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Abstract

Background/Aim. The risk assessment of heat illness and fatigue development is very important in military services. The aim of our study was to investigate the relationship between heat storage and various psychophysiological parameters of heat stress, as well as potential peripheral markers of fatigue in soldiers performing exertional heat stress test.

Methods. 15 young, healthy and unacclimatized men underwent exertional heat stress test (EHST) with submaximal work load in warm conditions (WBGT 29 °C) in climatic chamber. Every 5 minutes following parameters of thermotolerance were measured or calculated: core temperature (Tc), mean skin (Tsk) and body temperature (Tb), heart rate (HR), heat storage (HS), physiological strain index (PSI), as well as peripheral markers of fatigue (blood concentrations of ammonia, urea nitrogen (BUN), lactate dehydrogenase (LDH), cortisol and prolactin) and subjective parameters: thermal sensation (TS) and rate of perceived exertion (RPE).

Results. Tolerance time varied from 45-75 minutes (63±7,7 min). Average values of Tc, Tb, and HR constantly increased during EHST, while Tsk after 10 minutes reached the plateau. Concentrations of all investigated peripheral markers of fatigue were significantly higher after EHST compared to baseline levels (31,47±7,29 vs. 11,8±1,11 µmol/l for ammonia; 5,92±0,73 vs. 4,69±0,74 mmol/l for BUN, 187,27±28,49 vs.152,73±23,39 U/l for LDH, 743,43±206,19 vs. 558,79±113,34 mmol/l for cortisol and 418,08±157,14 vs. 138,79±92,83 μIU/mL for prolactin).

Conclusions. This study demonstrates the relationship between heat storage and Tc, HR, TS and RPE, but also with PSI. Concentrations of cortisol and especially prolactin showed significant correlation with parameters of thermotolerance.

Key words: exertional heat stress; hormonal response; fatigue; heat storage.
Apstrakt

Uvod/Cilj. Procena rizika od nastanka zamora i nekog oblika toplotne bolesti je od velikog značaja za vojnu službu. Cilj ovog istraživanja je da se utvrdi povezanost između stepena akumulacije toplote i različitih psihofizioloških parametara topotnog stresa, kao i mogućih perifernih markera zamora u populaciji vojnika izloženih toplotnom stresu kombinovanim sa fizičkim naporom. Metode. 15 mladih, zdravih, utreniranih i neaklimatizovanih muškaraca podvrgnuto je testu toplotnog stresa kombinovanog sa fizičkom aktivnošću pod submaksimalnim opterećenjem u klimatskoj komori. Na svakih 10 minuta registrovane su ili izračunate sledeće vrednosti parametara termotolerancije: unutrašnja (timpanična) temperatura (Tc), srednja temperatura kože (Tsk) temperature tela (Tb), frekvencja srčanog rada (HR), akumulacija toplote (HS), indeks fiziološkog napora (PSI), zatim perifernih markera zamora (koncentracije amonijaka, urea azota (BUN), laktatne dehidrogenaze (LDH) i kortizola, kao i subjektivnih parametara toplotnog osećaja (TS) i stepena napora (RPE). Rezultati. Vreme tolerancije je iznosilo 45-75 minuta (63±7,7 min). Prosečne vrednosti Tc, Tb, i HR konstantno su rasle tokom EHST, dok je Tsk dostigla plateau nakon prvih 10 minuta. Koncentracije svih ispitivanih perifernih markera zamora bile su značajno veće nakon EHST u odnosu na vrednosti pre testa (31,47±7,29 vs. 11,8±1,11 µmol/l za amonijak; 5,92±0,73 vs. 4,69±0,74 mmol/l za BUN, 187,27±28,49 vs.152,73±23,39 U/l za LDH, 743,43±206,19 vs. 558,79±113,34 mmol/l za kortizol i 418,08±157,14 vs. 138,79±92,83 μIU/mL za prolaktin). Zaključak. Rezultati ukazuju na povezanost između stepena akumulacije toplote i Tc, HR, TS i RPE, ali takođe i PSI. Koncentracije kortizola, a naročito prolaktna pokazuje značajnu povezanost sa parametrima termotolerancije.

Ključne reči: toplotni stres; hormonski odgovor; zamor; akumulacija toplote
**Introduction**

Fatigue is generally useful mechanism in prevention the harmful exertion and damage of organism. On the other hand, the development of fatigue/exhaustion during strenuous physical work is of major importance for performance in context of sports activities as well as in military services. There are several physiological and psychological factors related to the onset of fatigue: environmental conditions (especially high temperature and/or high humidity), duration and intensity of physical activity, supplementation, hydration status, motivation and level of physical fitness. The feeling of fatigue is triggered by complex processes resulted from peripheral and central factors. Peripheral fatigue occurs within the muscle and is related to impairment in neuromuscular and muscular structures and functions (1), while central fatigue considers alterations in efferent neurons and impaired neurochemistry in brain, such as interplay between dopamine and serotonin, which affects mood and motivation (2). Hence, some peripheral parameters in blood may serve as markers of fatigue, such as lactate, ammonia, stress hormones and pro-inflammatory interleukins (3).

The risk assessment of heat illness development is also very important in military services. There are numerous indices used in prediction of excess heat strain during physical activity in hot conditions. The most commonly used are environmental parameters or their combination such as WBGT (Wet Bulb Globe Thermometer) (4). Furthermore, physiological parameters of thermotolerance are also used, such as core and skin temperatures and heart rate, with models developed to predict heat stress such as physiological strain index (PSI). Estimation of heat storage in body is also used to evaluate the both fatigue and potential risk of overheating. Finally, some subjective parameters such as thermal sensation (TS) and rate of perceived exertion (RPE) may also serve in prediction of development of heat strain and fatigue (5).

Considering the importance of heat strain and fatigue in military personnel, the aim of our study was to investigate the relationship between heat storage and various psychophysiological parameters as well as potential peripheral markers of fatigue in soldiers performing exertional heat stress test.
Methods

Study population consists of 15 male soldiers aged 19-21 years, healthy, fit and unacclimatized. Investigation was conducted in Military Medical Academy (MMA) in Belgrade, designed as experimental study. The study was approved by the local Ethics Committee, and signed informed consent was obtained from each participant. The subjects performed exertional heat stress test (EHST) by walking on a treadmill with submaximal work load in warm conditions (40 °C, Wet Bulb Globe Thermometer (WBGT) 29 °C) in climatic chamber (Weiss Technik, Germany). Core (tympanic) temperatures (Tc), and mean skin temperatures (Tsk) as well as heart rates (HR) were continuously measured every 10 minutes using system for data acquisition MP 150 SKT100C (BIO PAC Systems Inc. USA) and Q4500 Exercise Test Monitor (Quinton Instruments, USA), respectively. Detailed descriptions of methods of temperature measurements are presented in our previous study (6). The protocol of EHST and criteria for termination were also previously presented (7). Before and immediately after EHST venous blood samples were collected for analysis of peripheral markers of fatigue: concentrations of ammonia, blood urea nitrogen (BUN), lactate dehydrogenase (LDH), cortisol and prolactin, and analyzed in Institute of Medical Biochemistry, MMA. At the beginning of the EHST and every 10 minutes during test as well as at the moment when the subjects finished their tests they assessed their subjective thermal sensation (TS) and rate of perceived exertion (RPE) using a modified Gagge 8-point scale (8) with verbal descriptions between “cool” (ranking 5) and “unbearably hot” (ranking 13) and Borg 15-point scale of RPE (9) with verbal descriptions of physical work load between “very, very light” (ranking 6) and “very, very hard” (ranking 20), respectively.

Calculations: Body temperature (Tb) was calculated as:

\[ Tb = K + Tc + (1 - K) \times Tsk \]

Where Tc represents core (tympanic) temperature and Tsk mean skin temperature. In warm conditions, K has constant value of 0.9 (10).

We used Tikuisis’ modification of Moran’s calculation for physiological strain index (PSI):

\[ PSI = 5 \times \frac{Tc - Tc(0)}{39.5 - Tc(0)} + 5 \times \frac{HR - HR(0)}{180 - HR(0)} \]


Where Tc and HR represent current values of tympanic temperature and heart rate while Tc (0) and HR (0) represent values of the same parameters at rest (5).

Heat storage (HS) was determined using Havenith’s calculation as follows:

\[ HS = ((0.8 \times (Tc - Tc(0))) + (0.2 \times (Tsk - Tsk(0)))) \times 3.49 \text{ J/g} \]

where Tsk represent current value of mean skin temperature and Tsk (0) initial mean skin temperature, and 3.49 J/g is specific heat of the body tissues (11).

Since some subjects had shorter exposures than others, we introduced the rate of change (ROC) in investigated parameters with calculation as follows (12):

\[ ROC(x) = \frac{x(\text{end}) - x(0)}{T} \]

Where x (end) is investigated parameter (Tc, HR, PSI, HS, TS, RPE) at the end-point of EHST, x (0) is value of the same parameter before the start, and T is total exercise time.

Statistical analysis: Normality of data was tested by Kolmogorov-Smirnov test. Data were presented as mean ± standard deviation (SD). The significance of differences between time points was tested using t-test and Tukey’s test for pairwise comparisons. The significance of relations was test using Pearson’s correlation. The statistical significance was accepted at p<0.05. All statistical analyses were performed using SPSS 18 package (Chicago, USA).

Results

Baseline anthropometric and ergometric characteristics of the participants are presented in Table 1.

Table 1. Anthropometric and ergometric characteristics of subjects

| Characteristic           | Mean ± SD      | Range              |
|-------------------------|----------------|--------------------|
| Body weight (kg)        | 76.14±7.12     | 65.86 – 88.32      |
| Body mass index (kg/m²) | 22.9±1.82      | 20.3 – 25.8        |
| Body surface area (m²)  | 1.97±0.09      | 1.84 – 2.14        |
| Body fat (%)            | 17.53±3.33     | 13.8 – 23.1        |
| Lean body mass (kg)     | 62.77±5.15     | 55.65 – 72.89      |
| VO₂max (ml/kg LBM)      | 68.22±12.16    | 52.13 – 89.98      |
Tolerance time before termination of EHST (due to reaching the ethical barrier for Tc of 39.5 °C or unbearable subjective discomfort) varied between 45 and 75 minutes (average time was 63±7.70 min). Average values of core and body temperatures were very close and constantly increased during EHST, while mean skin temperature reached the plateaux after first 10 minutes, i.e. when sweating occurred. Average hearts rate raised in the same manner (Fig. 1 and 2).

Figure 1.
Average values of core, skin and body temperatures during EHS

Tc=core (tympanic) temperature
Tsk=mean skin temperature
Tb=body temperature
Concentrations of all investigated peripheral parameters of fatigue were significantly higher after EHST compare to basal values (Table 2).

Table 2.

|                | Before EHST | After EHST | p     |
|----------------|-------------|------------|-------|
| Ammonia (µmol/l) | 11,8±1,11   | 31,47±7,29 | <0,001|
| BUN (mmol/l)     | 4,69±0,74   | 5,92±0,73  | <0,001|
| LDH (U/l)        | 152,73±23,39| 187,27±28,49| <0,001|
| Cortisol (mmol/l)| 558,78±113,34| 743,43±206,19| =0,001|
| Prolactin (µIU/mL)| 138,79±92,83| 418,08±157,14| <0,001|

BUN – blood urea nitrogen
LDH – lactate dehydrogenase

Average values of HS and levels of PSI constantly increased during EHST following almost identical pattern (Fig. 3). Subjective measures of TS increased in the first 40 minutes and after that we recorded the plateau closely to maximal values for given scale, while RPE values continue to rise up to the end of test, approaching the maximal value (Fig. 4).
HS=heat storage
PSI=physiological strain index

Figure 3.
Average values of heat storage and physiological strain index during EHST

TS=thermal sensation (modified Gagge’s scale)
RPE=rate of perceived exertion (Borg’s scale)

Figure 4.
Average values of thermal sensation and rate of perceived exertion during EHST
Heat storage strongly correlated with average levels of Tc in time points between 10 and 60 minute (r values varied between 0,6061; \( p<0,05 \) and 0,7894; \( p<0,01 \)). HS also correlated with average HR from 20 to 60 minute (r values between 0,54484 and 0,8498), and strongest correlation was recorded with PSI in time points between 10 and 60 minute (r values between 0,6778 and 0,8451). On the other hand, RPE showed significant correlation to parameters of thermotolerance (Tc, HR and PSI) only in second half of the test, i.e. between 40 and 60 minute (values of r coefficient varied between 0,5266 and 0,8498).

When analysing the end-point values (the last measured values at the moment of exhaustion), of all the parameters of thermotolerance, thermal sensation, and peripheral markers of fatigue, only values of RPE and prolactin significantly correlated with HS (r=0,59221; \( p<0,05 \) and r=0,5516; \( p<0,05 \), respectively). There was also significant correlation between end-point HS and \( \text{VO}_{2\text{max}} \) (r=0,564983; \( p<0,05 \)), but not with any anthropometric parameter whatsoever. Concentration of prolactin at the end of EHST significantly correlated with end-point values of thermotolerance: Tc, HR and PSI (r=0,5306; r=0,5758; r=0,6126, respectively; \( p<0,05 \)). The correlation was also highly significant between prolactin concentrations and RPE (r=0,750084; \( p<0,001 \)), but not with TS.

In order to incorporate the tolerance time, we calculated rates of change in parameters of thermotolerance and fatigue between values at the very end of the EHST and start values (Table 3).
| Parameter                      | ROC (Rate of Change) |
|--------------------------------|----------------------|
| ROC HS (J/g/min)               | 0.112±0.016          |
| ROC Tc (°C/min)                | 0.032±0.005          |
| ROC Tb (°C/min)                | 0.032±0.004          |
| ROC HR (/min)                  | 1.03±0.30            |
| ROC PSI                        | 0.135±0.028          |
| ROC TS                         | 0.064±0.009          |
| ROC RPE                        | 0.152±0.040          |
| ROC Ammonia (µmol/l/min)       | 0.315±0.142          |
| ROC BUN (mmol/l/min)           | 0.021±0.008          |
| ROC LDH (U/l/min)              | 0.522±0.203          |
| ROC Cortisol (mmol/l/min)      | 3.080±2.255          |
| ROC Prolactin (µIU/mL)         | 4.44±1.76            |

When we introduced the ROC values, we found even stronger correlation between HS and ROC RPE ($r=0.68311; p<0.01$), but the correlation between HS and ROC HR was also significant ($r=0.5915; p=0.05$).

We also analysed the relationship between rate of change in heat storage, i.e. the speed of increase in body heat and end-point values of other investigated parameters. The ROC HS showed statistically highly significant correlation with end-point values of HR and PSI ($r=0.636531; p<0.01$ and $r=0.570339; p<0.05$, respectively), as well as with concentration of prolactin after EHST ($r=0.51278; p<0.05$). The significance was borderline in relation between ROC HS and values after EHST of two other peripheral markers of fatigue:
concentrations of LDH and cortisol ($r=0.41812; \ p=0.054$ and $r=0.45442; \ p=0.051$, respectively).

**Discussion**

The high ambient temperature combined with physical activity plays important role for physically demanding occupations such as military services (13). The most useful method of alleviation of physiological strain in hot conditions is acclimatization (14,15). Unacclimatized persons are prone to operational mean error rate when engaged in hot conditions (16). In this study, we investigated physiological parameters of heat strain in relatively homogenous population of young, fit male soldiers, in order to establish relationship between heat storage during heat stress test and various markers of fatigue. Finding suitable models of heat exchange between human body and environment is important issue for more than 70 years. The problem is especially pronounced when physical activity is involved. Beside classical parameters of thermotolerance such as core and skin temperatures and heart rates, over 100 different heat stress indices have been explored (4). The study conducted by Cuddy et al. with 56 male participants performed EHST in the conditions similar to our study revealed several parameters which showed significant accuracy in assessing the risk of heat illness (17). The authors concluded that heart rate and skin temperature, as well as PSI may serve as predictors of heat risk. According to PSI values, subjects were divided in group “at risk” (PSI>7,5) and “not at risk” (PSI≤7,5). Subjects in “not at risk” group also showed significantly lower RPE, especially between 60 and 90 minutes of test, which coincided with lower values of Tc and HR. In our study end-point PSI was 8,35±0,70. In the first 30 minutes, all subjects showed PSI under 7,5 (“no risk”). After 40 minutes, 2 subjects have had PSI over 7,5 (“no risk”). At the end of test 12 of 15 have had PSI over 7,5.

In previously mention study (17), authors reported the significant relation between subjective perception of heat strain and total exercise time, which is in disagreement with our results. We found no significant correlation between RPE and time before exhaustion. Our results rather support the theory of “critical core temperature”, proposed by Gonzales-Alonso et al. (18). They suggested that absolute value of “critical core temperature” triggers the fatigue, regardless the total exercise time. In according to their results, we
found that exhaustion occurred at the similar Tc, when all participants rated their RPE at the almost same level, close to upper limit of scale.

Several studies reported that the rate of heat storage is well correlated with acute fatigue during physical work in hot conditions. This is confirmed in experiment conducted on animal model (19), but the results of given study are in disagreement with the hypothesis of “critical core temperature”. The rate of body heat storage is also related to body composition, i.e. content of body fat (20) which is expected due to different specific heat of tissues. Cumulative value of heat storage in our investigation showed constant increase, with significant correlation with Tc, HR and PSI from 10-60 minute. This confirms the findings of other authors (19,21).

Temperature sensation and thermal comfort may contribute to self-regulation of exercise intensity. In addition, acceptability and comfort were found to be closely correlated. Zhang et al. investigated local thermal sensation of different body parts, as well as overall thermal sensation in 30 subjects and reported the positive correlation between these factors and thermal comfort (22). Other authors reported the linear correlation between the thermal sensation and ambient temperature and suggested that physiological parameters such as core and skin temperature and heart rate may be used as predictors of thermal comfort (16). However, evaluation of thermal sensation is still a challenging issue (12). Assessment of individual perception of thermal state is commonly obtained by using several standard scales, with various number of points (23), which contributes the difficulties in comparison of results. In our investigation we used modified Gagge 8-point scale (8) with verbal descriptions between “cool” (ranking 5) and “unbearably hot” (ranking 13). Gagge et al. indicate that lower ambient temperature more affect subjective sensation of discomfort than higher temperatures and one may expect rapid increase of discomfort with lowering skin temperatures. However, values of subjective thermal comfort in our investigation showed constant linear increase from the beginning to the end of EHST, with average end-point value 12.2±0.6. At the end of EHST12 of 15 subjects showed values 12 and the rest ranked their thermal sensation as “unbearably hot” (rank 13). This relatively high ranking of thermal discomfort is in agreement with the results of the previously mentioned study by Davey et al, where the subjects reaching thermal tolerance limit ranked their subjective thermal sensation with average 18.8±1.3 using 20-points scale (12). Considering the similar
ambient temperature (WBGT 29 °C in our investigation and 28,79-31,85 in given study), similar results of thermal sensation are expected. RPE increase was faster between 40th and 70th minute compared to first 30 minutes. After 30 minutes, it correlates with TC, HR and PSI. Some authors suggest that participants who were allowed to self-select their exercise work by maintaining the RPE level mobilize an anticipatory mechanism with adjusting the work rate by regulating the degree of motor-unit recruitment to prevent harmful increase in core temperature and thus the onset of premature fatigue (21) which is confirmed by findings that RPE correlates with changes in EEG. Finally, in our study we wanted to investigate the potential importance of peripheral markers of fatigue and their relation to psychophysical parameters of thermotolerance. Of all the investigated markers, concentrations of prolactin showed the most prominent role. Prolactin is stress hormone which may indicate the rate of central fatigue, since it’s secretion is stimulated and inhibited by serotoninergic and dopaminergic neurons in the brainstem (2). Fatigue may be considered as an impairment of balance between brain secretion of serotonin and dopamine, which is reflected in prolactin concentration in blood. The increase in prolactin concentrations is expected in high-intensity and/or long duration exercise in both cool and warm environment, as well as in passive thermal stress. Manfredelli et al. found the correlation between increase in prolactin and lactate levels during high-intensity exercise (24). However, prolactin response has been more pronounced during exercise in heat compared to cool conditions (25). Investigation conducted on 21 young males exercising in the heat showed that concentrations of prolactin were more sensitive in indicating heat stress than cortisol and the most important stimulus to prolactin secretion was increase in core temperature (26). However, other authors did not find any significant increase in cortisol and prolactin levels during exercise-heat stress, but in their investigation 10 young and 10 older men performed short-time (30 minutes) bouts of physical activity (3). The acclimatization tends to alleviate the increase in prolactin during exercise in the heat (27).

Our results show the association between prolactin concentrations at the end of EHST and end-point values of core temperature, heart rate and PSI. Other authors also found the correlation between prolactin and parameters of thermotolerance. In their study, Wright et al. investigated peripheral markers of central fatigue in group of 23 healthy men of which 12 were well-trained, with average VO_{2max} 70±2 ml/kg LBM (similar to our participants).
They performed EHST under similar ambient conditions and similar work load (2). Values of Tc, HR and change in Tc at the moment of exhaustion were in agreement with our results. Same authors also reported a sudden decrease in circulating free tryptophan levels at Tc over 39.5 °C consistent with levels of thermal strain which may be the consequence of increased permeability of blood brain barrier. Nevertheless, the results of this study, as well as our findings indicate that the increase in prolactin concentrations may serve as peripheral marker of central fatigue reflecting increase in serotonin and decrease in dopamine secretion in brain.

**Conclusion**

Prevention of heat illness is one of the most important issues regarding physical activity in hot conditions. As expected, this study demonstrates the relationship between heat storage and physiological and psychological parameters of thermotolerance (core temperature and heart rates, as well as thermal sensation and rate of perceived exertion), but also demonstrates the suitability of using PSI as reliable index of thermal strain. The perception of heat strain was in consensus with physiological strain parameters. Concentrations of prolactin, and to some extent cortisol, showed the strongest correlation with these parameters, so may be considered as peripheral markers of fatigue.

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