge.

Participants underwent measurements at three time points: before the CTC, right after it, and after the one-month-long rest period. At each time point, peripheral blood (5 ml) was collected in blood collection tubes with EDTA. Blood samples were centrifuged at 13 000 rpm and +4 ºC for 15 minutes, plasma was separated and stored before the detection at -80 ºC. All blood samples were collected in the morning between 7 and 9 o'clock following an overnight fast.

The assessment of possible muscle damage was performed using the detection of the myoglobin concentration level in blood. The antioxidative system activity was investigated by detection of catalase activity (CAT), superoxide dismutase activity (SOD), and total antioxidants capacity (TAC) in plasma. OS was assessed by changes in the glutathione index. Myoglobin was measured by ELISA method (Myoglobin ELISA kit, Sigma-Aldrich, USA), and the results were expressed in ng/ml. CAT activity (Catalase Activity Assay kit, Abcam), SOD activity (SOD determination kit, Sigma-Aldrich, USA), TAC (Total Anti-oxidant Capacity Assay kit, Sigma-Aldrich, USA), and oxidized (GSSH) and reduced (GSH) glutathione (Quantification kit for oxidized and reduced glutathione, Sigma-Aldrich, USA) were detected by the colorimetric method. Absorbance at 490 nm was used for the measurement of CAT and TAC, absorbance at 450 nm was used for SOD, and absorbance at 405 nm was used for the measurements of GSSH and GSH. All measurements were performed following the manufacturer's instructions. Glutathione index was calculated as a ratio of oxidized (GSSH) to reduced glutathione (GSH).

Statistical analyses were performed by R-package 'nparLD' 2.1 [25]. A model of three repeated measures – Time 1, 2, and 3 – was applied for the analysis of the dynamics of markers. ANOVA-Type Statistics (ATS) was applied for the assessment of a longitudinal change. After revealing the effect of time, the Bonferroni post-hoc test was applied for multiple paired comparisons [26].

RESULTS

As a marker of body composition, cadets' BMI decreased significantly after CTC from 24.8±2.4 to 24.5±2.0, $z(41) = 2.80$, $p = .005$. It confirmed the significance of CTC-associated limitations.

A model for the repeated measures in a homogenous group of participants ('ld.f1' in terms of Noguchi et al. [23]) revealed significant changes in all but one marker of oxidative stress and muscle damage (Table 1). The level of myoglobin was relatively high at the baseline and after the training course (Figure 1). There were no outliers after the 10-day-long training course. Finally, myoglobin decreased significantly after the one-month-long recovery with only a few cases higher than zero. The level of CAT followed a different trend (Figure 2). It remained relatively unchanging after the training course (with some outliers with higher level of catalase) and visibly increased after the recovery. SOD remained relatively stable during the training course and after the recovery (Figure 3). It was a single marker unchanged during the study (Table 1).

TAC demonstrated no significant change during the training course and an increase after the one-month-long recovery (Figure 4). The interquartile range of the total antioxidant capacity was the highest after the training course, indicating high variability in this marker. Later, the recovery resulted in an increase of the capacity, compared to the measure before the special training course.

Table 1. Markers of oxidative stress and muscle damage before and after the intensive training course and after the one-month-long recovery (N = 42).

Marker	Time 1 (Before)	Time 2 (After)	Time 3 (Recovery)	ANOVA-Type
	Median (IQR)	Median (IQR)	Median (IQR)	Statistics
Myoglobin, ng/ml	35.75[a] (16.95 – 61.28)	59.49[a] (25.84 – 108.89)	0.00[b] (0.00 – 0.00)	166.24***
CAT, absorbance at 490 nm	0.07[a] (0.06 – 0.09)	0.07[a] (0.06 – 0.13)	0.39[b] (0.16 – 0.78)	148.98***
SOD, absorbance at 450 nm	0.65 (0.61 – 0.72)	0.68 (0.58 – 0.73)	0.68 (0.59 – 0.71)	0.04
TAC, absorbance at 490 nm	1.08[a] (1.04 – 1.11)	1.08[a] (1.05 – 1.23)	1.14[b] (1.10 – 1.21)	11.46***
Glutathione ratio	0.84[a] (0.76 – 1.00)	1.14[b] (0.96 – 1.24)	0.97[c] (0.91 – 1.04)	14.71***

Notes: CAT – catalase, SOD – superoxide dismutase, TAC – total antioxidant capacity, IQR – Interquartile range. Different superscripts indicate significant differences among measures in time.
*** $p < 0.001$.

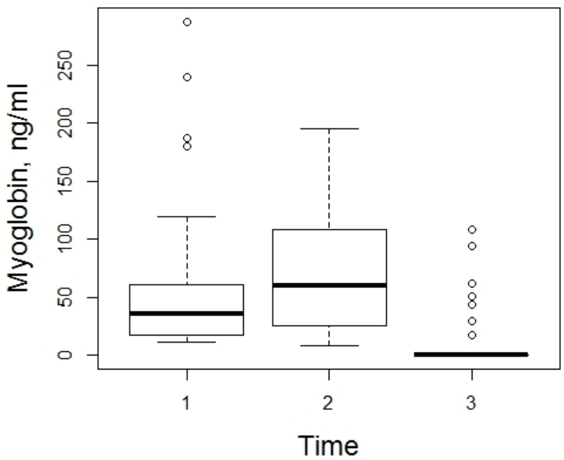

Figure 1. Level of myoglobin before (1) and after (2) the training course and after one-month-long recovery (3).

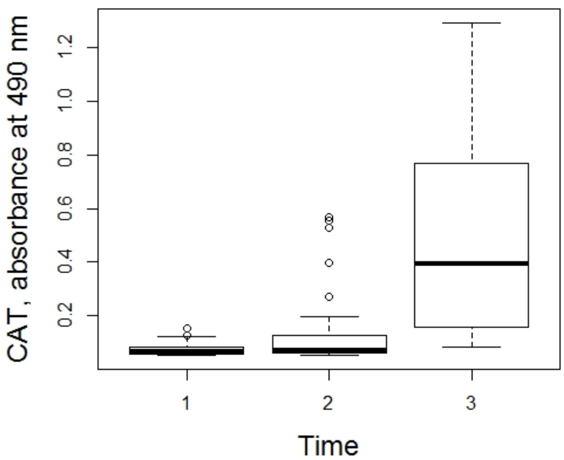

Figure 2. Level of CAT before (1) and after (2) the training course and after the one-month-long recovery (3).

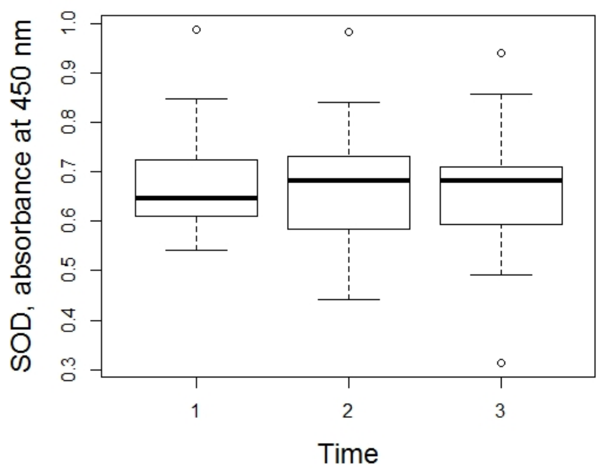

Figure 3. Level of SOD before (1) and after (2) the training course and after the one-month-long recovery (3).

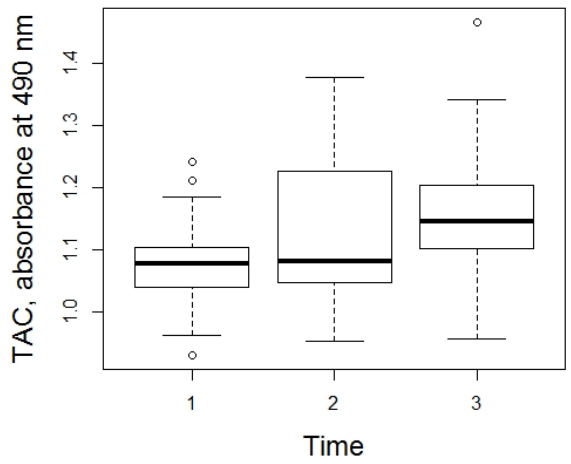

Figure 4. Level of TAC before (1) and after (2) the training course and after the one-month-long recovery (3).

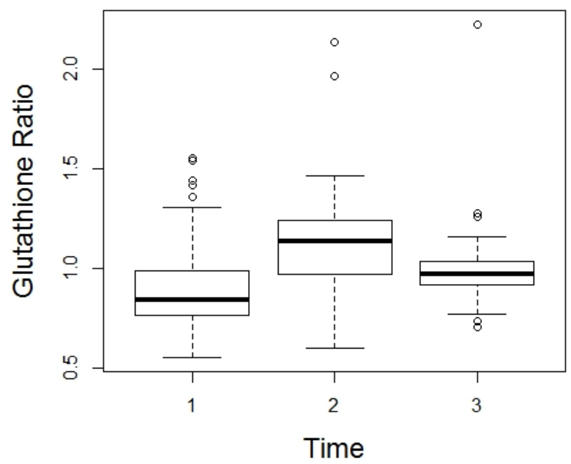

Figure 5. Glutathione ratio before (1) and after (2) the training course and after the one-month-long recovery (3).

Glutathione ratio increased significantly during the training course (Figure 5). Its decrease after the recovery was also significant. However, the ratio after the recovery remained relatively higher that before the training course.

DISCUSSION

The results revealed the increased level of myoglobin before and after the intensive training, which normalized after the recovery period. It indicated the skeletal muscle damage before the intensive physical load, which, probably, cumulated during cadets' basic training. Intensive physical training added to observed muscle damage. Previous studies focused on the investigation of serum myoglobin in shorter time intervals during high physical load [27,28] and demonstrated increasing of myoglobin within 30 min, which remained relatively high 5 days after exertion. Our study analyzed the one-month-long recovery. Normalizing of the level of myoglobin was expected, and some outliers could be associated with a continuous physical load within the period of recovery.

During the intensive training course, two parameters of the enzymatic proteins (CAT, SOD) and one parameter of the non-enzymatic proteins of the antioxidative system (TAC) did not change. After the recovery period, the activity of CAT and TAC was higher than before and after the training course, while SOD was changed nor after the training neither after the recovery. It concurs with a study on runners' recovery [28] when trained athletes demonstrated elevated activity of CAT during shorter recovery periods after a 21-km run. At the same time, SOD activity did not change throughout the recovery phase up to 24 hours [28]. Similar stability of SOD was observed during the dual (physical and mental) stress and after one-hour recovery in young healthy, trained men [16]. Our results support these findings even after a one-month-long recovery period.

Observed dynamics of TAC could be assessed regarding the findings of Margonis et al. [7], who focused on the investigation of OS biomarkers during 12 weeks of resistance training with a phase of overtraining. TAC demonstrated a rapid decrease associated with the overtraining and an increase when the load is decreasing. In our study, unchanged TAC level after the intensive training course did not reveal overtraining under the progressive load in ten days. Observed leverage of TAC after one month indicated a recovery in cadets that is also confirmed by changes in CAT. Simultaneously, relatively higher variability of TAC after the training course indicated possible differences in individual trajectories of change.

OS index – the glutathione index – showed a tendency similar to changes in myoglobin concentration. It increased during the intensive training course and decreased after the one-month recovery. However, the level observed before the intensive training was not reached. In a study by Margonis et al. [7], glutathione index indicated overtraining and normalized in 96 hours after the last exercise course. Based on a positive correlation between the glutathione index and training severity [7], we can conclude that its increase during the intensive training course can be associated with signs of overtraining.

Following Lushchak [12], increasing OS index during the training course and no change in markers of the antioxidative system during this course can be classified as low-intensity OS [12]. In our study, this grade of OS positively affected the capacity of the antioxidative system in cadets and caused an increase in these parameters during the recovery period. At the same time, a higher level of OS index after the recovery than before the intensive training course indicated a possible need for a longer period of rest. The further exploration of this change requires greater attention to individual differences in cadets' physical conditions [1]. The complex effect of overtraining, as it was explored by Tanskanen et al. [19], also remains a question for further research.

Undoubtedly, our study has several limitations. Measurement of three antioxidative markers is limited by the level of absorbance and did not present the level of their concentration in plasma. Military cadets presented a sub-population of healthy and specifically trained people. It included predominantly males (95%) and was not useful for the assessment of gender differences in OS during the physical load and recovery, pointed by Balci [17]. Therefore, generalization of our findings to other samples and conditions seems restricted because of possible effects of age, sex, training history, recovery, sleep and nutrition, as well as environmental, psychological, and social factors.

CONCLUSIONS

The results revealed the increased level of muscle damage and OS during the intensive training and no changes in three parameters of antioxidative system (CAT, SOD, and TAC). These findings can be classified as low-intensity OS. This grade of OS positively affected the capacity of the antioxidative system in cadets and caused an increase in CAT and SOD during the recovery period. Despite no signs of muscle damage after the recovery, a higher level of OS index after the recovery than before the intensive training course indicated a possible need for a longer period of rest. The further exploration of this change requires greater attention to individual differences in cadets' physical conditions.

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