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Serum Sex-Hormone Binding Globulin and Cortisol concentrations are Associated with Overreaching during Strenuous Military Training

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Running title: Association between Hormones and Overreaching

ABSTRACT

The purpose was 1) to study the effect of an 8-week Finnish military basic training period (BT) on physical fitness, body composition, mood state and serum biochemical parameters among new conscripts, 2) to determine the incidence of overreaching (OR), and 3) to evaluate whether initial levels or training responses differ between OR and noOR subjects. Fifty-seven males (19.7 ± 0.3 yrs) were evaluated before and during BT. OR subjects had to fulfill three of five criteria; decreased aerobic physical fitness ($VO_2\max$), increased RPE in 45-min submaximal test at 70% of $VO_2\max$ or sick absence from these tests, increased somatic or emotional symptoms of OR and high incidence of sick absence from daily service. $VO_2\max$ improved during the first 4 weeks of BT. During the second half of BT, a stagnation of increase in $VO_2\max$ was observed, basal serum sex-hormone binding globulin (SHBG) increased, and IGF-1 and cortisol decreased. Furthermore, submaximal exercise-induced increases in cortisol, maximum heart rate and post-exercise increase in blood lactate were blunted. Out of 57 subjects, 33% were classified as OR. They had higher basal SHBG before and after 4 and 7 weeks of training, and higher basal serum cortisol at the end of BT than noOR subjects. In addition, in contrast with noOR, OR subjects exhibited no increase in basal testosterone/cortisol ratio but a decrease in maximal La/RPE ratio during BT. As one third of the conscripts were overreached, training after BT should involve recovery training to prevent overtraining syndrome from developing. The results confirm that serum SHBG, cortisol, and testosterone/cortisol and maximal La/RPE ratios could be useful tools to indicate whether training is too strenuous.

Key words: biochemistry, training monitoring, testosterone, IGF-1, aerobic capacity

INTRODUCTION

The fitness level of young men entering Finnish compulsory military service has declined with a concomitant increase in body mass during the last 25 years (30). In order to improve the physical fitness of new conscripts, a new physical training program for an 8-week basic military training (BT) period was established in 1998 (35). However, the effect of the BT program on physical fitness and performance among current conscripts, who exhibit large inter-individual variations in physical fitness levels, is unknown.

Conscripts entering military service are intensively exposed to non-training stresses due to the totally new environment and the change in their working conditions. Most of these young men will have their first experience of demanding physical training, eating outdoors, and performing overnight exercises in a forest. In addition, the physical training involved in military service may exceed their previous training level, which could contribute to overreaching in the multi-stressor environment of the military service. Overreaching (OR) is an accumulation of training and/or non-training stress resulting in short-term decrements in performance capacity, which may be accompanied by related physiological and psychological signs and symptoms of maladaptation (22). OR could also be an early indicator of the development of overtraining syndrome (OTS) (22, 33), which is characterized by an unexpected decrease in performance, despite increased or maintained training load (38). There is some evidence to suggest that the development of OTS is not solely related to the stress of training, but also non-training (psychological) stress (15, 21).

In order to monitor responses to training and to avoid OR and OTS, there is still a clear need for reliable tools that facilitate the early diagnosis of OTS (37). Although there are no specific markers of impending OTS, testosterone (an anabolic hormone) and cortisol (a catabolic hormone) have often been used to represent the overall anabolic and catabolic activities in the body (2, 36). In addition, serum testosterone and cortisol concentrations have been identified as reliable markers of military training stress (27), and the testosterone/cortisol ratio has been used to represent the balance between anabolic and catabolic activity (2). It has also been suggested that acute exercise induced changes in serum hormones could be used to monitor training load and to avoid OTS (37). In particular, a diminished response of cortisol to an acute submaximal exercise (39), and reduced basal cortisol levels (31, 37) may be useful indicators of exhaustive training loads. In addition, insulin-like growth hormone (IGF-1) has recently been identified as a suitable marker for monitoring training load (26) and investigating OTS. Furthermore, an impaired mood state and subjective complaints are consistently described as sensitive and early markers of OTS (37).

The purpose of the present research was 1) to study effect of an 8-week Finnish military basic training period (BT) on physical fitness, body composition, mood state and serum biochemical parameters, 2) to determine the incidence of OR during BT among new conscripts, and 3) to evaluate whether the initial levels or training responses of physical fitness, body composition and serum biochemical parameters differ between OR and noOR subjects.

METHODS

Experimental Approach to the Problem

Purpose 1. Performance tests and physiological and psychological markers to monitor the training load of BT training were selected based on existing literature of OTS and training studies. Performance tests (maximal aerobic fitness [VO_2max] test and submaximal test) and physiological tests were performed three times, and psychological markers five times during the 8-week training period. In addition, acute responses to a submaximal test were studied. For the submaximal test, a marching test was selected because it closely resembles the routine activities of conscripts and enables a test to be performed for a large group of subjects. Furthermore, sick absences were followed over the entire BT, because poor VO_2max levels have been associated with training related sick absences during military service (17). The experimental protocol is presented in Table 1.

Table 1 close here

Purposes 2 and 3. No single symptom or set of symptoms have been found to be associated with OR or OTS. However, a decrease in performance and an increase in perceived exertion at the same subjective work load have been found to be related to OR and OTS (13, 14, 38, 39). In addition, impaired general health status, negative mood and feelings of fatigue have been found to be related to OR and OTS (13, 22, 38, 39). Therefore, subjects in this study had to fulfill three of five criteria to be classified as OR subjects. In addition, differences between subjects who

fulfilled a single OR criteria were evaluated to determine whether initial levels of physical fitness, body composition and serum biochemical parameters differed among these subjects.

Criteria 1. A reduced VO_2 max of greater than 5% (13, 31) 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 36 conscripts. It was assumed that illness itself reduces performance. Furthermore, overload training has been reported as a risk factor for upper respiratory track infections (32, 39).

Criteria 2. An increase in mean RPE during the submaximal marching test greater than 1.0 (13) from the lowest value at wk1 or wk4 and remaining more than 1.0 above the lowest value, or did not perform the test because of illness (see description above). All three submaximal marching tests were completed by a total of 35 conscripts.

Criteria 3. An increase in somatic symptoms of OTS 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 upper third.

Criteria 4. Admitted feeling physically or mentally overloaded at week 7 or 8.

Criteria 5. Sick absence more than 10% of daily service (upper third tertile based on the sick absences during BT).

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 direction of the Defense Command. The intensity level of physical activity in the daily program was low in the first week

of BT and increased thereafter. 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. The average sleeping time at the garrison was from 10 p.m. to 5:45 a.m. Military education included physically demanding activities such as marching, combat training and sport related physical training. The conscripts also carried combat gear weighing 20 kg including clothing, particularly during marching and combat training. In addition, the training included an overnight field exercise. Garrison training involved theoretical education in classroom settings, material handling, shooting and general military education, such as close order drills. Conscripts marched four times per day (approximately 5 km in total) for meals, and slept in dormitory-type rooms.

During the BT period, practical skills are trained daily. At the beginning of BT, the amount of physical training is approximately two hours per day, increasing to 3 to 4 hours during weeks 4 to 7. The BT schedule includes four longer (from two to eight hours) marching exercises with battle equipment. However, daily outdoor exercises, including transportation, are often performed by marching. Rifle marksmanship in the garrison is performed twice a week throughout the BT period.

Subjects

Male conscripts (N=57, aged 19.6 ± 0.3 yr) from the Signal Battalion of Northern Finland participated in the present study. They were selected from a total of 131 subjects who initially volunteered to participate. From these subjects, 47 were discarded based on cardiorespiratory or musculo-skeletal disorders, and incomplete fulfillment or willingness to perform special duties.

The remaining 84 were divided into three categories according to their level of voluntary physical activity before military service, which was determined by the International Physical Activity Questionnaire (IPAQ) (7). To ensure large variations in initial physical fitness levels, 19 conscripts were randomly selected from each category, making a total of 57 subjects. 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.

VO₂max test

To determine VO₂max (mL·kg⁻¹·min⁻¹), the conscripts performed a maximal treadmill test in week 1 (before training), and after 5 (wk5) and 8 (wk8) weeks of the BT period. The follow-up tests for each subject were always performed at the same time of day between 8.30 a.m. and 5 p.m., having consumed a similar diet before each test. The start of the test involved walking for 3-min at 4.6 km·h⁻¹ and walking/jogging at 6.3 km·h⁻¹ (1% slope) as a warm-up. Thereafter, exercise intensity was increased every 3-min to induce an increase of 6 mL·kg⁻¹·min⁻¹ in the theoretical VO₂max demand of running (1). This was achieved by increasing the initial running speed of 4.6 km·h⁻¹ by a mean of 1.2 km·h⁻¹ (range 0.6 – 1.4 km·h⁻¹), and by increasing the initial grade of 1 deg by a mean of 0.5 deg (range 0.0 - 1.0 deg) up to the point of exhaustion (1). Pulmonary ventilation and respiratory gas exchange data were measured on-line using the breath-by-breath method (Jaeger Oxygen Pro, VIASYS Healthcare GmbH, Hoechberg, Germany), and mean values were calculated at 1-min intervals for statistical analysis. The analyzer was calibrated before each test according to manufacturer specifications. Heart rate was

continuously recorded at 5-s intervals using a telemetric system (Polar810i, Polar Electro Oy, Kempele, Finland). Rating of perceived exertion (RPE) was assessed at the end of each workload. The criteria used for determining VO_2max were: a lack of increase in VO_2max and heart rate despite an increase in grade and/or speed of the treadmill, a respiratory exchange ratio (RER) higher than 1.1 and a post-exercise blood lactate value (determined 1 minute after exercise completion from a fingertip blood sample using a lactate analyzer; LactatePro®, Arkray, Japan) that was higher than $8 \text{ mmol}\cdot\text{L}^{-1}$ (1). All participants fulfilled these criteria.

Submaximal marching test

The 45-minute submaximal marching test (exercise) was performed after one week of adjustment to military service at the end of the first week (before training), and after four (wk4) and seven (wk7) weeks of the BT period. The time between the tests was three weeks and the follow-up exercise for each subject was always performed at the same time of day between 9 a.m. and 12 p.m. Heart rate (HR) was recorded in 5 s intervals during the entire exercise to define the mean HR. RPE was assessed three times, every 15 min using a 6- to 20-point scale. RPE was calculated as the average of these three values. The aim for determining marching speed was to divide subjects into three groups so that each group involved subjects marching at approximately 70% of their individual maximal workload. Exercise of this intensity and duration has been shown to result in increased serum cortisol levels (34) and to be tolerable for untrained subjects. Firstly, theoretical individual maximal workload ($\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was calculated based on the treadmill speed and grade in the VO_2max test using the ACSM estimation formula for VO_2 in running (1). Secondly, since in load carriage tasks (~20kg) the relationship between relative VO_2max and endurance time has been shown to be lower compared to an absolute VO_2max

value (3), theoretical individual maximal workload as $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ was converted into $\text{L}\cdot\text{kg}^{-1}$. Thirdly, the individual speed corresponding to 70% of maximal workload when carrying the 20 kg combat gear during the test on even terrain was determined. It was assumed that carrying the extra load required a similar level of effort to carrying extra body weight. As the ACSM formula for the estimation of VO_2max in walking yields unrealistically low values for brisk walking (above $5 \text{ km}\cdot\text{h}^{-1}$), a new equation for marching was determined that combines the ACSM equations for walking and running between 5 and 8 km/h. The equation is based on the earlier findings that walking and running are metabolically equivalent at a velocity of approximately $8 \text{ km}\cdot\text{h}^{-1}$ (20). Fourthly, the individually estimated marching speeds were rounded to the closest even number. 12 subjects were categorized into the group marching at $6 \text{ km}\cdot\text{h}^{-1}$, 43 subjects at $7 \text{ km}\cdot\text{h}^{-1}$ and 4 subjects at $8 \text{ km}\cdot\text{h}^{-1}$. An accurate and even marching speed was ensured by the group leader who walked in front of the group without extra weight and wore the Polar S625 (Polar Electro Oy, Kempele, Finland) running computer, which was equipped with an individually calibrated speed measuring device.

Body composition

Body composition measurements (body mass [BM], fat free mass [FFM], fat mass [FM] and percentage of body fat [F%]) were performed at the same time points as the marching tests using eight-point bioelectrical impedance (Inbody720, Biospace Co. Ltd, Seoul, Korea). For each subject, the measurements were performed at the same time between 6 a.m. 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. 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. Recent studies have shown that eight-polar bioelectrical impedance is a reliable method to detect changes in FFM (Δ FFM) and FM (Δ FM) when performed under strict standardized conditions (16). Accordingly, Δ FFM and Δ FM during the study were calculated using this method.

Blood samples and analyses

Blood samples were drawn from an antecubital vein after an overnight fast at rest (basal), 2 hours after a light breakfast before exercise (pre-exercise) and immediately after exercise (post-exercise). Basal samples were taken for serum total testosterone, cortisol and SHBG, pre-exercise samples for serum total testosterone, cortisol and total insulin-like growth factor-1 (IGF-1) and post-exercise samples for serum testosterone and cortisol. Fingertip lactate (La) samples were taken pre- and post-exercise. Circadian variability in blood parameters was minimized by collecting basal samples at the same time in the morning between 6.30 a.m. and 7.30 a.m., and by collecting individual pre- and post-exercise samples at the same time of day between 9 a.m. and 12 a.m. following similar patterns of food ingestion. The standardized breakfast before the test had a total energy content of 1860 kJ, 61 g of carbohydrate (56% of total energy intake), 16 g of protein (14% of total energy intake) and 15 g of fat (30% of total energy intake). All blood samples were taken in the same position (seated). Subjects were instructed not to ingest alcohol, coffee, tea, chocolate, cola drinks or bananas on the morning of the measurements, or the previous evening.

The percentage change in plasma volume ($\% \Delta PV$) was calculated from changes in hemoglobin and hematocrit according to the method of Dill and Costill (8). Serum post exercise values were adjusted for changes in plasma volume: Post-exercise adjusted = post-exercise + (post-exercise * $\% \Delta$ plasma volume /100). Exercise induced relative changes in serum hormonal concentrations were calculated using the equation; (post-exercise adjusted – pre-exercise)/pre-exercise *100, and expressed as $\Delta\%$. La was analyzed from fingertip blood samples (Lactate Pro, Arkray, Japan). Testosterone, cortisol and SHBG were analyzed by Immulite 1000 (Diagnostics Products Corporation, Los Angeles, USA) using commercial chemiluminescent enzyme immunoassays IMMULITE®/IMMULITE 1000 (Diagnostics Products Corporation, Los Angeles, USA). The intra-assay coefficients of variance for these assays were < 5.7%, < 4.8% and < 2.4%, respectively, and sensitivities of variance were $0.5 \text{ nmol} \cdot \text{L}^{-1}$, $5.5 \text{ nmol} \cdot \text{L}^{-1}$, and $0.2 \text{ nmol} \cdot \text{L}^{-1}$, respectively. Hematocrit and hemoglobin were analyzed using a Sysmex KX 21N-analyzer (Sysmex Co., Kobe, Japan). IGF-1 was analyzed with an R&D duoset ELISA kit (R&D Systems, Minneapolis, MN, USA). The intra- and inter-assay coefficients of variance and sensitivity for these assays were < 6.4 %, < 9.1 % and $26 \text{ pg} \cdot \text{ml}^{-1}$, respectively.

Questionnaires and sick absence

The questionnaire to assess the somatic symptoms of OR and OTS was formulated based on previously reported symptoms of OTS (12, 15, 22, 37). The somatic symptoms were subjective ratings of well-being; upper respiratory track infections (Cronbach's alpha 0.82), flu-like symptoms (Cronbach's alpha 0.74), digestive disorders and reduced appetite (Cronbach's alpha 0.45), musculo-skeletal disorders (Cronbach's alpha 0.70), physical complaints and sleep difficulties (Cronbach's alpha 0.61). The subjects rated how severely they experienced a list of

symptoms during the last week of training 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 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. Questionnaires were administered one day before and one week after the marching test at the same time of day in standardized conditions to avoid pre- versus post-exercise and morning versus evening variations (22). Sick absence was defined as an attendance / not-attendance in daily service because of illness or injuries examined by a physician.

Statistical Analyses

Purpose 1. Physiological and/or psychological responses to training (before, and after 4 and 7 weeks of BT) and exercise (pre and post submaximal marching test) were assessed using repeated-measures ANOVA. *Post hoc* analysis (LSD) was used to identify significant differences. In addition, the effect of training and exercise and their interaction were calculated. Pearson product-moment correlations were used to observe associations between variables. These analyses were performed for the 35 subjects who completed all submaximal marching tests, and thus had all results.

Purposes 2 and 3. Independent samples T-tests were used to identify significant differences between OR and noOR subjects. In addition, groups of subjects satisfying each single OR criteria (1-4) were evaluated independently by independent samples T-tests. For OR criteria 5 (sick absence), One-way ANOVA was used to identify significant differences between the groups. During the study, there were 42 military service days, and the average number of sick absences

was 3 days, ranging from 0 to 10 days (0 - 24%). Subjects were divided into tertiles based on sick absence; 0% sick absence (n=20), 2-7% sick absence (n=19), 10-24% sick absence (n=18). All data are presented as mean \pm SD. Where assumptions for normality were not met, the data were log transformed before statistical analysis. The untransformed values are shown in the text, tables and figures for more meaningful comparison. Statistical analyses were performed using SPSS (Version 14.0.1. 2005; SPSS Inc., Chicago, IL). The level of statistical significance was set at $p < 0.05$.

RESULTS

Purpose 1. The effect of the basic training period

VO₂max

All three submaximal marching tests and VO₂max tests were completed by a total of 21 conscripts. In 65% of cases, the reason for not participating in the test was upper respiratory tract infection (URTI). There was a main effect of training for VO₂max ($p < 0.001$), post-exercise lactate ($p < 0.05$) and maximum HR in VO₂max-tests ($p < 0.01$). Conscripts showed positive responses to training with an 11% increase in VO₂max after 5 weeks of training (before 45 ± 7 vs. wk5 49 ± 5 mL·kg⁻¹·min⁻¹, $p < 0.001$). From wk5 to wk8, VO₂max did not change significantly. In addition, the individual VO₂max values before training were negatively correlated with individual $\Delta\%$ VO₂max after 5 ($r = -0.61$, $p < 0.001$) and 8 ($r = -0.74$, $p < 0.001$) weeks of training. Post-exercise lactate was lower after 8 weeks of training (9.8 ± 2.1 mmol·L⁻¹)

compared to 5 weeks of training ($11.1 \pm 2.1 \text{ mmol} \cdot \text{L}^{-1}$, $p < 0.05$) and before training ($11.6 \pm 2.3 \text{ mmol} \cdot \text{L}^{-1}$, $p < 0.01$). Maximum HR was lower after 5 ($p < 0.01$) and 8 ($p < 0.001$) weeks of training compared to before training values (before $195 \pm 7 \text{ bpm}$, wk5 $190 \pm 7 \text{ bpm}$, wk8 $188 \pm 6 \text{ bpm}$).

Body composition

The initial average body height was $178.2 \pm 7.9 \text{ cm}$, BMI $24.7 \pm 4.5 \text{ kg} \cdot \text{m}^{-2}$ and F% $19.3 \pm 7.5\%$. The values before and after 4 and 7 weeks of training for BM, FM and FFM are presented in Table 2. From one subject, body composition measurement by eight-point bioelectrical impedance was not available at wk7, therefore the number of the subjects is 34 in Table 2. Both BM and FM exhibited a main effect of training ($p < 0.001$), decreasing from wk4 to wk7 ($p < 0.001$ and $p < 0.01$, respectively), with lower values compared to the before training values ($p < 0.001$). All subjects exhibited a decrease in FM after 7 weeks of training compared with the before training values (range from -28.0 to -0.4%). In contrast, the mean FFM was higher after 7 weeks of training compared with the before training values ($p < 0.05$) (Table 2).

Table 2 close to here

Basal serum hormone concentrations

There was a main effect of training for serum cortisol, SHBG and IGF-1 ($p < 0.001$) and for the testosterone/cortisol ratio ($p < 0.05$). Basal testosterone levels were similar at all three time points (Figure 1a). Basal SHBG increased, while basal cortisol and pre-exercise IGF-1 decreased from wk4 to wk7 ($p < 0.001$), being significantly different from the before training values ($p < 0.05$ - 0.001) (Figures 1b, 1c and 1d). Serum basal testosterone/cortisol ratio increased from wk4 to

wk7 ($p < 0.01$) (before 0.034 ± 0.013 , wk4 0.037 ± 0.016 , wk7 0.040 ± 0.012). However, 46% of the subjects showed an initial increase in basal testosterone during the first 4 weeks of training. Thereafter, basal testosterone decreased from wk4 to wk7 in these subjects. In contrast, 23 % of the subjects showed increases in basal testosterone throughout the study.

Figure 1 close to here

Responses to submaximal marching tests

There was a main effect of training for HR and RPE during submaximal marching ($p < 0.001$). HR was lower after 4 weeks ($p < 0.001$) compared with the before training values, and after 7 weeks of training compared with the wk4 and before training values ($p < 0.001$)(Table 3). RPE was lower after 7 weeks of training compared with the before training ($p < 0.001$) and wk4 values ($p < 0.01$)(Table 3). Blood La exhibited a main effect of training ($p < 0.001$) and exercise ($p < 0.001$), as well as a training and exercise interaction ($p < 0.001$) by increasing due to exercise before (179 ± 211 %, $p < 0.001$) and after 4 weeks of training (68 ± 91 %, $p < 0.001$), but not after 7 weeks of training (13 ± 49 %, n.s.). In addition, post-exercise La was lower after both 4 ($p < 0.001$) and 7 ($p < 0.001$) weeks of training compared with the before training values (Table 3). Serum testosterone and cortisol showed a main effect of exercise ($p < 0.001$). In response to exercise, serum testosterone increased significantly at each time point ($p < 0.05 - 0.001$) (before training 20 ± 23 %, $p < 0.001$; wk4 12 ± 27 %, $p < 0.01$; wk7 11 ± 25 %, $p < 0.05$). After 7 weeks of training, $\Delta\%$ testosterone induced by exercise was lower compared with the before training values ($p < 0.05$) (Figure 2). Serum cortisol increased significantly only after 4 weeks of training

(before $5 \pm 44\%$, n.s.; wk4 $16 \pm 35\%$, $p < 0.01$; wk7 $8 \pm 31\%$, n.s.). Individual variations in cortisol responses were large, ranging from -48 to 107 % (Figure 2).

Table 3 close to here

Figure 2 close to here

Questionnaire responses

The somatic symptoms associated with OTS exhibited a main effect of training ($p < 0.001$), whereby symptoms increased towards the end of BT. The greatest increase was observed between wk4 and wk7 ($p < 0.01$) (Figure 3). Concurrently, the proportion of subjects who answered “yes” to the question “Do you feel physically or mentally overloaded?” increased from 41% to 80% (Figure 4).

Figures 3 and 4 close to here

Purpose 2 and 3. Incidence of overreaching and differences between OR and noOR subjects

From a total of 57 subjects, OR criterion 1 was detected among 42% of subjects, criterion 2 32%, criterion 3 13%. According to the measured results before training, the subjects who fulfilled OR criteria 1, 2 or 3 were not significantly different from those who did not fulfill any OR criteria.

Criterion 4 was detected among 83% of subjects. Those who admitted to feeling physically or mentally overloaded in weeks 7 or 8 had lower $VO_2\max$ (3.3 ± 0.4 vs. 3.9 ± 0.5 L·min⁻¹,

$p < 0.001$), fat free mass (61.9 ± 0.8 vs. 68.7 ± 5.6 kg, $p < 0.05$) and higher HR in the submaximal marching test (152 ± 11 vs. 141 ± 10 , $p < 0.05$) before training compared to those who answered “no”.

Criterion 5 was detected among 32% of subjects. The subjects who had sick absences of more than 10% had lower VO_2max (46 ± 6 vs. 40 ± 7 $\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $p < 0.01$), basal testosterone (18.6 ± 4.3 vs. 14.9 ± 4.1 $\text{nmol} \cdot \text{L}^{-1}$, $p < 0.05$) and testosterone/cortisol ratio (0.036 ± 0.015 vs. 0.026 ± 0.007 , $p < 0.05$) before training compared to those who had no sick absences. In addition, subjects with more than 10% sick absence also had lower basal testosterone after 4 and 7 weeks of training (21.1 ± 7.5 vs. 17.0 ± 4.7 $\text{nmol} \cdot \text{L}^{-1}$ and 20.0 ± 6.1 vs. 15.5 ± 5.6 $\text{nmol} \cdot \text{L}^{-1}$, $p < 0.05$) (Figures 5a, 5b and 5c).

Figure 5 close to here

At least three of the five criteria were detected among 33% of the subjects, all of whom were classified as OR subjects. The remaining 67% were classified as noOR subjects. At the beginning of BT, only basal SHBG differed between OR and noOR subjects ($p < 0.01$) (Table 4). Similarly, OR subjects had higher basal SHBG than noOR subjects after 4 ($p < 0.01$) and 7 weeks of training ($p < 0.05$). Furthermore, OR subjects had higher basal serum cortisol than noOR subjects after 7 weeks of training ($p < 0.05$). Moreover, OR and noOR subjects differed in terms of the response of the basal testosterone/cortisol ratio to training from wk4 to wk7 ($p < 0.05$), and the maximal La/RPE ratio in the VO_2max test from baseline to the end of BT (Table 4).

Table 4 close to here

DISCUSSION

The purpose of the present study was to evaluate the training load of an 8-week Finnish military basic training period among new conscripts, to determine the incidence of OR during BT and to assess whether initial levels or training responses differ between OR and noOR subjects. The subjects' physical fitness characteristics in this study were representative of young Finnish men (30). However, there was a high incidence of dropouts from the performance tests; only 63% of the subjects completed all submaximal tests and 38% both submaximal and maximal tests. The dropouts did not mean that these subjects had more sick absence. However, serum testosterone, testosterone/cortisol ratio and $VO_2\text{max}$ were lower in "high incidence of sick absence" subjects than "no sick absence" subjects. Furthermore, subjects who admitted to feeling overloaded demonstrated low $VO_2\text{max}$, fat free mass and high submaximal test heart rate before training. These observations confirm the earlier finding that poor aerobic fitness is a risk factor for sick absence during military service (17), and highlight the importance of good physical fitness for overall wellbeing. A new finding was that testosterone had an impact on sustaining stress and maintaining health during the 8-week military training period.

For those conscripts who were able to perform all the tests, the increase in $VO_2\text{max}$ indicates an improvement in aerobic capacity. In addition, decreases in HR, post-exercise La and serum testosterone during the 45-min submaximal marching test indicate decreased exercise-induced metabolic stress (28, 40) during the first four weeks of BT. Furthermore, as expected, our data

showed an inverse relationship between initial VO_{2max} and relative changes in VO_{2max} , indicating that the training was most effective for the least fit subjects. This is comparable with studies performed in the Polish (9) and USA (28) armies.

During the second half of the 8-week training period, a stagnation of increase in VO_{2max} was observed. Concurrently, basal serum IGF-1 decreased and basal serum SHBG and testosterone/cortisol ratio increased with no change in basal serum testosterone. Furthermore, basal serum cortisol decreased with a concomitant increase in the number of subjects who felt overloaded. Previously, serum total testosterone concentration has been shown to decrease in response to demanding training periods (6, 37), as well as during extremely stressful military training periods (25, 26), and to increase with overload training (36). In contrast to the present study, basal SHBG has been found to increase (19, 23) and IGF-1 to decrease (23-26) after heavy training. A decrease in IGF-1 is suggested to indicate a diminished capacity for protein deposition. In addition, IGF-1 is sensitive to both energy intake and dietary protein content, and demonstrates significant decreases with inadequate dietary intake (23, 24). Furthermore, a 7 week intensive endurance overload training program (36) and combined endurance and strength overload training for 6 weeks (5) have been found to decrease testosterone/cortisol ratios. Even the biochemical changes of testosterone and testosterone/cortisol ratio are controversial compared to previous findings, a stagnation of increase in VO_{2max} in this study indicates that the training load during the second half of the 8-week training period may have been too exhaustive. This is also supported by a decrease in maximal post-exercise lactate and HR (14, 22, 38), as well as the fact that 46% of subjects showed a decrease in basal serum testosterone from wk4 to wk7.

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, and OR was detected among 33% of the subjects. The only previous study concerning OR/OTS among young men who entered military service and performed an 8-week BT training period was by Chicharro et al. (4). They found a 24% incidence of OTS during BT, determined by absolute decreases in the free testosterone/cortisol ratio, a classical criteria for OR/OTS (2, 5, 19). Similarly, in this study, OR subjects differed in response to training compared to noOR subjects. OR subjects exhibited a tendency towards a decrease in basal $\Delta\%$ testosterone/cortisol ratio, whereas noOR subjects exhibited an increase. Snyder et al. (31) defined five criteria for OR, one of which was a decreased maximal La/RPE ratio. In the present study, the maximal La/RPE ratio tended to decrease during the latter part of BT in OR subjects.

To our knowledge, no previous studies have a reported relationship between basal serum SHBG and the incidence of OR. In the present study, OR subjects had higher levels of SHBG, both before training and after 4 and 7 weeks of training. Although basal SHBG has been found to increase after heavy training (19, 23), the mechanism underlying the present findings is still not fully understood. Testosterone did not differ between noOR and OR subjects. However, most of the testosterone is bound to SHBG, which in turn decreases bioavailable testosterone in serum, and may thereby have an influence on training responses.

OR subjects had higher basal serum cortisol at the end of BT, which is in line with the OR scenario presented by Steinacker et al. (33), where an increase in basal cortisol was observed in

the OR state. However, a decrease in basal serum cortisol during the second half of BT was observed among the 35 conscripts who performed all submaximal tests. This may be a marker of overtraining, along with lower psychosocial stress (29, 31, 33, 37). Basal serum cortisol has been found to increase during extremely stressful military training periods (25, 26), but no change (6, 19, 36), or a decrease (29, 31) has been observed in endurance based overtraining studies. However, in response to strenuous resistance training, both no change (11, 18) and an increase (10) of basal serum cortisol have been reported. Glucocorticoids, like cortisol, exert many beneficial actions in exercising humans: increasing the availability of metabolic substrates for the energy requirements of muscles, maintaining normal vascular integrity and responsiveness, and protecting the organism from an overreaction of the immune system in response to exercise-induced muscle damage (29, 33). Differences in cortisol behavior between military and overtraining studies, as well as incoherent training responses of basal serum cortisol in this study, may be explained by the differences in energy availability, training mode and fitness levels of the subjects. The military training studies were performed under conditions of nutritional and sleep deprivation, in combination with high energy expenditure and physiological strain. Most of these studies involved relatively short (from 5 to 14 days) training periods, and the subjects were soldiers. In the overtraining studies, food and sleep were ad libitum, and the subjects were athletes, or at least had a good fitness level. Furthermore, the training period in the overtraining studies ranged from 2 to 7 weeks, consisting mostly of endurance or strength training. 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.

Recently, it has been proposed that the best indicator of OR and OTS is the physiological response to an acute stress, such as an exercise test (22). Only 47% of the OR subjects performed all submaximal tests because of sick absences. This may be the reason for the non-significant difference in cortisol responses to exercise between OR and noOR subjects. However, among the conscripts who were able to perform all submaximal tests, the exercise induced increase in cortisol in this study was only significant after 4 weeks of training. It has been suggested that increased basal levels of cortisol contribute to exhaustion of the hypothalamic-pituitary-adrenal axis, thus preventing an adequate cortisol response to such an acute stress (10). In the present study, the attenuated cortisol response before training could be explained by the higher basal values, although this seems unlikely as pre-exercise cortisol levels were not associated with cortisol responses to the submaximal 45-min marching test. In the taper period (recovery period) of training, adreno-corticotrophic hormone (ACTH) and cortisol increase in response to metabolic stress; during overreaching, the cortisol response is blunted along with an augmented ACTH response (33). Overtraining is characterized by a decrease in ACTH and cortisol responses (33), and exercise induced decreases in cortisol have been linked to intensive training (37). Thus, in this study, the increased response of cortisol to exercise after 4 weeks of training among the 35 conscripts who completed all tests indicates an improvement in performance, and an attenuated response after 7 weeks is indicative of overreaching.

PRACTICAL APPLICATIONS

The fitness level of Finnish young men has decreased with a concurrent increase in body mass (30). Currently, approximately 15% of new conscripts interrupt the compulsory military service within one month. Consequently, many of the conscripts have a low fitness level. However, the same physical training program has to be performed by all conscripts in the military environment. In the present study, improvements in physical performance were observed during the first 4 weeks of BT, such as an increase in maximal aerobic capacity and decreased submaximal exercise-induced stress. Although general diagnostic parameters to diagnose OR/OTS have not yet been found, several changes in biomarkers and physical performance may indicate that the training load during the second half of the 8-week training period was not well tolerated by all the conscripts. Furthermore, both emotional and somatic symptoms of OR/OTS increased.

Since 33% of the subjects were classified as overreached, the end of the BT period has to be taken under careful consideration. If demanding training is continued after BT, overtraining syndrome may develop. Thus, the weeks after BT should involve recovery type training. Furthermore, if the amount of sick absence is set as a criterion for the tolerance to exercise training, these results suggest that in order to enhance physical fitness and to avoid OTS, conscripts could be divided into different training groups according to their aerobic fitness level. Initial serum basal SHBG and testosterone, as well as testosterone/cortisol levels, would give additional information, but in practise, collecting the samples from a large group of subjects is

challenging. However, our results confirm that serum cortisol and the testosterone/cortisol and maximal La/RPE ratios could be useful tools for monitoring whether training is too stressful.

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FIGURE LEGENDS

FIGURE 1. Mean (\pm SD) basal serum testosterone (a), SHBG (b), cortisol (c) and IGF-1 (d) concentrations before, and after 4 and 7 weeks of basic military training in 35 conscripts. Difference compared to: baseline *** $p < 0.001$, * $p < 0.05$; week 4 $\alpha\alpha\alpha$ $p < 0.001$. There was a main effect of training for SHBG, cortisol and IGF-1 ($p < 0.001$). $n = 35$.

FIGURE 2. Mean (\pm SD) serum testosterone and cortisol responses (%) to the 45-minute submaximal marching test before, and after 4 and 7 weeks of basic military training. Increase due to exercise; $\wedge\wedge\wedge$ $p < 0.001$, $\wedge\wedge$ $p < 0.01$, \wedge $p < 0.05$. Difference compared to baseline; * $p < 0.05$. There was a main effect of exercise for testosterone and cortisol ($p < 0.001$). $n = 35$.

FIGURE 3. Mean (\pm SD) sum of the somatic symptoms scores associated with OTS, defined from five-point Likert scale questionnaires during the 8-week military basic training period. Significant difference; *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. There was a main effect of training for somatic symptoms ($p < 0.001$). $n = 35$.

FIGURE 4. The proportion of subjects who answered “yes” or “no” to the question “Do you feel physically or mentally overloaded?” ($n = 35$).

FIGURE 5. Mean (\pm SD) VO_2 max and (a) basal serum testosterone/cortisol ratio (b) before training and basal serum testosterone (c) before, and after 4 and 7 weeks of basic military training in conscripts who had a sickness absence of 0%, 2-7% and 10-24% of days. Difference compared to the 0% group; * $p < 0.01$. $n = 57$.

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 marching test	X			X			X	
Body composition	X			X			X	
Blood samples	X			X			X	
Questionnaire	X			X		X	X	X

TABLE 2. Body mass (BM), fat mass (BM) and fat free mass (FFM) before, and after 4 and 7 weeks of military basic training (n=34).

	Before		After 4 weeks			After 7 weeks				Before vs. after 7 weeks
	Mean	±SD	Mean	±SD	Δ%	Mean	±SD	Δ%	p	Δ%
BM (kg) [#]	78.7	17.7	78.5	16.9	0.0	77.0	15.9	-1.8	***, □□□	-1.8
FFM (kg)	62.6	9.4	62.9	8.8	0.7	63.0	9.3	0.1	*	0.8
FM (kg) [#]	16.1	10.7	15.6	10.7	-3.8	14.0	9.1	-7.5	***, □□	-12.6

Difference compared to; before *** p<0.001, * p<0.05; week 4 □□□ p<0.001, □□ p<0.01. Main effect of training, [#] p<0.001.

TABLE 3. Heart rate (HR), RPE and blood lactate (La) responses to the 45-min submaximal marching test at 70% of VO₂max before, and after 4 and 7 weeks of military basic training (n=35).

	Before		After 4 weeks				After 7 weeks				Before vs. after 7 weeks	
	Mean	±SD	Mean	±SD	p	Δ%	Mean	±SD	p	Δ%	Δ%	
HR (bpm) [#]	154	11	148	11	***	-4	140	12	***, □□□	-5	-9	
RPE [#]	13.1	1.7	13.2	1.7		1	12.1	0.9	** , □□□	-8	-7	
Post-exercise La (mmol·L ⁻¹) [#]	2.9	2.0	1.7	0.8	***	-29	1.1	0.5	***, □□□	-26	-48	

Difference compared to: before *** p<0.001, ** p<0.01; week 4, □□□. Main effect of training [#] p<0.001.

TABLE 4. Differences between OR and noOR classified subjects before and during military basic training.

	OR subjects		noOR subjects		p
	Mean	±SD	Mean	±SD	
Basal serum SHBG at baseline (nmol·L ⁻¹)	38.4	16.9	27.9	7.9	**
Basal serum SHBG after 4 weeks (nmol·L ⁻¹)	38.4	14.1	28.5	8.6	**
Basal serum SHBG after 7 weeks (nmol·L ⁻¹)	46.4	19.2	36.6	10.6	*
Basal serum Cortisol after 7 weeks (nmol·L ⁻¹)	545	145	465	97	*
Change (%) in basal testosterone/cortisol ratio from after 4 to after 7 weeks	-6	23	24	32	*
Change (%) in maximal La/RPE ratio from before to after 8 weeks	-30	18	-3	20	**

Difference compared to OR; ** p<0.01, *p<0.05.

