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Citation style: Duch Klaudia, Michnik Anna, Pokora Ilona, Sadowska-Krępa Ewa (2020). Modification of blood serum DSC profiles by sauna treatments in cross-country skiers during the exercise cycle. "Journal of Thermal Analysis and Calorimetry" Vol. 0, nr 0 (2020), s. 1-6. DOI:10.1007/s10973-020-10162-7



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Modification of blood serum DSC profiles by sauna treatments in cross-country skiers during the exercise cycle

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Received: 9 January 2020 / Accepted: 4 August 2020
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Abstract

The study has evaluated the effect of sauna treatments on the blood serum of professional cross-country skiers (3 women and 6 men) during two exercise sessions: the first without any treatments and the second after ten sauna treatments. Aqueous solutions of serum samples from the blood of athletes collected in four stages in both exercise sessions: at rest, after exercise, after 1 h and 24 h rest and additionally only at rest after the first sauna treatment, were measured by means of differential scanning calorimetry (DSC). DSC results allowed to register characteristic post-exercise changes in the profile of thermal denaturation transition of serum. A trend of these changes was similar regardless of the use of sauna, but their intensity was stronger in the exercise session after the sauna treatments. In particular, the differences relating to the use of sauna have revealed in the higher temperature range of serum denaturation transition. Regarding the recovery process after exercises, a large individual variability was observed in both sessions.

Keywords Cross-country skiers · DSC · Human blood serum · Protein thermal denaturation · Finnish sauna treatments

Introduction

Sauna is a physical treatment, originating from Scandinavian countries such as Finland, and now widely used throughout the world [1–3]. Sauna treatments are used very often in sports medicine in rehabilitation and recreation or biological regeneration due to its positive effect on the body of athletes [1–12]. It has been shown that sauna treatments positively influence the hemodynamics of blood pressure and pulse [1, 2, 10], stimulate athletes' immune system [4], help to maintain adaptive changes to physical effort [6, 7], favorably modulate heart rate variability [8], support circulation [9, 11], improve sleep [9], improve mental health [9, 11], increase "body detoxification" [9], relieve back and musculoskeletal pain [9], probably reduce the risk of sudden

cardiac death [11], improve lipid profile [12]. In some studies, there were no positive effects of the use of sauna treatments [5, 13, 14]. It is important to prevent any misuse of the sauna [3, 14].

Differential scanning calorimetry (DSC) has found wide application in medical and biological sciences [15–34]. Attempts to use the DSC method for clinical purposes have been made for several years [15–27]. DSC profiles of blood serum, plasma, synovial fluid, cerebrospinal fluid are very useful because they can be a valuable source of information about alterations in these physiological fluids caused by different diseases or treatments. DSC seems to be a very informative technique also in sports medicine [28–32].

In this paper, DSC has been employed to detect changes in the thermal behavior of blood serum solutions due to sauna treatments. The effect of one sauna treatment and a series of 10 sauna treatments on endothermic denaturation transition of cross-country skiers' serum has been analyzed and compared. Moreover, post-exercise changes and those in the recovery period were tracked in blood serum during two same exercise sessions, of which one was before and the other after ten sauna treatments.

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Materials and methods

Participants

Nine athletes (3 women and 6 men) well trained in cross-country skiing participated in this study. These athletes belong to the national team and take part in competitions in many countries around the world. A study was carried out during the transition phase of the annual training plan.

All athletes were informed about the purpose and the nature of the research before giving their written consent to participate in the experiment. The studies were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Jerzy Kukuczka Academy of Physical Education in Katowice (Certificate of approval No. 2/2016).

Experimental

The study consisted of pre-sauna (SI) and post-sauna (SII) stages. The athletes' $\dot{V}O_{2\max}$, peak HR, peak power output and HR at individual anaerobic threshold (AT) (HR-AT) were determined in the week prior to the pre-sauna period, using a graded running exercise test.

Experimental exercise test (EET) was carried out twice: before (SI) and after (SII) a series of ten sauna baths. EET test consists of ~60 min running with the intensity below the individual anaerobic threshold, on treadmill H/P/Cosmos Pulsar at 20 ± 1 °C ambient temperature, $56 \pm 3\%$ relative humidity and 985 ± 5 hPa atmospheric pressure, in Research Center for Sports, Academy of Physical Education in Katowice. More details can be found in our recently published paper [32].

Finnish sauna treatments

Participants were exposed to whole-body heating (WBH) treatment in the Finnish sauna at the Academy of Physical Education in Katowice in the afternoon after the part of daily training sessions in the transition phase. We decided to standardize the duration of sauna exposure rather than standardize the relative heat stress to minimize the potential impact on the athletes' daily life/training schedule. The exclusion criteria included acute respiratory tract disorders, hyperthyroidism or hypothyroidism, and purulent-gangrenous skin lesions. The average temperature and humidity in the sauna room were 90 ± 2 °C and $15 \pm 3\%$, respectively. A single sauna session consisted of three

WBH treatments, each lasting from 10 to 15 min, followed by breaks to cool down the body.

Blood serum samples

Venous blood samples were taken from athletes at rest: before sauna treatments, after 1 sauna treatment and after 10 sauna treatments which were held on weekdays over two weeks. Blood samples were always collected at the same time in the morning. Additionally, blood samples were collected during two exercise sessions: SI—before sauna treatments and SII—after 10 sauna treatments in 4 stages: before exercise (be), 3 min after exercise (ae), after 1 h of rest (r1h) and after 24 h rest (r24h).

Blood serum samples were prepared using the standard method and kept frozen at -20 °C until the measurement. Serum samples were thawed at room temperature for about 10 min to prepare 20-fold diluted solutions using the degassed water. The pH values of the tested aqueous serum solutions were 7.0 ± 0.5 .

DSC measurements

Differential scanning calorimetry (DSC) measurements of aqueous serum solutions were made using the VP-DSC microcalorimeter (MicroCal Co, Northampton, MA). Two scans were obtained for each sample in the temperature range 20–100 °C with a heating rate of 1 °C min^{-1} and a pressure of about $1.7 \cdot 10^5$ Pa. All raw serum DSC curves were corrected for the water–water instrumental baseline. The next steps were normalization for the gram mass of protein and subtracting the linear baseline. An apparent excess heat capacity C_p^{ex} ($\text{J } ^\circ\text{C}^{-1} \text{g}^{-1}$) versus temperature (°C) has been plotted.

The following thermodynamic parameters: temperatures (T_1 , T_2 , T_3) and excess heat capacities (C_{p1} , C_{p2} , C_{p3}) of local maxima, the enthalpy (ΔH) of thermal denaturation of serum as the area under the endothermic peak, the width of peak in its half height (HHW) and the first moment of the transition with respect to the temperature axis (T_{FM}) [20, 28].

Statistical analysis

Statistical analysis was carried out with the Statistica 13.1 program. After checking for normal distribution (tested by Shapiro-Wilk test), ANOVA with repeated measurements has been used. Mauchly's test for sphericity was included as a part of the procedure. When the ANOVA results were statistically significant, the post hoc Tukey's test was performed to determine which pairs of means had significant differences. If the distribution was not normal, we used the Friedman's test. The level of statistical significance was set at $p < 0.05$.

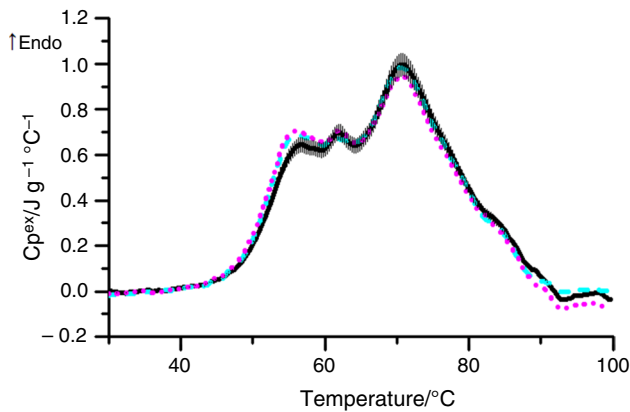


Fig. 1 Mean serum DSC curves: before sauna treatments (solid line), after 1 sauna treatment (short dots line), after series of 10 sauna treatments (short dash line); the shaded area added to the solid line is the standard error (SE) at each temperature

Results and discussion

The thermal denaturation of the proteins contained in the blood sera is observed in serum DSC profile as complex endothermic transition. At first sight, at least two or three peaks can be distinguished in this transition for healthy people, depending on the serum proteome and the solvent used [15–33]. Mean DSC curves of aqueous solutions of human blood sera samples collected from participants of our study at three time points (before sauna treatments, after 1 sauna treatment and after 10 sauna treatments) are shown in Fig. 1. All three curves are very similar to each other and slight differences between them seem to be within the limits of experimental error. The first peak at about 56 °C mainly represents the contribution of un-liganded albumin. The second sharp peak at about 62 °C comes from haptoglobin, the acute phase protein. It is not always clearly visible. The third peak at about 70 °C is derived from immunoglobulins and dominates over the less intense other two. Every of these peaks contains contributions from other serum proteins.

Average thermodynamic parameters of considered denaturation transition are listed in Table 1. In accordance with what is suggested in Fig. 1, Cp_1 and HHW values are higher while Cp_3 is slightly lower after sauna treatments. The value of T_{FM} parameter is lower after the sauna than before treatments. Surprisingly, the effect of 1 treatment seems to be stronger than 10 treatments. Particularly, statistically significant difference between T_{FM} before sauna and after 1 sauna treatment has been found ($p=0.017$ for main effect in ANOVA with repeated measure and $p=0.013$ in post hoc Tukey's test).

Figures 2 and 3 show the average serum DSC curves for four stages in exercises session SI and SII, respectively. Comparing the set of curves in both sessions, we can see

Table 1 Mean parameters (mean \pm SD) of serum denaturation transition before sauna (0 sauna), after 1 sauna (1 sauna) and after 10 sauna treatments (10 sauna)

	0 sauna	1 sauna	10 sauna
$T_1/^\circ\text{C}$	56.7 ± 0.8	56.0 ± 0.7	56.5 ± 1.1
$Cp_1/J \text{ g}^{-1} \text{ }^\circ\text{C}^{-1}$	0.653 ± 0.059	0.716 ± 0.063	0.703 ± 0.038
$T_2/^\circ\text{C}$	61.9 ± 0.7	62.0 ± 0.5	62.0 ± 0.6
$Cp_2/J \text{ g}^{-1} \text{ }^\circ\text{C}^{-1}$	0.716 ± 0.038	0.724 ± 0.075	0.699 ± 0.050
$T_3/^\circ\text{C}$	70.8 ± 0.4	70.6 ± 0.4	70.3 ± 0.5
$Cp_3/J \text{ g}^{-1} \text{ }^\circ\text{C}^{-1}$	1.000 ± 0.063	0.950 ± 0.059	0.988 ± 0.042
$\Delta H/J \text{ g}^{-1}$	23.4 ± 1.3	23.4 ± 1.3	23.4 ± 0.4
HHW/ $^\circ\text{C}$	25.4 ± 1.0	26.6 ± 0.9	26.3 ± 0.7
$T_{FM}/^\circ\text{C}$	67.5 ± 0.6	66.7 ± 0.4	67.1 ± 0.5

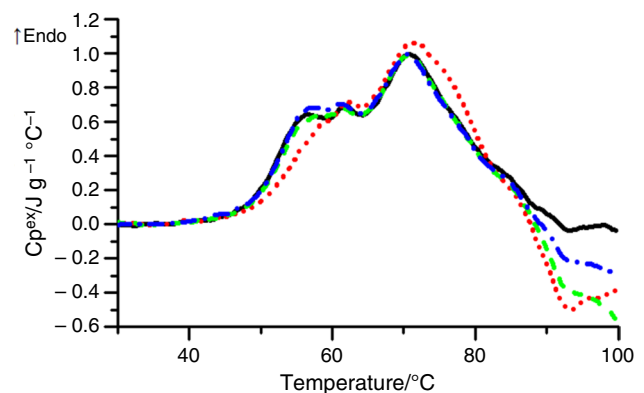


Fig. 2 Mean serum DSC curves in SI: be (solid line), ae (short dots line), r1h (short dash line), r24h (short dash dots line)

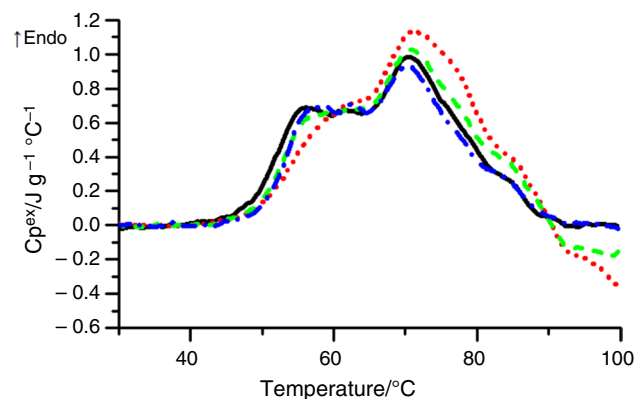


Fig. 3 Mean serum DSC curves in SII: be (solid line), ae (short dots line), r1h (short dash line), r24h (short dash dots line)

a similar trend of exercise related changes, but these in session II are more intense. In the temperature range of about 45–60 °C, we see a reduction of the first local maximum after the exercise. The excess heat capacity at the

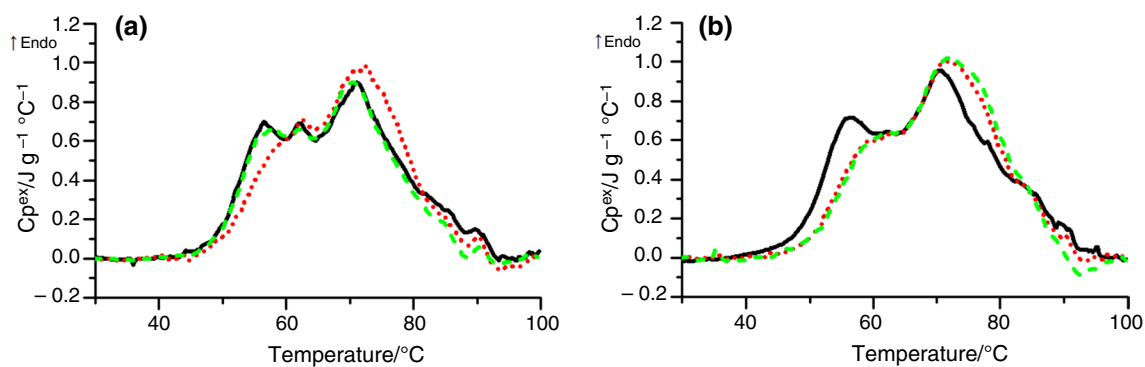


Fig. 4 The DSC curves in: be (solid line), ae (short dots line), r1h (short dash line) stages of session I **(a)** and II **(b)** for serum of the athlete whose regeneration process in session II was delayed

temperature of the first local maximum (56.7 °C in SI and 56.5 °C in SII) decreases by 0.162 J g⁻¹ °C⁻¹ (from 0.653 to 0.491 J g⁻¹ °C⁻¹) and by 0.193 J g⁻¹ °C⁻¹ (from 0.703 to 0.510 J g⁻¹ °C⁻¹) in SI and SII, respectively. This is accompanied by a marked increase in the intensity of the transition in the temperature range of about 65–85 °C. The local maximum in this temperature range shifts from 70.8 to 74.1 °C in SI and from 70.3 to 71.1 °C in SII because of the exercise done. The results of Friedman test showed statistically significant differences between the mean T_3 values in various stages for session I ($p=0.003$) and session II ($p=0.008$). The mean excess heat capacity at the temperature of the third local maximum increases due to the exercise from 1.000 to 1.070 J g⁻¹ °C⁻¹ (by 0.070 J g⁻¹ °C⁻¹) in SI and from 0.988 to 1.137 J g⁻¹ °C⁻¹ (by 0.149 J g⁻¹ °C⁻¹) in SII. The differences between pre- and post-exercise mean values of Cp_3 have been found statistically significant ($p=0.04$ and $p=0.0008$ in session I and II, respectively). Presented results suggest that post-exercise changes in serum proteome are stronger after a series of 10 sauna treatments.

In the regeneration phase, the return to the initial shape has been observed for the denaturation transition of serum.

A comparison of Figs. 2 and 3 suggests that after an hour of rest the post-exercise modifications disappear in SII only partially, while in SI practically completely. Additionally, a slight shift of the first local maximum to higher temperature can be observed after an hour as well as a day of rest. Moreover, the exothermic aggregation effect (stronger in session I than in session II), disappearing during recovery period, is well visible in the temperature range above 85 °C.

One possible explanation of the reduction of the first local maximum may be a decrease of ligand-free albumin fraction due to attaching ligands to albumin molecules. Albumin with bound ligands denatures at higher temperatures. Albumin binds many substances such as vitamins, metabolites, dyes and drugs [35–41]. Exercises can increase the binding of various ligands by albumin, especially when they were performed after a series of sauna treatments. It is in agreement with the more intense peak in the range of 65–85 °C, which is mainly derived from immunoglobulins and liganded albumin. It is believed that exercise can play a major role in enhancing the endogenous defense system against mechanical and metabolic muscle damage, which may explain the increased contribution from immunoglobulins.

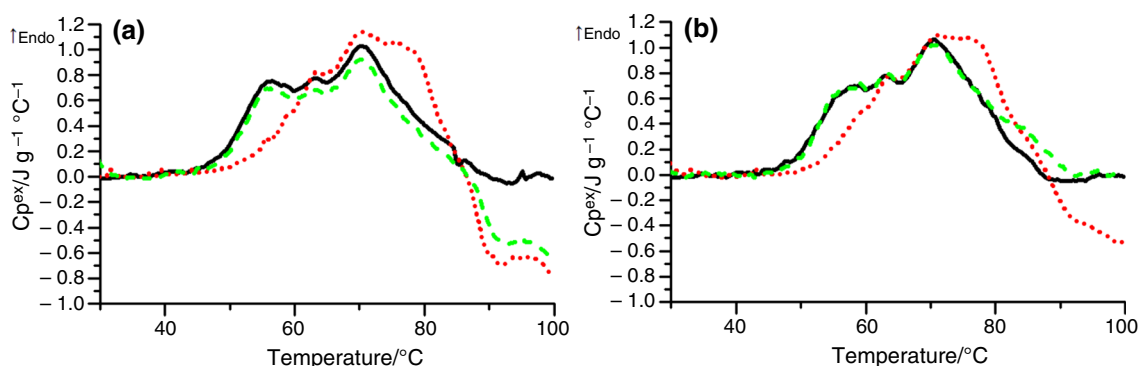


Fig. 5 The DSC curves in: be (solid line), ae (short dots line), r1h (short dash line) stages of session I **(a)** and session II **(b)** for serum of the athlete whose regeneration process took no more than an hour despite relatively large post-exercise changes in both sessions

It is known that individual metabolism is reflected in blood serum proteome modification. The data collected in this study showed that not everyone responds to the exercises in the same way and regenerates at the same time after exercise. Figures 4 and 5 show DSC curves for the athletes whose reaction was different from the one indicated by mean DSC curves presented in Figs. 2 and 3. Figure 4 presents a typical response to effort but no signs of regeneration during 1 h of rest in session II. This athlete needed more time to reverse the changes in serum caused by intense exercises performed after a series of 10 sauna treatments. Figure 5 suggests stronger than average response to exercise and despite that very fast recovery process in both sessions.

Conclusions

DSC results obtained in this study indicate that sauna treatments do not significantly modify serum denaturation transition. Although the statistically significant decrease in T_{FM} after 1 sauna treatment suggests a beneficial effect of sauna on athletes, a larger number of blood serum samples would be required for verification of this suggestion. The current DSC results did not notice gender dependence in response to sauna treatments, but only 3 women and 6 men were involved into the experiment. An important observation from our research is that the DSC method has allowed to register characteristic post-exercise changes in thermal transitions of serum denaturation. These changes are similar in nature, regardless of the use of sauna treatments before the exercise session, but enhanced by the sauna. A reduction of the first local maximum, connected mainly with the fraction of ligand-free albumin, has been observed after exercise in both sessions. Slightly larger decrease of excess heat capacity in this temperature region has occurred in the case of exercise after a series of 10 sauna treatments. The second post-exercise effect, also more intense in the exercise session after sauna treatments, was an increased intensity of the third local maximum, connected mainly with the fraction of gamma-globulins and albumin with bound ligands. So, changes in the athlete's blood serum proteome, conditioning the modification of serum DSC profiles in the session held after sauna treatments, seem to be stronger than those in the session not preceded by treatments. Comparison of sera DSC profiles indicates a diverse recovery process among athletes, especially in the post-sauna exercise session.

Funding The project has been financed by the Grant Ministry of Science and Higher Education/Nr 0050/RS4/2016/54.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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