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Association of military training with oxidative stress and overreaching

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ABSTRACT

We hypothesized that increased oxidative stress and disrupted redox balance may be predisposing factors and markers for overreaching (OR). **Purpose:** To examine whether oxidative stress markers and antioxidant status and physical fitness are related to overreaching during an 8-week military basic training (BT) period. **Methods:** Oxidative stress and antioxidant status were evaluated in the beginning, after 4 and 7 weeks of training in 35 males (age 19.7 ± 0.3 yrs) at rest and immediately after a 45-min submaximal exercise. Physical activity (PA) was monitored by accelerometer throughout BT. Indicators of OR were also examined. **Results:** From baseline to wk4 increased daytime moderate to vigorous PA led to concomitant decreases in the ratio oxidized to total glutathione (GSSG/TGSH) and GSSG. After 4 weeks of BT, GSSG/TGSH and GSSG returned to the baseline values at rest, while PA remained unchanged. At every time point acute exercise decreased TGSH and increased GSSG and GSSG/TGSH, while a decrease was observed in antioxidant capacity after 4 weeks of training. In the beginning of BT, OR subjects (11 of the 35 males) had higher GSSG, GSSG/TGSH and malondialdehyde (a marker of lipid peroxidation) at rest ($P < 0.01-0.05$) and lower response of GSSG and GSSG/TGSH ratio ($P < 0.01$) to exercise than noOR subjects. Moreover, OR subjects had higher PA during BT than noOR ($P < 0.05$). **Conclusion:** The sustained training load during the last four weeks of BT led to oxidative stress observable both at rest and after submaximal exercise. Increased oxidative stress may be a marker of insufficient recovery leading possibly to OR.

Keywords: training load; physical activity; exercise testing; military; male; young adult

INTRODUCTION

Paragraph 1. In competitive sports and military training the goal of the training is to improve performance. However, both prolonged aerobic and strength training with high intensity or volume together with inadequate recovery could lead to non-functional overreaching (OR) resulting in stagnation or decrements in performance capacity. (16). OR could also lead to the development of OTS, both of which are characterized by an unexpected decrease or at least stagnation in performance despite increased or sustained training load. The time required for the recovery from OR takes several days to several weeks, whereas the development of OTS and recovery from it may take much longer time.

Paragraph 2. Several factors and symptoms have been linked to OTS. However, there are no reliable and specific markers of impending OTS, nor knowledge on how to identify individuals who are more vulnerable to OR. Moreover, the precise mechanisms leading to OR and OTS have not yet been delineated. Recently, impaired antioxidant capacity and increased oxidative stress, a state in which the production of reactive oxygen species (ROS) overwhelms antioxidant defenses, was found to be related to OTS in elite athletes (28). Animal (18, 36) and human studies have provided evidence that a progressively increased training volume, which causes symptoms of OR or OTS, can increase oxidative stress and attenuate antioxidant capacity (15, 35). While a single bout of physical exercise of sufficient intensity and duration generate ROS, both aerobic (21) and anaerobic training (2) have been shown to enhance antioxidant status and decrease the generation of ROS. In contrast, overload training can lead to an impaired antioxidant defense and lack of expected adaptations to training (25), and distortion of the redox balance (8). Extreme increases in training volumes may lead to substantial rise in inflammation

and apoptosis markers (7). Therefore, overload training may induce inflammation, which is also associated with increased oxidative stress.

Paragraph 3. We hypothesized that increased oxidative stress and disrupted redox balance in response to heavy physical training may be predisposing factors and markers for overreaching (OR). For this purpose we aimed to study the association of oxidative stress markers and antioxidant status with physical activity during an 8-week military basic training (BT) period. BT comprises the first eight weeks of military service and includes both endurance and strength type of training. A particular aim was to evaluate whether the levels of oxidative stress markers and antioxidant status and physical activity differ between OR and noOR subjects.

METHODS

Paragraph 4. Subjects. Male conscripts (N=35, aged 19.6 ± 0.3 yrs) participated in the present study. They were a part of a larger group of 60 soldiers who initially volunteered to participate and fulfilled selection criteria (29). All subjects were fully informed of the experimental protocol, and gave their written consent to participate in the study. They were also advised of their right to withdraw from the investigation at any time. The study protocol was approved by the Finnish Defence Forces, and the Ethical Committees of the University of Jyväskylä and the Kainuu region of Finland. The study took place during winter in Finland, when daily outdoor temperatures ranged from -31°C to $+1^{\circ}\text{C}$, with an average of -13°C (data from the local weather station). “The overall physical load of the 8-week BT period was set according to the standard basic program (31). It included a total of 300 hours military training, of which 100 hours was military related physical training, 33 hours sports related physical training such as combat

training and marching, and the rest of hours were other military training such as shooting, material handling, skill training and general military education (23). The intensity level of physical activity in the daily program was planned to be low in the first week of BT and increased gradually thereafter. The conscripts slept from 10 p.m. to 5:45 a.m. in dormitory-type rooms at the garrison and marched four times per day (approximately 5 km in total) for meals. The BT schedule included four longer (from two to eight hours) marching exercises with a combat gear and two overnight field exercises from one up to three days. Food and water intake were in accordance with the standard army meal, and water intake was not restricted. However, subjects were not permitted to use any extra nutritional supplements throughout the study. ”

Paragraph 5. Experimental protocol. Performance tests (maximal aerobic fitness [VO_2max] test and submaximal exercise test) were performed and oxidative stress and antioxidant status determined three times and psychological markers (questionnaires) five times during the 8-week training period. In addition, acute responses to a submaximal exercise were studied. For the submaximal exercise, a marching test with a 20 kg backpack was selected because it closely resembles the routine activities of conscripts and enables the test to be performed for a large group of subjects. The experimental protocol is presented in Table 1.

Paragraph 6. Performance tests. An incremental test until voluntary exhaustion to determine maximal oxygen uptake (VO_2max) was carried out on a treadmill at baseline and on weeks five (wk5) and eight (wk8) as described earlier (29). The 45-minute submaximal exercise test was performed on an outdoor track at the 70% level of subject’s individual maximal workload in the beginning (baseline) of BT at baseline, on the weeks four (wk4) and seven (wk7). The follow-up tests for each subject were always performed at the same time of day (VO_2max -test between 8.30

a.m. and 5 p.m., submaximal exercise between 9 a.m. and 12 p.m.) having consumed a similar diet before each test with the same test protocol. The hormonal responses to submaximal exercise have previously been reported (30).

Paragraph 7. Body composition. Body composition measurements (body mass [BM], fat free mass [FFM], fat mass [FM] and percentage of body fat [F%]) were performed using eight-point bioelectrical impedance (Inbody720, Biospace Co. Ltd, Seoul, Korea). For each subject, the repeated measurements were performed at the same time between 6 and 7 a.m. after an overnight fast and after voiding, with no exercise for 12 hours before the test. The physical activities in the daily program were planned to be of a low intensity on the day preceding each measurement and fluid status was estimated to be in balance based on the dietary records of the subjects (29). The subjects were barefoot and wore T-shirts and trousers. Body height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as BM (kg) divided by height (m) squared.

Paragraph 8. Blood samples and analyses. Blood samples from an antecubital vein and fingertip were drawn 2 hours after a light breakfast at rest, before submaximal exercise, and immediately after exercise. Circadian variability in blood parameters was minimized by collecting individual pre- and post-exercise samples at the same time of day between 9 and 12 a.m. preceded by similar patterns of food ingestion. Subjects were instructed not to ingest alcohol, coffee, tea, chocolate or cola drinks since the previous evening of the measurements.

Paragraph 9. Blood samples were centrifuged at 1200 g and 4°C for 15 min immediately after collection to separate the plasma. Plasma samples were stored in multiple portions at -80°C until

analysis. Protein carbonyls, markers of protein oxidative damage, were measured using an ELISA method as previously described (19). Nitrotyrosine concentrations were determined with the ELISA method using a commercial kit (HyCult biotechnology b.v; Netherlands). Lipid peroxidation marker total malondialdehyde in plasma was measured according to the method of Gerard-Monnier et al. (9). Oxygen radical absorbance capacity (ORAC) was used for the measurement of antioxidant capacity, which was performed using a multi-well plate reader according to the methods described previously (13). The maximal intra-assay coefficients of variation for protein carbonyls, nitrotyrosine, malondialdehyde and ORAC were 5.9%, 10.0%, 6.2% and 8.1%, respectively, and the maximal inter-assay coefficients of variation were 9.2%, 12.7, 10.6% and 11.3%, respectively. Protein carbonyls and ORAC measurements were performed in triplicate. Total glutathione (TGSH) and an oxidative stress marker reduced glutathione (GSSG) concentrations were determined spectrophotometrically as described previously (24). The tissues were deproteinized with metaphosphorous acid (MPA) for TGSH and GSSG analysis. The maximal intra-assay coefficients of variance for these assays was 4.8 %, and inter-assay 6.4 %. Hemoglobin and hematocrit were analyzed using a Sysmex KX 21N-analyzer (Sysmex Co., Kobe, Japan). Blood lactate was analyzed from fingertip blood sample using Lactate Pro® –analyzer (Arkray, Japan). Submaximal exercise-induced changes in plasma volume was calculated from changes in hemoglobin and hematocrit (6) and malondialdehyde, nitrotyrosine and ORAC post exercise values are reported adjusted for these changes. Plasma protein carbonyl results were expressed in $\text{nmol}\cdot\text{mg}^{-1}$ protein. The BT and/or exercise induced relative changes are expressed as $\Delta\%$.

Paragraph 10. Questionnaires and sick leave. The subjects rated how severely they experienced a list of symptoms during the last week using a five-point Likert scale; 1 = not at all, 2 = one day, 3 = 2-3 days, 4 = 4-5 days, 5 = 6-7 days. The somatic symptoms were subjective ratings of well-being, upper respiratory track infections, flu-like symptoms, digestive disorders and reduced appetite, musculo-skeletal, physical complaints and sleep difficulties (30). The sum of the symptoms associated with OTS was determined as the sum of the symptom scores. In addition, the question “Do you feel physically or mentally overloaded?” was asked. Sick leave was defined as an attendance / not-attendance in daily service because of illnesses or injuries that were evaluated by a physician.

Paragraph 11. Physical activity (PA). Physical activity (PA) was measured from 18 subjects with customized version of Polar AW200 Activity monitor that was worn on the non-dominant wrist. AW200 has been found useful and accurate for measurement of EE during long-term exercise (3). AW200 contains a uniaxial accelerometer. The acceleration signal is band pass filtered (0.3-3.0 Hz), and device has reduced sensitivity to repeated low-intensity hand movements. The device counts hand movements if acceleration exceeds $1.0 \text{ m}\cdot\text{s}^{-2}$ (12). Epoch length was set at one minute and a curvilinear equation was used to transform activity counts to metabolic equivalents (1 - 16 MET), which were further adjusted by body height. Among the same subjects, AW200 weekly physical activity energy expenditure correlated well ($r = 0.78$) to that obtained with doubly labeled water (unpublished data, see (29)). Daytime was determined from 6:00 a.m. to 9:00 p.m. and nighttime from 9:00 p.m. to 6:00 a.m. Periods that contained no single movement during 30 min in daytime, or during 6 hours or more in nighttime, were classified as non-wear time and excluded from analysis. PA by each minute was classifying into either (1) no activity = REST ≤ 1.0 MET, (2) very light to light activity = VLPA, 1.0-3.9 MET or

(3) moderate to vigorous physical activity = MVPA \geq 4 MET. REST (during daytime) or sleep (during nighttime) was selected if accelerometer showed no hand movements within more than 50% of a 10 minute moving window. Each subject's daily and nightly data were included for analysis only if activity recording covered more than 80% of daytime and 90% of nighttime. Measures of PA were adjusted by the proportion of time recorded. The activity watches were collected every evening between 9 to 10 p.m. for data download, and redistributed within 30 minutes. Out of a total of 41 days, we included days and nights when at least two thirds of subjects had enough data. In all, 33 days and 28 nights were included in the analysis. Data were pooled into two week periods; weeks 1-2, weeks 3-4, weeks 5-6 and weeks 7-8.

Paragraph 12. Criteria for overreaching (OR). In this study subjects had to fulfill three of five criteria to be classified as OR subjects (30). Criteria 1; A reduced maximal aerobic fitness ($VO_2\max$) of greater than 5% (11, 26) or did not perform the test because of illness. Absence from the test was set as an additional criterion, because all three $VO_2\max$ tests were completed by a total of 21 of 35 conscripts. It was assumed that illness itself reduces performance. Furthermore, overload training has been reported as a risk factor for upper respiratory track infections (27, 34). Criteria 2; An increase in mean RPE during the submaximal exercise greater than 1.0 (11) from the lowest value at wk1 or wk4 until the end of BT. Criteria 3; An increase in somatic symptoms of OTS (16, 32) greater than 15% from wk4 to wk7, and remaining the same or increasing from wk7 to wk8. Subjects were divided into tertiles based on an increase in somatic symptoms of OTS from wk4 to wk7; 15% was the cut-off for the upper third. Criteria 4; Admitted feeling physically or mentally overloaded (16, 33, 34) at week 7 or 8. Criteria 5; Sick leave more than 10% of daily service. Subjects were divided into tertiles based on sick leave during BT; 10% was the cut-off for the upper third.

Paragraph 13. Statistical Analyses. Statistical analyses were performed using SPSS (Version 16.0.1. 2005; SPSS Inc., Chicago, IL). The level of statistical significance was set at $p < 0.05$. Assumptions for normality were not met for protein carbonyls, malondialdehyde and nitrotyrosine, and data were log transformed before statistical analysis. The untransformed values are shown in the text, tables and figures for more meaningful comparison, except for nitrotyrosine. Responses to submaximal exercise (before and after) and BT period (baseline and wk4 and wk7) were assessed using repeated-measures ANOVA. Mixed-design factorial ANOVA [group (noOR vs. OR) x exercise x BT] was used to identify differences between and within the OR and noOR subjects. Bonferroni as the *Post hoc* analysis was used to identify significant differences. In addition, the effect of exercise and BT and their interaction were calculated. Pearson product–moment correlations were used to observe associations between variables. All data are presented as mean \pm SD.

RESULTS

Paragraph 14. VO₂max and body composition. There was a main effect of BT for VO₂max ($P < 0.001$) with an increase in VO₂max after 5 wk of BT ($P < 0.001$). From wk5 to wk8, VO₂max did not change significantly (baseline 45 ± 7 mL·kg⁻¹·min⁻¹; wk4 49 ± 5 mL·kg⁻¹·min⁻¹; week 7 49 ± 5 mL·kg⁻¹·min⁻¹). The initial mean body height was 178.2 ± 7.9 cm, BMI 24.7 ± 4.5 kg·m⁻² and F% $19.3 \pm 7.5\%$. Both BM and FM exhibited a main effect of BT ($P < 0.001$), decreasing from wk4 to wk7 ($P < 0.001$ and $P < 0.01$, respectively), with lower values compared to the before BT values ($P < 0.001$)(BM baseline 78.7 ± 17.7 kg; wk4 78.5 ± 16.9 kg; wk7 77.0 ± 15.9 kg; FM baseline 16.1 ± 10.7 kg; wk4 15.6 ± 10.7 kg; wk7 14.0 ± 9.1 kg). In contrast, the mean FFM was

higher after 7 wk of BT compared to baseline ($P<0.05$) (baseline 62.6 ± 9.4 kg; wk4 62.9 ± 8.8 kg; wk7 63.0 ± 9.3 kg).

Paragraph 15. Oxidative stress markers and antioxidant capacity. There was a main effect of exercise for TGSH ($P<0.001$), GSSG ($P<0.001$), and the GSSG/TGSH ratio ($P<0.001$), a main effect of BT for TGSH ($P<0.05$) and ORAC ($P<0.01$), and exercise x BT interaction for TGSH ($P<0.05$), GSSG ($P<0.001$), the GSSG/TGSH ratio ($P<0.01$) and ORAC ($P<0.05$). TGSH and ORAC (Figure 1) and protein carbonyls, malondialdehyde and nitrotyrosine at rest remained unchanged during BT. After 4 wk of BT a decrease at rest were observed in GSSG ($P<0.01$), an increase in GSSG and GSSG/TGSH ratio at the latter part of BT (Figure 1). At every time point submaximal exercise induced a decrease in TGSH and an increase in GSSG and GSSG/TGSH ratio (Figure 1). However, a significant submaximal exercise induced decrease in ORAC was observed only at wk4 (Figure 1). In addition, at wk4 TGSH and ORAC were lower after submaximal exercise than at baseline (TGSH $P<0.01$; ORAC $P<0.01$) and wk7 (TGSH $P<0.001$; ORAC $P<0.001$). GSSG and the GSSG/TGSH ratio after submaximal exercise were higher at wk4 (GSSG $P<0.05$; GSSG/TGSH $P<0.01$) and GSSG at wk7 ($P<0.001$) than at baseline (Figure 1). No significant changes were observed due to the submaximal exercise in protein carbonyls, malondialdehyde and nitrotyrosine.

Paragraph 16. Differences between noOR and OR subjects. At least three of the five criteria were detected in 31% (N=11 of 35) of the subjects, all of whom were classified as OR subjects. The remaining 69% (N=24) were classified as noOR subjects. From OR subjects, nine subjects fulfilled criteria 1, six criteria 2, eight criteria 3, nine criteria 4 and six subjects fulfilled criteria

5. There was a group x exercise x BT interaction only for GSSG ($P<0.001$) and GSSG/TGSH ratio ($P<0.05$). At baseline, GSSG ($P<0.01$) and the GSSG/TGSH ratio ($P<0.05$) was higher in OR than in noOR at rest (Figures 2a and 2c, respectively). In OR, no response of GSSG and GSSG/TGSH to acute submaximal exercise was seen, in contrast to noOR subjects (Figures 2b and 2d, respectively). Furthermore, OR subjects had higher malondialdehyde at baseline ($P<0.05$) than no OR (Table 2). VO_2 max and body composition did not differ between the groups.

Paragraph 17. In response to BT at rest, OR subjects exhibited decreased GSSG ($P<0.01$) and a trend of decreased GSSG/TGSH ratio ($P=0.058$) from baseline to wk4 (Figures 2a and 2c). Compared to baseline OR had a higher relative increase in GSSG due to submaximal exercise both after 4 ($P<0.01$) and 7 ($P<0.05$) wk of BT, and in the GSSG/TGSH ratio after 4 wk of BT ($P<0.05$) (Figures 2b and 2d). Among the noOR subjects exercise induced decrease in TGSH was lower after 7 wk of BT than after 4 wk of BT ($P<0.01$) (Table 2). However, only the OR subjects exhibited a significant exercise-induced decrease in ORAC after 4 wk of BT ($P<0.05$), which was significantly lower compared to wk7 ($P<0.05$) (Figure 2f). There were no differences between the groups either neither in TGSH, ORAC, protein carbonyls, nitrotyrosine at rest, nor in the relative responses to exercise at baseline or during BT (Table 2). The relative changes at rest during BT in TSGH, GSSG, GSSG/TGSH ratio, ORAC, protein carbonyls, malondialdehyde or nitrotyrosine also did not differ between the groups. However, OR had a higher incidence of sick leave only during the last week of BT compared to the noOR subjects (mean difference 5.5%, 95% CI 1.8 to 9.0%, $P<0.004$).

Paragraph 18. Physical activity. During the entire BT period, the average daytime MVPA was $2:07 \pm 0:24$ h:min, LVPA $11:36 \pm 0:30$ h:min and REST $0:30 \pm 0:18$ h:min. During the nighttime between 9:00 p.m. and 6:00 a.m., the average MVPA was $0:05 \pm 0:02$ h:min, VLPA $1:00 \pm 0:18$ h:min and sleeping time $7:30 \pm 0:18$ h:min. There was a main effect of BT for daytime MVPA ($P < 0.001$), VLPA ($P < 0.05$), REST ($P < 0.001$) and nighttime MVPA ($P < 0.001$). Daytime MVPA was higher ($P < 0.001$)(Figure 3) and VLPA ($P < 0.05-0.001$)(Figure 3) and REST lower ($P < 0.001$) during the latter part of BT compared to the weeks 1-2 (REST; weeks 1-2 $2:00 \pm 0:06$ h:min; weeks 3-4 $0:24 \pm 0:12$ h:min weeks 5-6 $0:30 \pm 0:24$ h:min; weeks 7-8 $0:30 \pm 0:24$ h:min). However, nighttime MVPA was higher during the first four weeks of BT compared to the latter half of BT ($P < 0.05-0.01$)(Figure 3). Nighttime VLPA and sleeping time remained the same during the entire BT.

Paragraph 19. Physical activity among noOR and OR subjects. PA was monitored from 9 OR and 9 noOR subjects. These subjects did not differ in incidence of sick leave during BT, thus there were no need to take account the influence of sick leave on physical activity. There were main effect of group for daytime VLPA ($P < 0.05$) and nighttime MVPA ($P < 0.05$). OR had higher nighttime MVPA compared to noOR during the weeks 3-4 ($P < 0.05$) and weeks 5-6 ($P < 0.05$) (Figure 3) and less daytime VLPA during weeks 1-2 ($P < 0.05$) and weeks 5-6 ($P < 0.05$) (Figure 3). However, OR had higher daytime MVPA compared to noOR during the weeks 1-2 ($P < 0.05$) and weeks 5-6 ($P < 0.05$) (Figure 3). There were no differences between the groups in REST, nighttime VLPA and sleeping time. However, REST was less during weeks 5-6 than weeks 7-8 ($P < 0.01$; weeks 5-6 $0:36 \pm 0:24$ h:min; weeks 7-8 $0:42 \pm 0:24$ h:min,) in OR, but not in noOR.

DISCUSSION

Paragraph 20. In this study we showed that increased oxidative stress is associated with overreaching. To our knowledge, this is the first study showing that increased oxidative stress prior to the strenuous 8-week combined aerobic and strength training period was related to the incidence of OR during the training period. Oxidative stress markers, including GSSG, the GSSG/TGSH ratio and malondialdehyde at rest, were higher among OR subjects than in noOR. In addition, OR subjects had no increase in GSSG and GSSG/TGSH ratio in the submaximal exercise, which might be a consequence of high resting levels. Recently we have reported that impaired antioxidant capacity and increased oxidative stress at the state of OTS (28). However, whether increased oxidative stress is a cause for OTS or an outcome of training protocol with inadequate recovery remains unclear.

Paragraph 21. The average training load during 8-week BT was two hours per day with only half an hour of total rest during the daytime. Otherwise the day included low physical activity from sitting and standing to slow walking. This kind of high training load could be a risk factor for OR for subjects with a low fitness level. Surprisingly, OR and noOR subjects did not differ from each other according to VO_2 max or body composition at the beginning of BT. This suggests that fitness or fatness in this study are not strong underlying factors for overtraining. On the other hand, OR were more physically active both during the day and night time than noOR. These results support the hypothesis that the consequences of physical training are not only influenced by the intensity and duration of training, but also by the duration of recovery time. Among the OR subjects high physical activity during the day- and nighttime increased the accumulated training load at the end of BT, which may have contributed to the symptoms and indicators of OR. However, the OR and noOR subjects did not differ regarding to tasks of the

military service. Thus higher physical activity might more likely be an individual property rather than an external factor.

Paragraph 22. Classification of OR/OTS is challenging, and it is rarely classified by a single criterion. In this study, OR subjects had to fulfill three of five criteria, including a decrease in aerobic physical performance. Absence from VO₂max tests or submaximal exercise was set as an additional criterion for a decreased performance. In 71 % of the cases the main reason for sick leave during BT was an upper respiratory track infection (URTI). Sick leave itself could affect training responses, even resulting in a detraining effect. However, OR and noOR did not differ according to sick leave during the first 7 weeks of BT. Thus, the decrease in performance probably was mainly caused by an excessive training load. Furthermore, we have recently reported that these OR subjects had higher basal sex-hormone-binding-globulin (SHBG) than noOR subjects at baseline and also after 4 and 7 weeks of training (30). In addition, OR subjects had higher basal serum cortisol than noOR subjects after 7 weeks of training (30). Moreover, OR subject had a decrease in the testosterone/cortisol ratio at rest from wk4 to wk7 and the maximal La/RPE ratio in the VO₂max test from baseline to the end of BT, while noOR subjects did not (30).

Paragraph 23. In this study, day activity time increased and rest time decreased during the first 4 weeks of BT, with a concomitant increase in aerobic performance, while body mass remained the same. Among all 35 subjects, decreased oxidative stress at rest, evident as lower glutathione oxidation (GSSG), was observed after the first four weeks of training. The decreased oxidative damage can be explained either by attenuated generation of ROS (caloric restriction) or by enhancement of tissue protection and antioxidant systems, as a consequence of adaptation to

regular exposure to a small amount of ROS (such as from exercise) (20). Aerobic training itself has been found to decrease oxidative stress at rest also without changes in body mass (5). Thus, the decreased oxidative stress observed in this study may be a consequence of an enhanced antioxidant defense system in response to a tolerable training load (10, 14), also among OR subjects. The high capacity to consume oxygen (enhanced VO_2max) and consequently to produce a larger amount of ROS are accompanied by high erythrocyte glutathione peroxidase activity and high glutathione concentration which both serve to protect the organism from lipid peroxidation and cell membrane damage (14).

Paragraph 24. In contrast to the decreased oxidative stress at rest, four weeks of BT increased the susceptibility to acute exercise-induced oxidative stress evident as higher glutathione oxidation and lower antioxidant capacity after a submaximal exercise. At baseline the subjects were not familiar with an acute physical load that may lead to increased oxidative stress. In contrast we observed a higher exercise-induced oxidative stress at wk4 than at baseline in the trained subjects. In previous studies both aerobic (8) and strength (17) training has been found to reduce post-exercise oxidative stress at the same physical task. However, Vollaard et al. (35) found that neither tapering nor overload endurance training affected the exercise-induced increase in GSSG among endurance-trained athletes. Our results may indicate overreaching due the intensive BT period. In the entire group of 35 subjects and in the OR subjects a U-shape curve of oxidative stress at rest was observed during BT; GSSG decreased at rest from baseline to week 4, increasing back to baseline values at week 7. Interestingly, submaximal exercise decreased ORAC at wk4. This might be explained by the increased consumption of body's endogenous antioxidant resources. While coping with oxidative stress, plasma antioxidants are mobilized into the tissue (15).

Paragraph 25. During the second half of the 8-week training period, although day activity time and rest time remained the same, GSSG and the GSSG/TGSH ratio at rest increased and submaximal exercise resulted in a higher induction of GSSG/TGSH ratio. Also stagnation in VO_2 max was observed and all the subjects had a decrease in fat mass. All these findings indicate that that training load was too strenuous during the second half of BT causing oxidative stress (10, 15). However, ORAC did not response to submaximal exercise at the end of BT, as was the case at wk4. This could be a marker of OR, since diminished antioxidant protection has been observed among overtrained athletes (28).

Paragraph 26. Plasma nitrotyrosine and protein carbonyls, markers of oxidative protein modification, remained the same during the entire 8-week training period, both at rest and after exercise. There were also no differences between the OR and noOR subjects. Plasma nitrotyrosine levels had a wide variation, which may partly explain the observed findings. The present results also support the previous findings that plasma nitrotyrosine level may not be a sufficiently sensitive marker to assess training or acute exercise-induced changes in oxidative stress (22, 28). Proteins are an important target for oxidative challenge. Reactive oxygen species modify amino acid side chains of proteins to form protein carbonyls (1). Serum protein carbonyls have been found to increase after an intensive 12-week resistance training period (15), decrease after exhaustive marches (50 and 80 kg) in well trained soldiers (4) and increase after an exercise test to exhaustion in athletes (28). The turnover time for protein carbonyls varies from many hours to days (4). The 8-week training period might have been too short and submaximal exercise load in the present study may not be strenuous enough to modify proteins. Exercise may also have activated a mechanism that removes the oxidatively modified proteins from the

circulation, or alternatively, activation of antioxidant mechanisms that remove the ROS (4). In addition, differences among studies in oxidative stress responses to training and exercise may be explained by the differences in energy availability, training mode and length of the training period, fitness levels and genetic background of the subjects. The subjects in the present study were low to moderately fit conscripts in the compulsory army, and training consisted of both strength and endurance training.

Paragraph 27. In conclusion, the present results suggest that increased oxidative stress may be associated with overreaching. Although the training had favorable effects on oxidative stress markers during the first 4 weeks, sustained heavy training blunted this effect at the latter part of BT. At the end of the study military training increased oxidative stress at rest back to pre-training levels, and beyond the pre-training levels in response to submaximal exercise. Therefore, we may speculate that oxidative stress may also be a marker of insufficient recovery resulting in overreaching. Since one third of the subjects were classified as overreached, the variability of the BT program has to be taken into careful consideration. Especially, the program should involve more recovery phases, both daily and periodically.

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FIGURE LEGENDS

FIGURE 1. Mean (\pm SD) plasma a) TGSH, b) GSSG, c) GSSG/TGSH and d) ORAC concentrations before and after the 45-minute submaximal exercise at baseline, and at week 4 and 7 of basic training. Difference compared to: before exercise *** $P < 0.001$, ** $P < 0.01$; baseline \$\$\$ $P < 0.001$, \$\$ $P < 0.01$, \$ $P < 0.05$; week 4 ^^^ $P < 0.001$, ^^ $P < 0.01$. N=35.

FIGURE 2. Mean (\pm SD) plasma GSSG, GSSG/TGSH and ORAC concentrations at rest (a, c, e) and changes (%) due the 45-minute submaximal exercise (b, d, f) at baseline, and at week 4 and 7 of basic training among noOR (N=24) and OR (N=11) subjects. Difference compared to: noOR ### $P < 0.01$, # $P < 0.05$; baseline \$\$ $P < 0.01$, \$ $P < 0.05$; week 4 ^ $P < 0.05$. Significant change due the exercise *** $P < 0.001$, * $P < 0.05$.

FIGURE 3. Mean (\pm SD) a) daytime very low (VLPA) and b) moderate to vigorous (MVPA) physical activity and c) nighttime moderate to vigorous (MVPA) physical activity during the basic training period among noOR and OR classified subjects. Difference compared to no OR # $P < 0.05$; weeks 1-2 \$\$\$ $P < 0.001$, \$\$ $P < 0.01$, \$ $P < 0.05$; weeks 5-6 □□□ $P < 0.001$, □□ $P < 0.01$, □ $P < 0.05$.

TABLE 1. Experimental design during the 8-week military basic training period.

Week	1	2	3	4	5	6	7	8
VO ₂ max –test	X				X			X
Submaximal exercise test	X			X			X	
Body composition	X			X			X	
Blood samples	X			X			X	
Questionnaire	X			X		X	X	X

TABLE 2. TGSH and oxidative stress markers among noOR (N=24) and OR (N=11) subjects at rest and relative change due to the submaximal exercise ($\Delta\%$ ex.)

			Baseline		Week 4		Week 7	
			Mean \pm SD	P	Mean \pm SD	P	Mean \pm SD	P
TGSH	At rest	noOR	773 \pm 108		756 \pm 83		776 \pm 100	
	(μmol)	OR	801 \pm 140		754 \pm 91		767 \pm 115	
	$\Delta\%$ ex.	noOR	-11 \pm 11	***	-15 \pm 8	***	-8 \pm 7	***, ^^
		OR	-12 \pm 7	***	-12 \pm 11	***	-6 \pm 16	***
Malondialdehyde	At rest (μmol)	noOR	0.99 \pm 0.28		0.93 \pm 0.29		1.10 \pm 0.32	
		OR	1.24 \pm 0.29	#	1.11 \pm 0.32		1.11 \pm 0.16	
	$\Delta\%$ ex.	noOR	3 \pm 24		7 \pm 26		0.3 \pm 23	
		OR	-11 \pm 27		4 \pm 26		-10 \pm 21	
Protein carbonyls	At rest	noOR	0.09 \pm 0.02		0.09 \pm 0.02		0.09 \pm 0.02	
		($\text{nmol} \cdot \text{mg}^{-1}$)	OR	0.10 \pm 0.02		0.09 \pm 0.02		0.10 \pm 0.02
	$\Delta\%$ ex.	noOR	0.03 \pm 5		-1 \pm 4		1 \pm 4	
		OR	0.32 \pm 5		1 \pm 4		1 \pm 5	
Nitrotyrosine	At rest	noOR	6.53 \pm 2.31		6.56 \pm 2.18		6.53 \pm 2.18	
		(log adj.)	OR	6.45 \pm 1.84		6.45 \pm 1.78		6.52 \pm 1.79
	$\Delta\%$ ex.	noOR	4 \pm 15		-3 \pm 11		2 \pm 16	
		OR	9 \pm 23		-1 \pm 11		-6 \pm 12	

Difference; compared to noOR # $P < 0.05$; within the group compared to week 4 ^^ $P < 0.01$. Significant change due the exercise *** $P < 0.001$.