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## **Research Article**

## Heart Rate Variability Recordings are a Valid Non-Invasive Tool for Evaluating Soldiers' Stress

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## Abstract

The purpose of the present study was to investigate physiological responses and to evaluate heart rate variability as a non-invasive stress indicator during a 72-hour military field training (MFT). Ten healthy male soldiers (age 20 ± 1 yr.) participated in MFT. They slept approximately 2 h/day and ate only army field rations. During MFT, the soldiers' mean (±SD) energy expenditure was 4646 ± 674, energy intake 2200 ± 326, and energy deficit (ED) 2405 ± 890 kcal·day-1. Throughout the entire training period, serum total testosterone (TES) reduced from 19.0  $\pm$  3.0 to 12.6  $\pm$  6.2 nmol·l<sup>-1</sup> (p<0.001). Mean HR during the entire MFT was  $85 \pm 6$  bpm. RMSSD, which reflects cardiac vagal activity, decreased from 54  $\pm$  19 to 42  $\pm$  12 ms (p<0.05). No changes (p>0.05) were observed in HF and LF power, but changes in HF power correlated with baseline serum TES (r=-0.72, p<0.05). Based on the present findings, working for 72 h at a relatively low level of cardiovascular strain with ED and sleep deprivation can individually modify hormonal responses in association with cardiac vagal outflow. This suggests that HRV can be used as a non-invasive tool to measure stress in soldiers during MFT.

## Keywords

Hormone; Physiological stress; Military field training; Stress

## Introduction

Modern military operations are still very physically demanding for soldiers. Although soldiers' equipment has developed, the increased load of combat gear has resulted in greater physiological strain on individual soldiers [1]. To a dismounted infantry squad, this is a relevant part of daily life, as foot patrolling requires soldiers to carry all of their mission equipment and food in their backpacks. Investigation of the operational environment and physical loading affecting soldiers requires consideration of the multistressor environment [2,3].

Military operational stress generally consists of sustained physical and mental exertion combined with sleep and energy deprivation [3-5]. This has been shown to induce disturbances in hormonal sympatovagal balance, as indicated by increased susceptibility to infections, diminished physical and cognitive performance

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capabilities and longer recovery times [5-7]. Intense military field exercises have been reported to induce decreases in basal concentrations of circulating testosterone (TES) and free testosterone (TES<sub>free</sub>) [8]. In military circumstances, dehydration is also a common phenomenon [9] leading to weakened physical performance [10]. In addition, soldiers often suffer sleep and caloric deprivations during military field exercises. These factors have also been shown to decrease physical performance [11-14].

Often it is quite difficult to measure a level of physiological strain. Therefore, new methods which are easy to use and to evaluate objectively overtraining or stress reactions during military service are warranted. Heart rate variability (HRV) is a relatively new method used to study physiological strain and body homeostasis via autonomic nervous system (ANS) activity [15]. In a military environment, it has been shown to be a practical tool for screening initial fatigue [5]. In particular, RMSSD (the square root of the mean of the sum of the squares of differences between adjacent R-R intervals) is the most frequently used time domain method in HRV analysis, and has been shown to reflect vagus-mediated HRV. Power spectral densities have been used to study cardiac vagal activity (HF, 0.15-0.40 Hz), sympathetic and vagal outflow (LF, 0.04-0.15 Hz), and sympathovagal interaction (LF/HF ratio) [16,17]. Recently, it has been shown in military environment that there is a relationship between changes in cardiac vagal regulation during a long-term mentally stressful condition and the serum TES-to-cortisol ratio [18]. Later, this finding was confirmed by demonstrating that individual changes in heart rate and HRV were strongly associated both with changes in aerobic fitness and with changes in anabolic hormone concentrations during the stressful military training [19].

The purpose of the present study was to investigate further associations between HRV and anabolic hormones in a more stressful condition. Thus changes in TES,  $\text{TES}_{\text{free}}$ , SHGB, and sympathovagal balance were studied during a 72-hour dismounted infantry squad. The unique part of this study was to associate TES and HRV data during a field training of soldiers. It was hypothesized that soldiers exposed to considerable energy and fluid deficits, sleep deprivation, diminished recovery time, and prolonged physical exercise may undergo stress-related physiological changes.

## Methods

## Subjects

Ten healthy male soldiers (age  $20 \pm 1$  yr., height  $1.79 \pm 0.07$  m, body mass  $74.5 \pm 7.9$  kg, body fat  $12.3 \pm 1.7\%$ ) participated in a 72-hour dismounted infantry squad training. They were randomly selected from a larger group of volunteers, and they were informed of all test procedures. Subjects were physically fit maximal oxygen uptake, (VO<sub>2</sub>max 55.9  $\pm$  3.8 ml·kg<sup>-1</sup>·min<sup>-1</sup>) and provided written informed consent to participate in this study. The present study was approved by the Ethical Committee of the University of Jyväskylä.

## Military field training (MFT)

The day before MFT (0 day) consisted of lessons and short military drills in a garrison. MFT, which lasted 72 h, was a simulated two-

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sided battle designed to practice reconnaissance patrolling against an opposing force in forest. Terrain was nearly flat but rough because of brushwood, many ditches and swamps. In the beginning of MFT, soldiers' equipment, supplies and clothing weighed 29.0  $\pm$  6.5 kg, corresponding to an average of 39% of their body weight. During the missions, they moved 7.3-10.7 km/day [recorded by FRWD W600 (FRWD technologies, Oulu) based on Global Positioning System (GPS)] in forest terrain and slept 1.7  $\pm$  1.1 h-night<sup>-1</sup>. Participants only consumed army field rations having a daily mean energy content of 3426  $\pm$  112 kcal. They also carried daily water supply in their backpacks, and their water intake was 2.3  $\pm$  0.9, 3.2  $\pm$  0.7 and 3.3  $\pm$  0.9 l-day<sup>-1</sup> in the three consecutive MFT days. The weather was rainy on the first day, but remained dry thereafter, with a temperature of approximately +13°C.

## Procedure and measurements

Initial measurements were performed one day before and in the beginning of MFT, while follow-ups were performed at each of the next three days. Blood samplings from the antecubital vein, questionnaires and weight measurements were implemented daily between 08:00 and 09:30 am.

For studying energy and fluid intake (EI), the subjects kept daily food diaries. This process was carefully instructed and supervised. Sleep duration was inquired individually every morning. Heart rate (HR) and heart rate variability (HRV) were continuously recorded with a portable heart rate monitor (SUUNTO Smart Belt, Suunto, Vantaa, Finland). The recordings conformed to Task Force [16] standardized 24-hour recordings and were only interrupted while downloading data from the device to a computer.

#### Analysis

Testosterone (TES) was analyzed by Electrochemiluminescence immunoassay (ECLIA) with a Modular E170 (Roche Diagnostics, Mannheim, Germany) Immuno analyzer. Sex hormone binding globulin (SHBG) was analyzed by Immunoluminometric assay (Modular E170, Roche Diagnostics, Mannheim, Germany). Free testosterone was calculated using Andersson's formula: TES  $(p \cdot mol \cdot l^{-1})$ =TES (nmol · l^{-1}) x {2.28 - 1.38} x log [SHBG (nmol · l^{-1}) x 0.1] x 10 [20]. The sensitivity and intra-assay coefficient of variance for the assays were reported earlier [20]. The percentage change in plasma volume (PV) was calculated from changes in haemoglobin and haematocrit according to the method of Dill and Costill [21].

HR and HRV were analyzed with heartbeat analysis software (FIRSTBEAT Technologies Oy, Jyväskylä, Finland). The software estimates  $VO_2$  from HR, respiratory rate and on/off-dynamics information, while calculation of energy expenditure (EE) was based on  $VO_2$ , respiratory quotient and caloric equivalent. A validation study of the method was reported earlier by Smolander et al. [22].

#### Statistical analysis

The data were analyzed statistically by ANOVA for repeated measures and its nonparametric equivalent, the Friedman test, when normality assumptions were not met. The HRV values were transformed to the natural logarithms of the absolute values. In addition, either Pearson's or Spearman's correlation analyses were used to study associations between hormones and HRV variables. The level to achieve statistical significance was p<0.05. All data are presented as mean ±SD.

## Results

#### Energy balance and body mass alterations

Mean EE during the 72-hour MFT was  $4646 \pm 674$  kcal/day. Based on EE, the most physically demanding training phase was day 2 (5170  $\pm$  603 kcal), whilst EE was lowest during the first 24 hours ( $4112 \pm 773$  kcal). On the average, the total daily energy intake (EI) was  $2200 \pm 326$  kcal on each day of the training. However, individual variations were considerably high. Energy balance calculations (energy expenditure–energy intake) revealed that the mean energy deficit (ED) was  $2405 \pm 890$  kcal/day, with the greatest value occurring at day 2 ( $2978 \pm 1270$  kcal/day), when EE was also highest.

Before MFT, the mean body mass of the subjects was  $74.5 \pm 7.9$  kg, which decreased to  $72.3 \pm 7.8$  kg (p<0.001) after the training. Thus, mean body mass decreased by  $2.2 \pm 0.8$  kg ( $2.9 \pm 1.0\%$ , p<0.001). Plasma volume decreased by -4.1% (p<0.01), -3.2% (p<0.05), and -4.3% (p<0.01) in three consecutive days, respectively.

## **Testosterone Responses**

The hormonal responses to MFT are shown in Figure 1. Mean serum TES (p<0.01) and TES<sub>free</sub> (p<0.01-0.05) decreased after 48 and 72 hours of MFT. During the entire MFT, mean TES reduced by 34% from 19.0  $\pm$  3.0 to 12.6  $\pm$  6.2 nmol·l<sup>-1</sup>(p=0.008) and TES<sub>free</sub> by 31% from 56.0  $\pm$  19.2 to 38.9  $\pm$  21.8 pmol·l<sup>-1</sup> (p=0.04).

## Heart rate (HR) and heart rate variability (HRV)

The mean HR during MFT was  $85 \pm 6$  bpm, and the mean HR relative to the maximal individual level (%HR max) was  $44 \pm 4$ %. The highest HR values were measured on the second day, when the mean daily HR increased from  $76 \pm 9$  to  $90 \pm 6$  bpm (p<0.01), and %HR max from  $40 \pm 5$  to  $46 \pm 4$ % (p<0.01). Ambulatory measurements of HRV revealed no significant changes in HF power ( $6.93 \pm 0.50$  at day 0,  $6.51 \pm 0.82$  day 1,  $6.62 \pm 0.87$  day 2, and  $6.33 \pm 0.50$  ln ms<sup>2</sup> at day 3). The situation was the same for LF power, while the RMSSD values decreased from  $54 \pm 19$  to  $39 \pm 12$  ms (p<0.05) by the second day of MFT, and remained at  $42 \pm 12$  ms (p<0.05) on the third day. Throughout the entire MFT, the average value of RMSSD decreased from  $54 \pm 19$  to  $43 \pm 11$  ms (p<0.05). In spite of insignificant changes in HF power, individual changes were related to the baseline serum TES concentrations(r=-0.72, p<0.05) (Figure 2).

## Discussion

The present results clearly demonstrate that even a relatively low level of cardiovascular strain in a military environment, involving





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several stressors, can modify hormonal functions. In addition, it seems that prolonged military operational stress activates the autonomic nervous system, and that the cardiac vagal activity cannot be fully activated without sufficient sleep. However, high interindividual variability is evident in HRV data. Individual changes in HF power induced by MFT were strongly associated with baseline serum TES concentrations in this study. This finding may indicate an association between cardiac vagal activity and TES concentration. In other words, HF power seems to reflect altered regulation of both neural and hormonal mechanisms. Thus, due to the fact that the measurement of HRV is a non-invasive and reliable method, it can be utilized to determine individually suitable physical loads in military environment.

According to Friedl et al. [2], in prolonged military training, soldiers' body weight decreased by 7.8% and TES by 74%, compared to 2.8% and 34% in the present study. A noteworthy finding in that study was the immediate endocrinologic response to restoration of the energy balance. This kind of severe energy deficit also negatively affects physical and psychological performance [7] as well as resistance against diseases and infections [6]. The present results also indicate slight dehydration of the subjects. Mean daily water intake  $(2.9 \pm 0.8 \text{ l})$  did not correspond to the calculated water requirement, the minimum being 4.6 l according to EE (1ml/kcal). The decreased plasma volume supports this interpretation.

This study also indicates that physically fit soldiers are capable of training well with a 2400 kcal/day energy deficit in dismounted infantry squad missions lasting up to three days, although this resulted in reduced body weight. Castellani et al. [23] have also shown that soldiers experiencing a relatively short duration caloric deficit could maintain high EE and activity levels. In the present study, however, it is impossible to separate different stressors affecting decreases in TES and TES<sub>free</sub> concentrations but energy deficit and sleep deprivation might be the main candidates in this regard.

In the present study, the alterations of TES and TES<sub>free</sub> support the findings of previous studies. In military field exercises, where restricted recovery combines with physical and mental stress, the concentrations of TES and TES<sub>free</sub> have been shown to decrease [24]. According to Nindl et al. [3], 84-hours of military operational stress caused a 24% decrease in TES and a 30% decrease in TES<sub>free</sub>. During

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a 3-week military field exercise performed in similar conditions, Kyröläinen et al. [14] reported decreases of 27% and 26% in TES and TES<sub>free</sub>, respectively. In the present study, a 72-hour MFT resulted in a 34% decrease in TES and a 31% decrease in TES<sub>free</sub>, which were similar to the values reported previously. Nevertheless, the physiological mechanism causing the long-term decrease in TES levels is partly unclear. Nindl et al. [3] have reported that short-term military operations increased luteinizing hormone concentration, the signalling molecule for testosterone, which regulates TES production.

In conclusion, 72-hours of military operational stress due to dismounted infantry squad training is related to ED and sleep deprivation. Thus, working for 72 h at a relatively low cardiovascular strain level in a multistressor environment can modify cardiac vagal outflow, which may reflect individual adaptations of the autonomic nervous system to the exercise task. Interestingly, these changes seem to be associated with changes in serum TES levels. This suggests that HRV can be used as a non-invasive tool to measure stress in soldiers during MFT.

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