The effects of acclimatization on blood clotting parameters in exertional heat stress

Uticaj aklimatizacije na pokazatelje hemostaze u toplotnom stresu usled fizičkog napora

Zoran Vesić*, Milica Vukašinović-Vesić†, Dragan Dinčić‡, Maja Šurbatović‡, Sonja S. Radaković‡†

*Ministry of Defence, Belgrade, Republic of Serbia; †Association of Sport Medicine of Serbia, Belgrade, Serbia; ‡Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia

Abstract

Background/Aim. Exertional heat stress is a common problem in military services. Considering the coagulation abnormalities are of major importance in development of severe heat stroke, we wanted to examine changes in hemo-static parameters in soldiers during exertional heat stress test as well as the effects of a 10-day passive or active acclimatization in a climatic chamber. Methods. A total of 40 male soldiers with high aerobic capacity performed exertional heat stress test (EHST) either in cool [20°C, 16°C wet bulb globe temperature (WBGT)], or hot (40°C, 29°C, (WBGT) environment, unacclimatized (U) or after 10 days of passive (P) or active (A) acclimatization. Physiological strain was measured by tympanic temperatures (Tty) and heart rates (HR). Platelet count (PC), antithrombin III (AT), and prothrombin time (PT) were assessed in blood samples collected before and immediately after the EHST. Results. EHST in hot conditions induced physiological heat stress (increase in Tty and HR), with a significant increase in prothrombin time in the groups U and A. Platelet counts were not affected. Platelet counts were increased after EHST in all groups. A 10-day passive or active acclimatization in climatic chamber showed no effect on parameters investigated.

Key words:
physical exertion; heat stress disorders; acclimatization; blood coagulation; military personnel; serbia.

Apstrakt

Uvod/Cilj. Toplotni stres usled fizičkog napora predstavlja čest problem u vojsci. Pošto su poremećaji koagulacije veoma značajni za razvoj teškog toplotnog udara, cilj istraživanja bio je da se ispitaju promene parametara koagulacije tokom toplotnog stresa usled fizičkog napora, kao i uticaj desetodnevne pasivne, odnosno aktivne aklimatizacije. Metode. Cetvrtdeset vojnika muškog pola, visoke aero-bne sposobnosti, izloženo je fizičkom naporu submaksimalnog intenziteta i to: grupa C u termoneutralnoj sredini: 20°C, ili 16°C WBGT (indeks vlažnog i globus termometra), a ostali u toploj sredini (40°C, 29°C WBGT), i to neaklimatizovani (U), nakon 10-dnevne pasivne (P) ili aktivne (A) aklimatizacije u klimatskoj komori. Fiziološko opterećenje određeno je preko timpanične temperature (Tty) i frekvencije srčanog rada (HR). Serumske vrednosti protrombinskog vremena (PT), antitrombina III (AT) i broja trombocita (PC) određene su iz uzoraka krvi uzetih pre i odmah nakon testa. Rezultati. Fizički napor u toploj sredini izazvao je značajno povećanje vrednosti PT u neaklimatizovanoj i aktivno aklimatizovanoj grupi. Broj trombocita bio je značajno povećan nakon testa i to u svim grupama, bez obzira na temperaturne uslove tokom testa i na stanje aklimatizacije ispitanika. Vrednosti AT nisu se promenile ni u jednoj grupi. Zaključak. Kod utereniranih vojnika se fizičkim naporom u toplotnom stresu dovedo do promene nekih parametara koagulacije (produženja PT), dok nema uticaja na druge parametre, kao što je vrednost AT. Povećanje broja trombocita nakon testa može se pripisati dejstvu fizičkog napora, bez dodatnog uticaja toplotnog stresa, a 10-dnevna pasivna ili aktivna aklimatizacija ne utiče na ove promene.

Ključne reči: napor, fizički; stres uzrokovan toplotom; aklimatizacija; krv, koagulacija; vojnici; srbija.

Correspondence to: Sonja Radaković, Faculty of Medicine of the Military Medical Academy, University of Defence, Cmotravska 17, Belgrade, Serbia. E-mail: sonja.radakovic@vma.mod.gov.rs
Heat stress can be a significant problem in military services. Common preventive measures, such as restriction of physical activity, taking off clothes, and moving into shade, are usually suppressed by a strong motivation to accomplish the task. Heat stress can impair both physical and mental performance, but at the same time, can influence the vital body functions, with heat stroke as the most severe consequence.

Heat stroke is a life-threatening syndrome characterized by multiple organ dysfunction, including arterial hypotension, hyperthermia, and central nervous system disorders. Excessive activation of systemic inflammation and hypercoagulable state may contribute to multiple organ failure and dysfunction in heat stroke. The heat illness considers a certain continuum, so it is of major importance to understand the processes lying underneath. The heat illness syndrome is typically depicted as a series of discrete events, characterized by pathophysiological responses that increase in severity as one moves from the mildly impaired functioning such as heat cramps to heat exhaustion and heat stroke. Heat exhaustion is the most common heat syndrome, but, unrecognized, can progress to heat stroke.

Methods

Forty male soldiers (20.1 ± 0.9 years) participated in the trial after being informed of the purpose and details of the trial, any known risks and discomforts, and their right to terminate participation at will. After briefing, the soldiers gave their written informed consents to participate. The experimental protocol was approved by the Ethical Committee of the Military Medical Academy in Belgrade. Medical supervision of the subjects was conducted according to international standards. Standard anthropometric measurements were conducted, baseline levels of maximal aerobic power (VO2max) was indirectly determined on treadmill.

The investigation was conducted during wintertime (late November and December) in Military Medical Academy, Belgrade. The soldiers were randomly divided into four equal groups. The first group were unacclimatized controls, who performed the exertional heat-stress test (EHST) in cool environment (C). Another unacclimatized group performed the EHST in hot environment (U), and the rest two groups performed the same test, but after 10 days of acclimation in a climatic chamber (3 hours each day, at 35°C, relative humidity 40%, wind speed < 0.1 m/s); acclimation was in one group conducted passively (P), and in the other actively (A), with 1 hour walking on a treadmill, 5.5 km/h. The EHST included walking on a motorized treadmill (5.5 km/h) either in a cool [20°C, wet bulb globus thermometer temperature (WBGT) 16°C – group C] or hot (40°C, WBGT 29°C – group U, P, and A) environment, while wearing a normal combat uniform, with a backpack filled with 20 kg of sand in order to simulate regular weight burden. EHST duration was maximally 90 min; the criteria for termination were: tympanic temperature (Tty) 39.5°C, heart rate (HR) 190 beats/min, or intolerable subjective discomfort. The subjects were allowed to drink tap water at will, up to 1.5 L. Blood samples were collected before the EHST and immediately after it.

The soldiers were closely monitored up to 5 hours after finishing the trial and medically examined after 2 days (ECG, blood pressure and routine blood analysis).

Environmental conditions (dry bulb-temperature, WBGT, relative humidity and wind speed) were measured by MiniLab Light Laboratories, Brighton, England. Core Tty was continuously measured using contact probes (Elektro-laboratorium, Denmark) with a transducer introduced into the auditory canal and placed toward the eardrum. The temperature was registered every 5 minutes. Heart rate was continuously telemetrically monitored (Quinton instruments, USA), and recorded every 5 minutes. Atithrombin III (AT), prothrombin time (PT), and platelet count were assessed by standard laboratory methods in the Institute for Medical Biochemistry, Military Medical Academy, Belgrade.

Data are presented as means ± SD. The difference was assessed by the Student’s t-test and Wilcoxon’s Signed Rank test for paired samples. The normal distribution was tested by the Shapiro Wilk’s test. SPSS 11.5 was used to process statistical material and the 0.05 level of significance was used.

Results

Table 1 shows the physical characteristics of the subjects. All the groups were similar in all the investigated characteristics. None of 40 soldiers showed any symptom of heat stroke or severe heat exhaustion during or after the EHST. No results of any medical exams showed any sign of serious dysfunction. All the soldiers in the group C completed the EHST. However, only one soldier in the group U successfully completed the EHST, in the rest cases tests were terminated between 45 and 70 minutes, mostly due to reaching the ethical barrier for Tty 39.5°C, or intolerable subjective discomfort. In the acclimatized groups, most of the soldiers managed to finish the test (3 soldiers in the group P and 1 in the group A terminated the test between 60 and 80 minutes, reaching the Tty barrier). Even so, their subjective sensation of discomfort was tolerable, and they were willing to continue the test.

The mean Tty and HR values are presented in Figures 1 and 2. In the first 20 minutes (before sweating onset) there was an increase in Tty in all the groups, and after that in the group C Tty remained constant. Tty raised steadily in all groups performed EHTS in hot environment, with slightly lower values recorded in the acclimatized groups. Heart rates in the group C were steady, while in hot conditions there was a permanent increase in HRs in all the groups similarly, but the limit of 190 beats/min was never reached. A maximal recorded HR was 163 beats/min.

Table 2 summarizes the plasma levels of AT, PT, and platelet count (PC) for the controls, unacclimatized, passively, and actively acclimatized soldiers performing EHST (the groups U, P, and A).

The table reveals that PT values were significantly increased after the EHST compared to the basic values in the group U and A (0.94 ± 0.08 s before vs 1.01 ± 0.04 s after the EHST; Wilcoxon Z = -4.583; p = 0.009, and 0.97 ± 0.04 s before vs 1.03 ± 0.05 s after the EHST; Z = -2.373; p = 0.018, respectively). Platelet counts were significantly increased after the EHST compared to basic levels in all investigated groups, but did not differ among the groups, neither before, nor after the EHST: 242.6 ± 42.82 × 10^5/mm^3 before vs 267.0 ± 45.02 × 10^5/mm^3 after the EHST (Z = -2.703; p = 0.007) in the group C; 257.3 ± 38.0 × 10^5/mm^3 before vs 300.8 ± 49.97 × 10^5/mm^3 after the EHST (Z = -2.701; p = 0.007) in the group P, and 225.9 ± 55.81 × 10^5/mm^3 before vs 313.89 ± 83.26 × 10^5/mm^3 after the EHST (Z = -2.666; p = 0.008) in the group A. The EHST did not influence plasma AT levels regardless environmental conditions, and acclimatization state whatsoever.

**Discussion**

Impaired working efficiency is a well-known consequence of heat strain. This is particularly important in military services. Core temperature is considered a relevant indicator of thermal strain. Military training guidelines for continuous physical work times are based on achieving core temperature of 40ºC in acclimatized individuals with appropriate fluid replacement 11. Well-trained athletes and soldiers may tolerate hyperthermia without adverse side effects due to training-induced heat acclimatization effects on cellular protective mechanisms 12.

---

In our study, at high degree of heat strain, the majority of soldiers did not approach the levels at which their activity should be reduced. The HR values were well within the predicted maximum for their age, indicating that the workload had not exceeded their physical capabilities, considering their high baseline levels of VO2max.

In 2002, a new definition of heat stroke was introduced suggesting that multi-organ system failure was due to the combined effects of heat cytotoxicity, coagulopathies, and the systemic inflammatory response syndrome. During heat stress, systemic inflammation and activated coagulation are displayed, evidenced by increased prothrombin time, activated thromboplastin time, and D-dimer, and decreased platelet count, and protein C. Hypercoagulable state, along with systemic inflammation, can result from oxidative stress and thus may contribute to organ failure and dysfunctioning in heat stress. The inflammatory response is an important local defence mechanism against infection and injury. Because the inflammatory response is inseparable from the coagulation process, coagulation disorders are often associated with severe inflammatory disease. In experiments conducted on rodents, during heat stroke, leukocytosis, coagulation abnormalities and abnormalities of prothrombin consumption, thromboplastin generation, clotting time, one-stage prothrombin and clot retraction are common.

The major physiological response to heat stress considers primary cardiovascular adaptation, resulting in increased skin blood flow, in order to increase heat loss. Prolonged redistribution of blood flow into the skin leads to progressive reduction of splanchic blood flow, which is followed by nitrosative and oxidative stress, and production of reactive oxygen species. These products cause leakage at the sites of intestinal tight junctions, leading to increased permeability for Gram-positive and Gram-negative bacteria and their endotoxins. Intestinal tissue damages due to ischaemic environments contribute to local tissue inflammation and activation of inducible nitric oxide synthase and generation of reactive nitrogen species. These pathophysiological mechanisms are proposed to set a foundation to inflammation and coagulation abnormalities in heat stress.

Disseminated intravascular coagulation is a common complication of heat stroke that is initiated following thermal injury to the vascular endothelium and is regarded as an important mechanism of heat stroke morbidity and mortality. In vitro studies have shown that heat (43–44ºC) directly activates platelet aggregation and causes irreversible hyperaggregation following cooling. Early in heat stroke, widespread activation of coagulation stimulates excess deposition of fibrin in the arterioles and capillaries along with platelet aggregation that leads to microvascular thrombosis. Although rapid cooling of the heat stroke patient may normalize fibrinolysis, coagulation often persists until platelets and coagulation proteins are consumed at a faster rate than they are produced. Consumptive coagulation may lead to excessive, prolonged bleeding from multiple tissue sites (venipuncture sites, gums) and is associated with fatal outcome. The host inflammatory and hematostatic responses are closely associated not only with fatal heat stroke but also with severe heat stress, especially when combined with physical strain.

The prothrombin time provides information about the extrinsic (tissue factor) and common coagulation pathways. In our study, heat stress combined with intensive physical activity led to increase in prothrombin time values in soldiers performed EHST in hot condition. We observed no difference in the control group, which suggests that the same intensity of physical strain did not result in increasing of prothrombin time. Hence, this effect on prothrombin time can be attributed to heat stress, regardless physical activity.

### Table 2

<table>
<thead>
<tr>
<th>Statistical parameters for groups</th>
<th>Platelet count (10^3/mm^3)</th>
<th>Prothrombin time (s)</th>
<th>AntiThrombin III (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
<td>before EHST</td>
<td>after EHST</td>
<td>before EHST</td>
</tr>
<tr>
<td>mean</td>
<td>242.60</td>
<td>267.00</td>
<td>1.04</td>
</tr>
<tr>
<td>SD</td>
<td>42.82</td>
<td>45.02</td>
<td>0.46</td>
</tr>
<tr>
<td>C SE</td>
<td>13.54</td>
<td>14.24</td>
<td>0.01</td>
</tr>
<tr>
<td>t-test</td>
<td>-5.036; p = 0.001</td>
<td>-0.802; p = 0.443</td>
<td>0.350; p = 0.734</td>
</tr>
<tr>
<td>W</td>
<td>Z = -2.703; p = 0.007</td>
<td>Z = -0.816; p = 0.414</td>
<td>Z = -0.051; p = 0.959</td>
</tr>
<tr>
<td>mean</td>
<td>257.30</td>
<td>300.80</td>
<td>0.94</td>
</tr>
<tr>
<td>SD</td>
<td>38.00</td>
<td>49.97</td>
<td>0.08</td>
</tr>
<tr>
<td>U SE</td>
<td>12.02</td>
<td>15.80</td>
<td>0.02</td>
</tr>
<tr>
<td>t-test</td>
<td>-3.779; p = 0.004</td>
<td>-4.583; p = 0.001</td>
<td>-0.835; p = 0.425</td>
</tr>
<tr>
<td>W</td>
<td>Z = -2.701; p = 0.007</td>
<td>Z = -2.626; p = 0.009</td>
<td>Z = -0.818; p = 0.413</td>
</tr>
<tr>
<td>mean</td>
<td>225.90</td>
<td>267.30</td>
<td>1.07</td>
</tr>
<tr>
<td>SD</td>
<td>55.81</td>
<td>69.43</td>
<td>0.04</td>
</tr>
<tr>
<td>P SE</td>
<td>17.65</td>
<td>21.96</td>
<td>0.01</td>
</tr>
<tr>
<td>t-test</td>
<td>-3.156; p = 0.012</td>
<td>1.545; p = 0.157</td>
<td>2.090; p = 0.046</td>
</tr>
<tr>
<td>W</td>
<td>Z = -2.194; p = 0.028</td>
<td>Z = -1.425; p = 0.154</td>
<td>Z = -1.737; p = 0.082</td>
</tr>
<tr>
<td>mean</td>
<td>252.56</td>
<td>313.89</td>
<td>0.97</td>
</tr>
<tr>
<td>SD</td>
<td>56.37</td>
<td>83.26</td>
<td>0.04</td>
</tr>
<tr>
<td>A SE</td>
<td>18.79</td>
<td>27.75</td>
<td>0.01</td>
</tr>
<tr>
<td>t-test</td>
<td>-3.956; p = 0.004</td>
<td>-3.773; p = 0.005</td>
<td>1.789; p = 0.111</td>
</tr>
<tr>
<td>W</td>
<td>Z = -2.666; p = 0.008</td>
<td>Z = -2.373; p = 0.018</td>
<td>Z = -1.540; p = 0.123</td>
</tr>
</tbody>
</table>

C = cool environment; U = hot environment (unacclimatized); P = passive acclimatization; A = active acclimatization; EHCT = exertional heat-stress test; W – Wilcoxon’s Signed Rank test.

These findings can indicate the tendency of prothrombin consumption when exposed to heat stress.

Antithrombin is synthesized by the liver. Human and animal studies have shown that antithrombin behaves as a negative acute-phase protein. According to our results, heat stress combined with strenuous physical work does not influence the antithrombin values, because we found no difference between levels before and after the EHST, regardless of environmental conditions and acclimatization state. These findings suggest that the exertional heat stress of given intensity and duration could not challenge the acute-phase reaction reflected in antithrombin disturbances.

Finally, according to the results obtained in our study, exertional heat stress induced increase in platelet counts in all investigated groups, regardless of environmental conditions and acclimatization state. These findings are in disagreement with the proposed mechanisms of platelet aggregation and consumption during heat stress. We suggest that inflammatory triggers for coagulation onset were not activated at the given intensity of heat stress combined with hot environmental conditions. The subjects in our investigation were healthy, fit young males, well-trained and well hydrated, with fully mobilized protective mechanisms (both acclimatization and acquired tolerance to heat), which made them resistant to endothelial injury that lays beneath the hemostatic disturbances in heat stress. The common risk factors for heat coagulopathy such as preexisting illness, drug use, alcohol, amphetamines, ecstasy abuse, were also absent in the investigated population.

**Conclusion**

This study demonstrated the effects of physical activity in a hot environment on physiological parameters, as shown by an increase in Tty and HR. This physiological heat stress cause mild changes in serum parameters of blood clotting such as prothrombin time, while antithrombin values were not affected by the stress. Contrary to the expected, platelet count increased during exertional heat stress.

A 10-day acclimatization, either passive or active, showed no effect on parameters investigated, possibly due to a preexisting high level of aerobic capacity and tolerance to heat, which could not be additionally improved.

**References**

18. Dokladny K, Mosley PL, Ma TY. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. Am J Physiol Gastrointest Liver Physiol 2006; 290(2): G204–12.